QUANTIFYING THE BURDEN OF ANTIBIOTIC RESISTANCE IN THE NETHERLANDS

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Quantifying the Burden of Antibiotic Resistance in the Netherlands

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(met een samenvatting in het Nederlands)

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CHAPTER 1

General introduction

Antibiotic resistance has been a concern since the introduction of antibiotics in human healthcare. In the 1990s, the issue gained prominence due to the spread of resistant variants of Gram-positive micro-organisms within the hospital environment, most notably methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE). In some countries, including the Netherlands, policies were implemented in hospitals to prevent the dispersal of MRSA from one patient to another [1]. These efforts may have been particularly effective, as there is a clear distinction between countries facing MRSA endemicity in their healthcare environments, and countries where the MRSA problem is confined to individual patient clusters [2]. On the other hand, *E. faecium* has had the opportunity to spread in hospital environments much more inconspicuously. First, it emerged as hospital-adapted lineages of amoxicillin-resistant *E. faecium* (ARE), and with this phenotype as the omnipresent backbone in possibly all hospitals worldwide, VRE made its appearance in this environment [3]. In many countries, VRE now has since become a dominant hospital-associated phenotype of *E. faecium* [2]. Others, including the Netherlands, are in a continuous struggle to prevent the definitive settlement of VRE in hospitals [4].

The issue of antibiotic resistance has further escalated due to the dispersal of resistant Gram-negative bacteria in many different reservoirs, including hospitals, the open population, livestock and the environment [5]. The main micro-organism implicated in this spread is *Escherichia coli*, a commensal of the human and animal gut, which is also abundant in the surroundings of humans and animals. Successful multidrug-resistant (MDR) *E. coli* clones have acquired antibiotic resistance determinants colocated on plasmids. The backbone of these plasmids is generally formed by the presence of so-called extended-spectrum β-lactamases (ESBL) of the CTX-M type, or less often of AmpC cephalosporinases [6]. Both confer resistance to an array of β-lactam antibiotics considered essential in human healthcare. The dynamics driving the dispersal of these *E. coli* clones are largely unclear, but include selective pressure in reservoirs due to antibiotic exposure on the microlevel [7] and socioeconomic factors on the macrolevel [8].

MDR *E. coli* poses problems for treatment of infections emerging in the community, and manifests with an influx into the hospital environment [9]. The hospital population, and more widely the population exposed to the healthcare environment, is further confronted with additional threats from MDR Gram-negative bacteria. This threat generally involves the more traditional hospital-associated Gram-negatives including *Klebsiella pneumoniae* (similar to *E. coli* a member of the Enterobacterales order) and non-fermenters such as *Pseudomonas*

aeruginosa and *Acinetobacter baumannii*. These particularly affect the more immunocompromised patients, exposed to invasive devices and antibiotic therapy [10,11]. This is also the setting where the issue of carbapenemases, a β-lactamase with an even wider spectrum of activity than the aforementioned ESBLs and AmpCs, has first been noted [12]. Due to the continuous exchange between the hospitalized patients, open population and other reservoirs, there is a serious concern that carbapemenases may disseminate more widely into the community and in the end, may become as abundant as ESBLs [13].

Reasons for a burden of antibiotic resistance

Ultimately, antibiotic resistance in the form of pan-drug resistance may result in the complete unavailability of effective antibiotics. Yet, effective and safe antibiotics are currently available for the most common forms of antibiotic resistance, e.g. in the form of carbapenems in case of ESBL-producing pathogens [14]. This is less certain in the case of carbapenemase-producing pathogens with extended co-resistance, but in recent years, several alternative β-lactam/β-lactamase inhibitor combinations, e.g. ceftazidime/avibactam, have reached the market and offer safer alternatives for e.g. colistin [15]. Notably, in low-resource settings, effective and safe therapy for ESBL- or carbapenemase-producing pathogens may not always be available [16].

Other mechanisms by which antibiotic resistance may impact patients have been postulated, and one of them revolves around virulence. This connection is directly brought in mind by the term 'superbug', which is often used to portray highly resistant bacterial pathogens. The underlying assumption for this term is that these pathogens are not only more resistant but are also more prone to cause (severe) disease. Yet, until now, there is no clear evidence that resistant bacterial pathogens are systematically more virulent than non-resistant pathogens [17,18]. This may be due to the fact that the most relevant resistance problems involve bacteria that constitute the physiological human flora. These bacteria are opportunistic, facultative pathogens and spent most of their lifetime in relative harmony with their hosts, in contrast to professional pathogens, e.g. the causative pathogen of anthrax, *Bacillus anthracis* [19]. (*Mycobacterium tuberculosis* is a relevant exception in which antibiotic resistance is an issue of major importance in a professional pathogen.) Antibiotic resistance is a very relevant property to acquire for these opportunists as this offers an advantage for maintaining oneself within the flora of the host, especially when antibiotics are used [20]. In contrast, as they do not depend on causing disease to spread from human to human or otherwise, selective processes are less likely to affect their virulence. Most reports on the association between antibiotic resistance and virulence in fact focus on maintenance within the host instead of disease-

causing capacity in case of sepsis [21]. To sum up, the spread of bacterial clones combining multidrug resistance and above average virulence is theoretically possible, but until now, there are no indications that such clones form important resistant Gram-negative subpopulations.

At the same time, there are indications however that the spread of antibiotic resistant clones may directly add to the number of infections occurring, instead of replacing infections caused by their susceptible counterparts. For example, it was shown that hospitals struggling with endemicity of resistant bacteria had similar secular trends in infections caused by antibiotic-susceptible strains as hospitals not facing endemicity of resistant bacteria, while infections by antibiotic-resistant bacteria were on the rise [22]. Also in the community or in the entire body of infections occurring, infections caused by resistant bacteria increase faster than infections caused by susceptible bacteria [23,24]. Yet, in this case, it is harder to prove whether resistant bacteria truly drive this increase or whether they just hold a competitive advantage to exploit an already existing potential for increasing numbers of infection, for example due to changing prevalences of patient risk factors.

A final, and in many instances probably the most important way in which patients are affected by antibiotic resistance, has to do with the fact that detection of antimicrobial resistance in infections is generally delayed, whereas treatment is generally indicated instantly. To overcome this discrepancy, the principle of empiric antibiotic therapy is applied in treatment of infection. Based on clinical parameters observed at presentation, such as the suspected source of infection, patient characteristics, severity of infection, prior antibiotic exposure and prior microbiology results, an initial antibiotic regimen is chosen with a high likelihood of providing appropriate coverage of expected pathogens and their resistance profiles [25]. Later during the course of infection, antibiotics may be tailored to culture results, including the antibiogram of isolated micro-organisms. Empiric therapy, however, involves a trade-off between an as high as possible rate of appropriate coverage, and an as low as possible provision of overly 'broad' therapy. The latter is necessary to minimize the occurrence of adverse effects and the exertion of selective pressure on the patient's microbiome [26]. This means that empiric therapy can never be appropriate in 100% of cases – i.e. all pathogens implied in the infection are tested susceptible to the regimen – even if stratification schemes are applied to provide alternative, 'broader' regimens to patients at high risk of infection with resistant pathogens.

In the Netherlands, the issue of empiric therapy mainly revolves around ESBL-producing Enterobacterales. Traditionally, in many types of infection in which Enterobacterales may play a role, second- or third-generation cephalosporins are prescribed as empiric therapy [27]. These antibiotics are not effective for ESBL-producing Enterobacterales, in which case the optimal antibiotic therapy consists of a carbapenem [14]. Since these ESBL-producing Enterobacterales constitute a minority of infections in which Enterobacterales are involved (5–10% of Enterobacterales produce ESBLs in the Netherlands [28]), second- or third-generation cephalosporins remain the empiric antibiotics of choice, and empiric carbapenems should be restricted to those patients with known risk factors for ESBL-producing Enterobacterales, such as prior colonization. As these risk factors do not have a 100% sensitivity, it is generally observed that patients infected by resistant pathogens are provided appropriate antibiotics later than those infected by the more usual susceptible pathogens. In case of severe infection, the result may be further worsening of sepsis, and ultimately a higher probability of death [29].

Aims of this thesis

The main aim of this thesis is to quantify the burden of resistance problems that the Netherlands is confronted with, specifically within the hospital setting. There will be a particular focus on appropriateness of empiric antibiotic therapy, as this is *a priori* the most likely pathway by which a burden of antibiotic resistance manifests itself. When establishing the burden of resistance, the studies in this thesis will take an approach in which infections with resistant bacteria are replacing their susceptible, more usual counterparts. Any contribution of antibiotic resistance to increasing numbers of infections will be outside the scope of this thesis.

Quantifying the burden of antibiotic resistance is important for several reasons. Widespread knowledge of the societal consequences of antibiotic resistance may impact guidelines and practicing physicians when making decisions regarding antibiotic therapy. Quantifications may also serve as input for policy makers when allotting resources to issues in competition for attention. Finally, understanding of mechanisms in which antibiotic resistance leads to worse patient outcomes, may spur the search for strategies to cope with the issue.

As such, many studies have been performed worldwide on the patient burden of several antibiotic resistance problems, including methicillin-resistant Staphylococcus aureus (MRSA), different types of MDR Gram-negatives, and vancomycin-resistant enterococci [30,31]. As will be elaborated upon below, effects of antibiotic resistance cannot always be generalized from

one setting to the other. Compared to some other settings, the medical microbiology infrastructure in the Netherlands may be particularly well-developed with a close clinical involvement of medical microbiologists, early adoption of rapid molecular diagnostics, national efforts to shape and minimize human antimicrobial use, and the coordinated search-and-destroy policy to prevent the spread of MRSA [32]. Quite possibly as a result, the Netherlands has lower levels of antibiotic resistance in the traditional hospital-associated pathogens compared to neighboring countries [2], but as described, is not exempted from problems with MDR Gram-negatives and VRE. Apart from this, causal inference with regard to the burden of antimicrobial resistance is troublesome, as exemplified below and many of the published studies contain methodological weaknesses [33–35]. Therefore, it is necessary to investigate the burden of antimicrobial resistance in this setting while simultaneously applying an optimized study methodology.

Causal inference with regard to the burden of antibiotic resistance

We apply causal inference techniques in this thesis to establish the extent to which infection outcome (mainly mortality) is truly attributable to the exposure *antibiotic resistance*. Importantly, the burden of antibiotic resistance cannot feasibly be studied in controlled experiments in humans for ethical reasons. As such, any evidence stems from non-experimental studies, in which the course of infections in clinical practice is observed, including the naturally occurring diversity in resistance profiles of causative pathogens and management strategies of treating physicians.

To perform appropriate causal inference studies, variables other than exposure and outcome need to be recorded as well. The reason for this is that confounding needs to be controlled by means of study design (e.g. matching) or analytic methods (e.g. multivariable models or the use of propensity scores) [36]. By making use of so-called directed acyclic graphs (DAG), the interplay between these variables can be studied, and an appropriate selection of confounders can be made, while other variables that generally should not be controlled for (i.e. colliders and mediators), can also be identified [37]. In **Figure 1**, a DAG is presented for studying the effect of antibiotic resistance on mortality. Several issues become apparent:

- Confounding is an essential issue. Patients infected with resistant bacteria have generally been exposed more to antibiotics than patients infected with susceptible bacteria, as such selective pressure creates a microbiome niche for colonization by and outgrowth of resistant bacteria. They also have higher healthcare exposure rates,

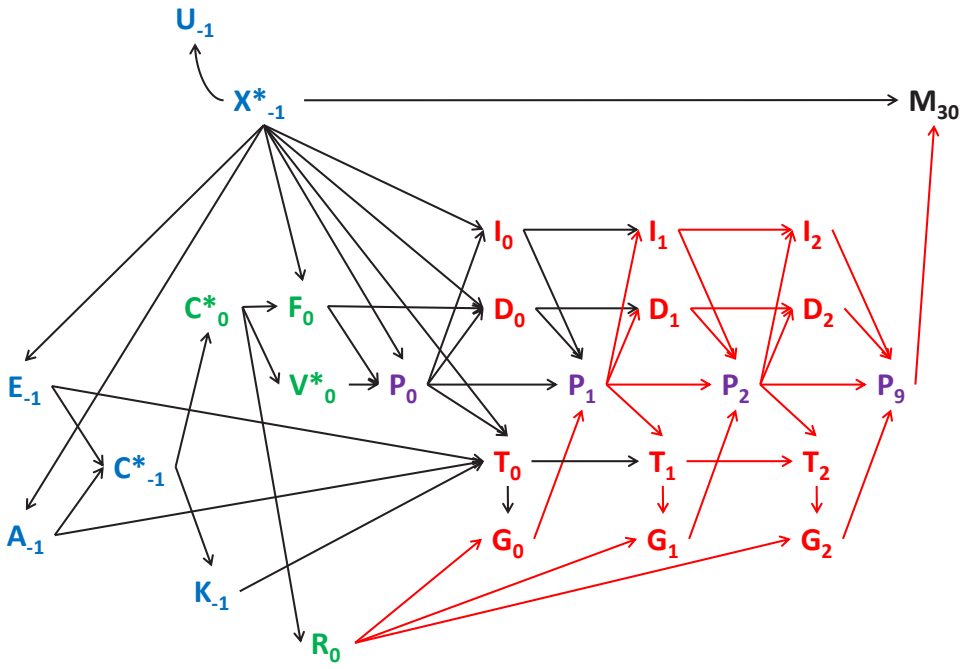

Figure 1. Directed acyclic graph depicting the causal web of variables related to studying the effect of resistance on mortality. Numbers indicate the time relation to the infection, with 0 being the day of onset of infection. For simplicity, states after day 2 of the infection are not further specified. Variables with an asterisk cannot be measured to their full extent with existing methodology.
Confounding variables: X^*_{-1}, underlying disease before onset of infection; U_{-1}, measurable underlying disease before onset of infection; E_{-1}, exposure to healthcare before onset of infection; A_{-1}, exposure to antibiotics before onset of infection; C^*_{-1}, colonizing bacterial strain before onset of infection; K_{-1}, known colonization with bacterial strains before onset of infection.
Infection-related variables: C^*_0, bacterial strain causing infection; F_0, infection source; V^*_0, virulence of bacterial strain; R_0, resistance of bacterial strain.
Mediating variables: I_0 – I_2, supportive care on days 0 through 2 of the infection; D_0 – D_2, source control procedures on days 0 through 2 of the infection; T_0 – T_2, antibiotic therapy on days 0 through 2 of the infection; G_0 – G_2, appropriateness of antibiotic therapy on days 0 through 2 of the infection; P_0 – P_2, disease severity on days 0 through 2 of the infection; P_9, disease severity later during the course of infection.
Outcome: M_{30}, 30-day mortality.

as this is the environment where the probability of becoming colonized with resistant bacteria is the highest. Exposure to antibiotics and healthcare are correlated with the underlying health state of a patient. This implies that patients infected with resistant bacteria already have a higher propensity of dying after the infection, independent of the occurrence of the infection. This confounding pathway needs to be controlled for. However, despite the availability of the Charlson comorbidity index [38], or the

APACHE scoring system [39], it remains very difficult to accurately quantify the underlying disease state of a patient directly prior to infection.

- It is important to discern whether one would like to study the effect of resistant clones in general with all of their associated characteristics including virulence and a predilection for specific infection sources, or whether one would like to focus solely on the fact that the pathogen is resistant. As exemplified before, in the latter case, the only relevant causal pathway involves the delay in appropriate antibiotic therapy. This delay, however, is setting-specific as it depends on local antibiotic treatment guidelines and it depends on how microbiology diagnostics, relay of culture results to the clinic, and subsequent management are shaped locally. If such a narrow focus is applied, variables pertaining to infection severity and source (which are affected by other characteristics of resistant pathogens) should be handled with care, as these variables are not only confounders, but also colliders, and controlling for them might bias the analysis [40].
- If the broader approach to the effect of antibiotic resistance is applied, effects mediated through infection severity and source are included and should not be controlled for. This also implies that apart from the local circumstances described before, the local epidemiology of resistant clones may be a further contributing factor to effects that are specific to a setting.
- It is even harder to establish the causal effect of a delay in appropriate therapy on infection outcome. This is because antibiotic therapy is a time-varying factor with strong latency effects, and there is a continuous interplay between antibiotic therapy and disease severity. Treating physicians generally escalate antibiotic therapy when patients deteriorate. Therefore, the appropriateness of antibiotic therapy at a specific moment during the course of an infection carries information on both the infection severity and resistance profile of the pathogen. Because of this causal structure, including appropriateness of therapy in an analysis may introduce collider stratification bias [40]. A further complication is that mortality and appropriate therapy are so-called competing events [41].

Outline of this thesis

The studies in this thesis start off with a meta-analysis of studies comparing mortality in bacteremia caused by ESBL-producing Enterobacterales to mortality in bacteremia caused by ESBL-negative Enterobacterales (**Chapter 2**). This meta-analysis was performed to evaluate

the state of the literature before the onset of the primary studies in this thesis, and to evaluate how methodological choices affect estimates of the association between ESBL production and mortality. **Chapter 3** then describes a retrospective pilot study on ESBL bacteremias from several Dutch hospitals in the years 2008–2010. It provides an overview of the epidemiology of these infections and analyzes the contribution of initial antibiotic therapy to mortality after infection onset. Then, in **Chapter 4**, the main study of this thesis is described. In a so-called parallel matched cohort study, approximately 2,000 patients with Gram-negative infection (both bacteremic and non-bacteremic) in eight Dutch hospital between 2013 and 2016 are compared to 2,000 patients without infection, and it is assessed whether MDR Gram-negatives are associated with an increased mortality. **Chapter 5** focuses on the consequences of another resistance problem, namely VRE, including data from both the Netherlands and Denmark. VRE bacteremias are compared with regard to mortality to matched ARE bacteremias, and it is evaluated whether an increase in mortality is caused by a delay in appropriate antibiotic therapy.

The final two studies have a different focus. They assess how ESBL-producing Enterobacterales can be anticipated when a patient presents with infection and empiric antibiotic therapy has to be started. In **Chapter 6**, the current stratification scheme applied in the Netherlands is evaluated, which incorporates known colonization status and recent antibiotic use. **Chapter 7** then describes a study in eight Dutch hospitals in which it is investigated whether incorporation of additional clinical parameters available at infection onset in a scoring system can improve on the prediction of ESBL-producing Enterobacterales as causative pathogen in infection. Finally, in **Chapter 8**, the general discussion puts all findings in perspective.

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CHAPTER 2

Effects of confounders and intermediates on the association of bacteremia caused by extended-spectrum β-lactamase-producing Enterobacterales and patient outcome: a meta-analysis

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Abstract

Background and objectives: Bacteremia caused by Enterobacterales (EB) producing extended-spectrum β-lactamase (ESBL+) has been associated with higher mortality compared with non-ESBL-producing (ESBL-) EB bacteremia in observational studies. We conducted a systematic review and meta-analysis of these studies to assess how adjusting for confounding in multivariable analyses affects the pooled estimate, and whether multivariable analyses that include intermediates in the causal pathway of outcome (sepsis severity and inappropriate empiric therapy) have lower estimates of attributable mortality.

Data sources: PubMed search on 23 November 2010 followed by manually searching reference lists of included studies.

Study eligibility criteria: Cohort studies published in English with separate mortality rates for ESBL+ and ESBL- EB bacteremia.

Synthesis methods: Random-effects pooling of unadjusted and adjusted odds ratios (ORs) followed by subgroup analyses to explore effects of adjustment procedures on adjusted ORs.

Results: The pooled OR for the unadjusted mortality associated with ESBL production was 2.35 (95% confidence interval (CI) 1.90–2.91, I^2 = 42%, 32 studies). The pooled adjusted OR was 1.52 (95% CI 1.15–2.01, I^2 = 32%, 15 studies). Adjustment for more intermediates was associated with decreasing ORs. The pooled OR for the analyses adjusting for inappropriate empiric therapy was 1.37 (95% CI 1.04–1.82).

Conclusions: ESBL production in EB bacteremia is associated with a higher mortality compared with bacteremia with ESBL- EB, although the estimate of this association is affected by adjustment procedures. Adjustment for inappropriate empiric therapy leads to a reduction in ORs, indicating that higher mortality is likely to be mediated through this phenomenon.

Introduction

Production of extended-spectrum β-lactamases (ESBLs) renders Enterobacterales (EB) resistant to third-generation cephalosporins, which are the antibiotics that are deployed most often to treat infections caused by these bacteria. As *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. are important pathogens in community- and hospital-onset infections [1], the increasing prevalence of ESBL-producing (ESBL+) bacteria may have serious consequences for patient outcome, especially since ESBL production is associated with co-resistance to other classes of antibiotics [2].

A worse outcome of infections caused by antibiotic-resistant bacteria could result from: (i) a delay between onset of infection and initiation of appropriate therapy; (ii) associations between resistance genes and the presence of virulence genes; and (iii) differences in effectiveness and side effects between antibiotics used for resistant and susceptible pathogens. Differences in outcome from infections caused by antibiotic-resistant pathogens can only be derived from observational studies, which are highly susceptible to confounding. Patients with a higher severity of illness generally require longer hospitalization and more antibiotics, which is associated with higher rates of colonization and infection by resistant bacteria. This implies that the prognosis of such patients, compared with patients infected with susceptible pathogens, is already worse before the onset of the infection [3]. Therefore, adjustment for the relevant confounders is crucial when investigating the causal relationship between antibiotic resistance and patient outcome.

In many studies the presence of inappropriate empiric therapy and septic shock were included as confounders [4]. Yet if antibiotic resistance increases mortality it is likely to be mediated through higher rates of inappropriate empiric therapy and the development of septic shock [4,5]. Such determinants, therefore, are not confounders but intermediates, and adjustment (as if they were confounders) might obscure the true causal relationship. We aimed to quantify the effects of adjustment for true confounders and intermediates on the attributable mortality of bacteremia caused by ESBL+ EB using a systematic review and meta-analysis approach.

Methods

Literature search and study selection

On 23 November 2010 the following search was performed in the PubMed database, applying tags for free text in titles and abstracts:

(esbl* OR extended spectrum beta lactamase*) AND (blood stream infection* OR bloodstream infection* OR bacteraemia* OR bacteremia* OR septicaemia* OR septicemia*) AND (mortal* OR fatal* OR lethal* OR death* OR dead OR surviv* OR alive OR outcome*).

No limits were set. Abstracts of all references identified were reviewed by W.C.R. and potentially relevant studies were reviewed in full. Reference lists were checked in an attempt to identify additional studies. Studies were included if they were observational cohorts (whether prospective or retrospective) providing separate mortality rates for patients that had developed bacteremia caused by ESBL+ and non-ESBL-producing (ESBL-) EB. Reducing the number of cases, specifying a domain or matching ESBL- cases to ESBL+ cases was allowed, as long as no cases were omitted based on resistance properties. Studies had to be written in English. The preferred definition of mortality was day 30 all-cause mortality, but if not available, other definitions were used with preference for the one closest to day 30 all-cause mortality. Hence, all-cause mortality took preference over infection-related mortality.

Data extraction

From the studies matching our inclusion criteria, the following data were extracted by W.C.R. with the help of structured data forms: characteristics of the study (location of study; period of study; hospital type(s) included; study design; inclusion of hospital-onset infections, community-onset infections or both, and definition thereof; ages of patients included; patient wards included; other inclusion and exclusion criteria; pathogens studied; and definitions of mortality, inappropriate empiric therapy and septic shock); ESBL+ and ESBL- group sizes; ESBL+ and ESBL- mortality rates; and characteristics of the study population (ESBL prevalence; mean age; and proportions of patients with infections being nosocomial, with the urinary tract as bacteremia source, with septic shock, treated in the intensive care unit at bacteremia onset, in each McCabe-Jackson category, with neutropenia and with polymicrobial infections). Mean length of stay before onset of bacteremia was also extracted where possible.

Odds ratios (ORs) for ESBL production from adjusted analyses (referred to as aORs) were also collected, including information on whether corrections were performed for inappropriate empiric therapy, underlying disease severity and/or sepsis severity (by means of severe sepsis/septic shock or scoring systems used at onset of bacteremia). Adjustments for inappropriate empiric therapy and sepsis severity were classified as adjustments for intermediates. Adjustment for underlying disease was defined as adjusting for at least one of

the following six variables: (i) a range of separate comorbidities; (ii) more than two comorbidities from that range; (iii) the Charlson comorbidity index; (iv) the McCabe-Jackson score; (v) a scoring system used before onset of bacteremia; and (vi) length of stay before onset of bacteremia [6]. In the absence of relevant data, authors were requested to provide additional information.

We developed a modified Newcastle-Ottawa scale to judge the quality of included studies (see the **Supplementary Material**) [7]. The total score (ranging from 0 to 9) was split into a selection-outcome score (ranging from 0 to 7) and a comparability score reflecting adjustment, selection or matching procedures (ranging from 0 to 2). Furthermore, in the case of multivariable analyses, we collected data on the covariate to event ratio in the final model and the explicit reporting of the procedure behind the model and of the variables eligible for inclusion in the model.

Data analysis

The meta-analysis was performed using Comprehensive Meta Analysis version 2 (Biostat). A random-effects model was applied, as heterogeneity was assumed *a priori* to be high. Heterogeneity was reported using the *Q* statistic (including its significance) and the I^2 measure. A funnel plot of standard errors against log unadjusted ORs (uORs) was used to assess publication bias. Subgroup analyses were performed using a mixed-effect analysis. Mixed-effect meta-regressions were performed using the maximum likelihood method. *P*-values <0.05 were considered significant.

The uORs and 95% confidence intervals (CIs) for mortality rates using ESBL as the independent variable were calculated and pooled. Sensitivity analysis using outliers in study size or uOR were performed. The effects of study characteristics and population characteristics on uORs for mortality were assessed by means of subgroup analyses and meta-regression.

The aORs and 95% CIs for ESBL production from studies that included a multivariable analysis of mortality were pooled. If the aOR with its 95% CI was not available, but ESBL was reported not to be significantly associated with mortality, an aOR of 1 was imputed, and the standard error of the unadjusted analysis was used as the measure of dispersion [8]. Variables were categorized as having been adjusted for if they were in the final multivariable model, but also if they had been included in a stepwise selection procedure (e.g. univariable testing), but had not ended up in the final model. The effects of decisions to correct for particular variables were assessed using subgroup analyses.

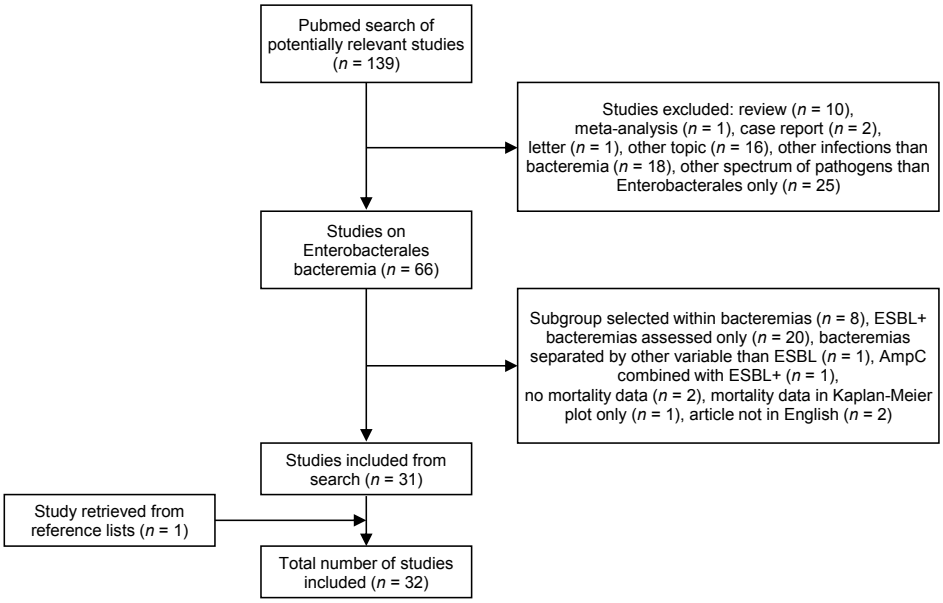

Figure 1. Flow chart of the study.

Subgroup analyses were also performed to relate scores on the Newcastle-Ottawa scale and quality indicators of the regression analysis to either uORs or aORs. This meta-analysis was reported according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guideline [9].

Results

In the PubMed search, 139 articles were identified, of which 31 met the inclusion criteria for meta-analysis (**Figure 1**) [10–40]. One other study was identified in reference lists of selected articles [41], increasing the number of included studies to 32 (**Table 1**).

Pooling of the 32 uORs yielded a pooled uOR for mortality due to ESBL+ EB bacteremia of 2.35 (95% CI 1.90–2.91), with moderate heterogeneity (Q = 53.68, p <0.01, I^2 = 42%; **Supplementary Figure 1**). A funnel plot revealed the possibility of publication bias, as small studies showing small effects were missing (**Figure 2**). Five studies in the lower right were not balanced by studies on the left side of the funnel plot, and exclusion of these changed the pooled uOR to 2.18 (95% CI 1.79–2.65; Q = 38.97, p = 0.05, I^2 = 33%). Exclusion of the largest study with 4,758 patients hardly changed the uOR (2.40, 95% CI 1.91–3.01).

Table 1. Study characteristics

Ref	First author	Year	Country	Study period	Study design	Study population	Pathogens	Mortality definition	ESBL+ group size, *n*[a]	ESBL- group size, *n*[a]	ESBL+ mortality, *n* (%)	ESBL- mortality, *n* (%)
10	Ariffin	2000	Malaysia	01/1996–12/1997	prospective	≤12 years on pediatric oncology unit with neutropenia	*K. pneumoniae*	related	16	15	8 (50)	2 (13)
11	Blomberg	2005	Tanzania	08/2001–08/2002	retro- or prospective	≤7 years	*E. coli* *Klebsiella* spp. *Salmonella* spp.	in hospital	19/14	106/85	10 (71)	33 (39)
12	Borer	2002	Israel	01/1997–08/1997	retrospective	>18 years CO only	EB	?	6	113	5 (83)	16 (14)
13	Cordery	2008	UK	03/2004–03/2006	retrospective incomplete cohort[b]	adults on ICU[c]	*E. coli* *Klebsiella* spp.	in ICU	16	39	11 (69)	14 (36)
14	Daikos	2007	Greece	11/2003–06/2005	prospective	not restricted	EB	14 day	23[d]	210[d]	4 (17)	24 (11)
15	Du	2002	China	01/1997–12/1999	retrospective	HO only	*E. coli* *K. pneumoniae*	in hospital	23	62	3 (13)	18 (29)
16	Endimiani	2005	Italy	01/1997–06/2004	retrospective	not restricted	*P. mirabilis*	1 month? (related)	11/9	14	3 (33)	2 (14)
17	Gudiol	2010	Spain	01/2006–10/2008	prospective	adult cancer patients and HSC transplant recipients	*E. coli*	(7 day) 30 day	17	118	6 (35)	23 (19)
18	Ho	2002	China	01/1996–12/1998	retrospective incomplete cohort[e]	not restricted	*E. coli*	30 day	50	100	9 (18)	7 (7)
19	Kang	2010	South Korea	10/2006–09/2007, 09/2008–04/2009	multicenter retrospective	CO only	*E. coli*	30 day	82/40	783/516	6 (15)	39 (8)
20	Kim BN	2002	South Korea	07/1999–06/2000	retrospective	≥15 years	*K. pneumoniae*	related	44/43	118/115	10 (23)	23 (20)
21	Kim YK	2002	South Korea	11/1993–12/1998	retrospective	≤17 years	*E. coli* *K. pneumoniae*	related	49/45	93/87	12 (27)	5 (6)

Table 1 (continued)

Ref	First author	Year	Country	Study period	Study design	Study population	Pathogens	Mortality definition	ESBL+ group size, *n*[a]	ESBL- group size, *n*[a]	ESBL+ mortality, *n* (%)	ESBL- mortality, *n* (%)
22	Marchaim	2010	Israel	11/2006–02/2008	multicentre prospective incomplete cohort[f]	>18 years CO only	EB	in hospital (related)	205/185	242/216	55 (30)	23 (11)
23	Marra	2006	Brazil	01/1996–05/2001	retrospective	HO only	*K. pneumoniae*	15 day	56	52	18 (32)	8 (15)
24	Melzer	2007	UK	06/2003–11/2005	prospective	≥16 years	*E. coli*	30 day	46	308	28 (61)	73 (24)
25	Memon	2009	Saudi Arabia	01/2006–12/2007	retro- or prospective	adults	*E. coli* *K. pneumoniae*	30 day (related)	29	80	6 (21)	18 (23)
26	Menashe	2001	Israel	01/1997–08/1997	retro- or prospective	>18 years HO only	EB	in hospital + 28 day after discharge	26	29	13 (50)	11 (38)
27	Mosqueda-Gómez	2008	Mexico	01/1993–12/2002	retrospective	adults	*K. pneumoniae*	all-cause	17	104	6 (35)	28 (27)
28	Ortega	2009	Spain	01/1999–12/2007	prospective	not restricted	*E. coli*	30 day	211	4547	33 (16)	413 (9)
29	Panhotra	2004	Saudi Arabia	07/2001–07/2003	retrospective	HO only	*K. pneumoniae*	related	10	16	6 (60)	1 (6)
41	Paterson	2004	inter-national	01/1996–12/1997	multicentre, prospective	>16 years HO only	*K. pneumoniae*	14 day	78	175	21 (27)	40 (23)
30	Peña	2001	Spain	05/1993–06/1995	prospective	adults HO only	*K. pneumoniae*	in hospital (related)	49	43	16 (33)	12 (28)
31	Rodríguez-Baño	2010	Spain	10/2004–01/2006	multicentre prospective incomplete cohort[g]	>14 years CO only	*E. coli*	14 day	95	188/187	16 (17)	15 (8)
32	Schwaber	2006	Israel	01/2000–12/2003	retrospective incomplete cohort[h]	adults	*E. coli* *Klebsiella* spp. *Proteus* spp.	in hospital (related)	99	99	35 (35)	18 (18)
33	Superti	2009	Brazil	06/2004–03/2006	retrospective	≥19 years HO only	*E. coli* *K. pneumoniae*	60 day	51	94	26 (51)	28 (30)

Table 1 (continued)

Ref	First author	Year	Country	Study period	Study design	Study population	Pathogens	Mortality definition	ESBL+ group size, *n*[a]	ESBL- group size, *n*[a]	ESBL+ mortality, *n* (%)	ESBL- mortality, *n* (%)
34	Szilágyi	2009	Hungary	01/2005–12/2008	multicentre retrospective incomplete cohort[b]	HO only	*K. pneumoniae*	in hospital (related)	100	100	36 (36)	23 (23)
35	Trecarichi	2009	Italy	01/2000–12/2007	retrospective	≥15 years on hematology ward	*E. coli*	30 day	26	36	11 (42)	2 (6)
36	Tsai	2010	Taiwan	01/2005–12/2006	retrospective	DM patients	*K. pneumoniae*	in hospital[i]	27[j]	166[j]	11 (41)	35 (21)
37	Tumbarello	2006	Italy	01/1999–12/2003	retrospective	not restricted	*K. pneumoniae*	(7 day) 21 day	48	99	25 (52)	29 (29)
38	Tumbarello	2010	Italy	01/2006–12/2006	retrospective	≥18 years	*E. coli*	21 day in hospital	37	97	11 (30)	6 (6)
39	Tuon	2010	Brazil	01/2006–01/2009	retrospective	>12 years	*Enterobacter* spp.	30 day	28	30	14 (50)	14 (47)
40	Zaoutis	2005	USA	05/1999–09/2003	retrospective incomplete cohort[b]	children	*E. coli* *Klebsiella* spp.	in hospital	35	105	8 (23)	14 (13)

The table shows characteristics of the 32 studies that were retrieved in the PubMed search and by checking reference lists of included studies. Mortality definitions in brackets are not used in the analyses presented.

Abbreviations: CO, community-onset; DM, diabetes mellitus; HO, hospital-onset; HSC, hematopoietic stem cell; ICU, intensive care unit; ref, reference.

[a] The number before a forward slash indicates the total number of episodes included and the number after a forward slash indicates the number taken into account in the univariable analysis of mortality.

[b] Random selection from all ESBL- cases.

[c] Including patients 72 h post-ICU discharge, excluding neurosurgical and cardiothoracic patients.

[d] Designed as integron+ versus integron-.

[e] ESBL- cases matched on specialty, sex, age and isolation date.

[f] ESBL- cases matched on date in the same hospital.

[g] Random selection from all ESBL- cases the month following the ESBL+ case in the same hospital.

[h] Matched on pathogen.

[i] Including critical discharge against medical advice.

[j] Designed as community-acquired versus nosocomial.

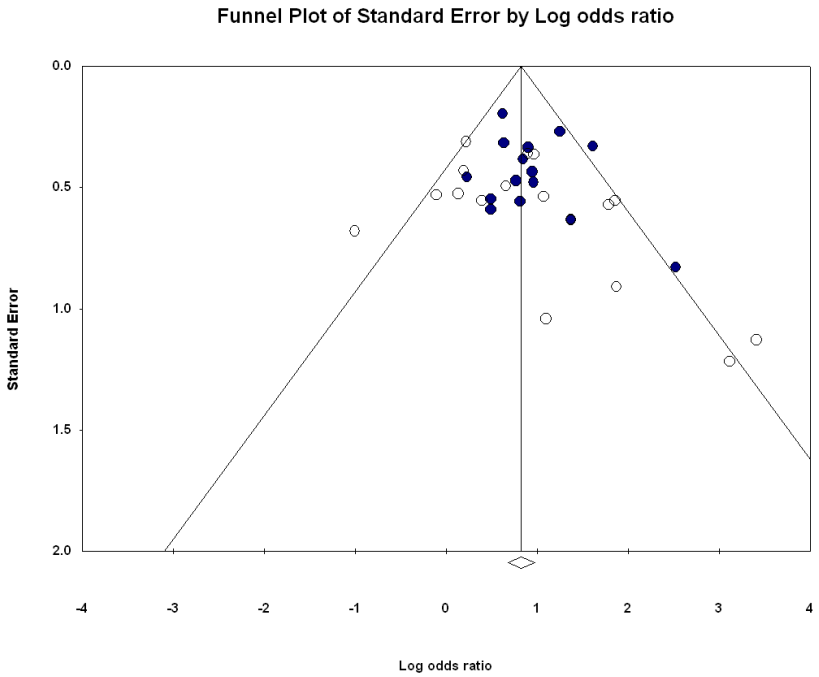

Figure 2. Funnel plot for uORs. The 15 studies from which the aOR was pooled are indicated by filled circles.

Results of subgroup analyses and meta-regression

Subgroup analyses of unadjusted results based on study design, definitions of mortality, pathogens included, patient groups included and origin of bacteremia did not yield statistically significant differences in pooled uORs (**Table 2**). Multivariable analyses were performed in 17 studies. From eight of these, aORs for mortality due to ESBL were available, either published (n = 7) or obtained after contacting authors (n = 1). For the eight studies that reported aORs, the pooled uOR for mortality was higher than for the nine studies that performed multivariable analyses without reporting aORs (3.02 (95% CI 2.21–4.13) versus 1.83 (95% CI 1.35–2.49), p = 0.03; **Table 2**).

In meta-regression, including 13 studies of adults, the uOR for mortality due to ESBL+ EB bacteremia was associated with the mean age of the population studied, with an increase in uOR of 0.03 per year increase in mean age (p = 0.02; **Supplementary Table 1**; patient population characteristics are shown in **Supplementary Table 2**). Each 1% increase in patients

Table 2. Subgroup analyses of uORs

	No. of studies	No. of patients	uOR (95% CI)	I^2, %	p^a
All studies	32	9,612	2.35 (1.90–2.91)	42	
Direction of design					
prospective	9	6,539	2.29 (1.61–3.27)	51	
retrospective	20	2,810	2.51 (1.87–3.39)	42	0.66
unknown	3	263	1.70 (0.75–3.84)	38	
Design					
incomplete cohort	7	1,426	2.59 (1.96–3.43)	0	
prospective complete cohort	7	5,856	2.10 (1.35–3.26)	54	0.67
retrospective complete cohort	15	2,067	2.66 (1.74–4.05)	55	
unspecified complete cohort	3	263	1.70 (0.75–3.84)	38	
Definition of mortality					
all-cause fixed <28 days	6	1,157	2.26 (1.48–3.45)	35	
all-cause fixed 28–31 days	8	6,096	2.64 (1.70–4.09)	46	
all-cause in hospital	8	1,408	2.08 (1.41–3.09)	46	0.79
other	6	604	2.21 (1.17–4.19)	50	
related	4	347	4.41 (1.3–14.94)	68	
Included pathogens					
E. coli/Klebsiella spp.	24	8,426	2.29 (1.79–2.94)	46	
E. coli/Klebsiella spp. and others	6	1,105	2.90 (1.82–4.63)	32	0.36
species other than *E. coli/Klebsiella* spp.	2	81	1.39 (0.55–3.49)	0	
Ages included					
adults	17	2,731	2.36 (1.71–3.25)	55	
all	11	6,479	2.08 (1.56–2.78)	19	0.27
children	4	402	3.58 (1.97–6.48)	0	
Origins included					
both	20	7,290	2.53 (1.95–3.28)	33	
community-onset only	4	1,358	3.18 (1.76–5.76)	45	0.16
hospital-onset only	8	964	1.68 (1.09–2.59)	46	
Multivariable analysis					
not performed	15	1,746	2.41 (1.64–3.56)	50	0.95
performed	17	7,866	2.38 (1.85–3.06)	36	
OR available	8	2,108	3.02 (2.21–4.13)	25	0.03
OR not available	9	5,758	1.83 (1.35–2.49)	12	

The table shows a subgroup analysis of study characteristics that may have had an effect on the outcome reported, i.e. the uOR for the association between ESBL production and mortality.

[a] *P*-value of mixed-effect analysis.

classified as having rapidly fatal underlying disease by the McCabe-Jackson score was also significantly associated with an increase in uOR of 0.03 (p = 0.05), although just nine studies could be included in the meta-regression. Three other characteristics tended to be associated with the uOR for mortality: the percentage of patients with the urinary tract as source of bacteremia (0.01 increase in uOR per 1% increase, p = 0.08), the percentage of patients suffering from neutropenia (slope 0.01, p = 0.07) and the percentage of patients developing septic shock during bacteremia (slope −0.09, p = 0.07). However, data on septic shock were available from only seven studies.

Results after adjustments

The association between ESBL+ EB bacteremia and mortality was investigated through multivariable analysis in 17 studies [11,13–15,17,19,22–24,26,28,30–32,34–36], three of which included separate multivariable analyses with and without adjustment for inappropriate empiric therapy [22,24,31]. One multivariable analysis was excluded, as only the variable 'treatment failure' was in the final model, a variable not used in any of the other multivariable analyses [15]. This resulted in 16 multivariable analyses that were analyzed in more detail, and aORs for mortality were available from 8 analyses [13,19,22,24,31,32,34,35]. In seven of the eight studies that did not provide an aOR for mortality, it was reported that ESBL was not statistically significantly associated with mortality, and an aOR of 1 was imputed [14,17,23,26,28,30,36]. In the remaining study ESBL reportedly was significantly associated with mortality, but an aOR was not available [11].

Pooling of 15 studies yielded an aOR of 1.52 (95% CI 1.15–2.01) with moderate heterogeneity (Q = 20.49, p = 0.12, I^2 = 32%; **Figure 3**). From the three studies that presented two aORs, the aOR closest to 1 was taken. Without the seven imputed aORs, this pooled aOR would have been 2.27 (95% CI 1.64–3.13). In the funnel plot of the uORs (**Figure 2**), the 15 studies had a distribution pattern similar to the entire set of studies.

Adjustment procedures applied were considerably distinct among the studies (**Supplementary Table 3**). Of the 18 multivariable analyses (including three studies with two multivariable analyses each), 2 did not adjust for intermediates (i.e. sepsis severity and inappropriate empiric therapy), 6 adjusted for one of these two variables and 10 adjusted for both variables. Pooled aORs were 2.87 (95% CI 1.57–5.26), 2.11 (95% CI 1.41–3.16) and 1.39 (95% CI 1.01–1.92), respectively, and this decrease was nearly statistically significant (p = 0.07)

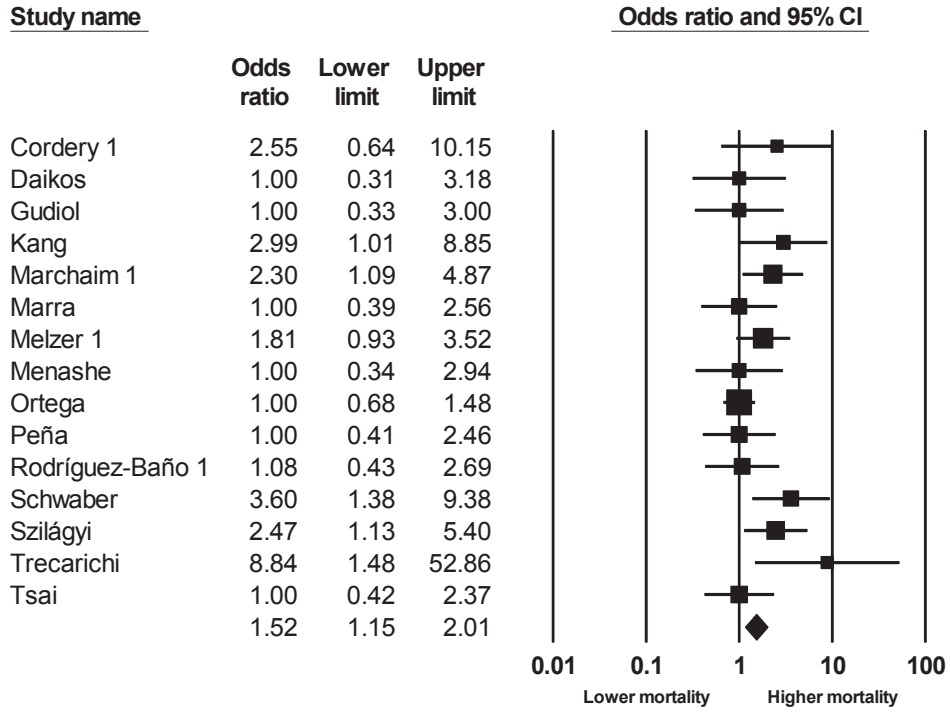

Figure 3. Meta-analysis of aORs. The aORs for the effect of ESBL production on mortality reported in each study were pooled. For studies reporting ESBL as not significantly associated with mortality on multivariable analysis, and not presenting an OR, an OR of 1 was imputed with the standard error copied from the unadjusted analysis. ORs >1 indicate a higher mortality in the ESBL+ group.

(**Table 3**). Adjustment for inappropriate empiric therapy, performed in 12 studies, was associated with lower aORs (1.37 (95% CI 1.04–1.82) versus 2.77 (95% CI 2.13–3.60), $p < 0.001$).

In two analyses, adjustment for underlying disease was incorporated without adjustment for any intermediate variables. The pooled aOR for these studies was 2.87 (95% CI 1.57–5.26). The pooled aOR for the five studies that adjusted for one intermediate in addition to adjusting for underlying disease was 1.90 (95% CI 1.20–3.02), still significantly higher than 1.

Study quality assessment

uORs were not affected by the selection-outcome score calculated from our modified Newcastle-Ottawa scale or the completeness of follow-up (**Supplementary Table 4**). However, studies including several episodes per patient reported significantly lower ORs than studies not explicitly doing so (1.60 (95% CI 1.09–2.35) versus 2.53 (95% CI 2.00–3.21), p =

Table 3. Effects of method of adjustment on aORs

	No. of analyses	No. of patients	aOR (95% CI)	I^2, %	p^a
All studies[b]	15	7,682	1.52 (1.15–2.01)	32	
Adjustments for intermediates					
none	2	398	2.87 (1.57–5.26)	0	
inappropriate empiric therapy or sepsis severity	6	1,340	2.11 (1.41–3.16)	37	0.07
inappropriate empiric therapy and sepsis severity	10	6,981	1.39 (1.01–1.92)	30	
Adjustment for inappropriate empiric therapy[c]					
no	5	1,435	2.77 (2.13–3.60)	0	<0.001
yes	12	7,229	1.37 (1.04–1.82)	22	

Subgroup analysis of adjustment procedures that may have had an effect on the reported or imputed aOR for the association between ESBL production and mortality.

[a] *P*-value of mixed-effect analysis.

[b] Three studies presented two multivariable analyses, one with inclusion of inappropriate empiric therapy and one without. The aOR of the multivariable analysis with inclusion of inappropriate empiric therapy was incorporated into this pooled aOR.

[c] One analysis was excluded, as it was unclear whether correction for inappropriate empiric therapy occurred.

0.05). Relating the comparability score to aORs did not lead to significant results. Explicit reporting of the procedure of the multivariable analysis and the variables eligible for inclusion did not influence the aORs found, and studies with a covariate to event ratio >10 did not have different aORs when compared with studies having lower ratios.

Discussion

This meta-analysis provides evidence that ESBL+ EB bacteremia is associated with increased mortality, even after adjustment for some obvious confounders. The finding that lower ORs for mortality are derived from studies that adjust for inappropriateness of initial antibiotic therapy supports the concept that this contributes to mortality. Furthermore, many investigators have adjusted for parameters that act as intermediates rather than confounders, which may well underestimate true associations between ESBL+ EB bacteremia and outcome. Moreover, there was evidence for publication bias, but there was no evidence that this markedly affected our study results. Finally, there is considerable heterogeneity among unadjusted study results, which can be explained partly by the association between, on the one hand, the outcome of ESBL+ EB bacteremia and, on the other hand, the mean age of the study population and the proportion of the study population qualified as rapidly fatal with the

McCabe-Jackson score. This suggests that bacteremia with an ESBL+ pathogen has more severe consequences in elderly patients and in patients with severe comorbidities.

Our estimate of ESBL-associated mortality (pooled uOR 2.35 (95% CI 1.90–2.91)) based on uORs from 32 studies is comparable to the relative risk of 1.85 (which can be converted into an OR of 2.33 [42]) obtained in a previous meta-analysis that included 16 studies [43].

The primary outcome of our study, the aOR including as many data as possible (pooled aOR 1.52 (95% CI 1.15–2.01)), was intentionally biased towards 1, as we used imputation of non-significant aORs in multivariable analyses and included the lowest aOR if studies presented multiple adjusted aORs. Nevertheless, even with these intentional biases and the fact that underlying disease was not adjusted in some studies, whereas other investigators adjusted for intermediates, the pooled aOR remained above 1. We consider this a strong indication that ESBL+ EB bacteremia is associated with a worse outcome than episodes with ESBL- EB. This is further supported by the finding that five studies adjusting for underlying disease still had a pooled aOR significantly higher than 1, although adjustment for one intermediate was simultaneously incorporated.

Adjustment for inappropriate empiric therapy greatly reduces the association between ESBL production and higher mortality, and this finding supports the hypothesis that higher mortality in infections with highly resistant microorganisms is mediated through this phenomenon. In a large meta-analysis of studies on septic patients, inappropriate empiric therapy was shown to increase mortality rates significantly [8]. This has also been reported for methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia [44], although reported findings are inconclusive. For instance, two recent studies failed to identify either methicillin resistance or inappropriate empiric therapy to be associated with mortality [45,46].

Our study also identified important inconsistencies and omissions in published papers. ESBL production is often not forced into the multivariable model, even when its association with outcome is the primary aim of the study. Furthermore, occurrence of polymicrobial bacteremia and multiple episodes in individual patients are frequently not described, as is true for details of the timing of assessment of variables (see also McGregor *et al.* [4]). For instance, the McCabe-Jackson score can be used as a measure of underlying disease, but also as a measure of sepsis severity when determined at the onset of bacteremia. Furthermore, there is a large amount of heterogeneity between definitions for nosocomial infections, appropriateness of therapy, septic shock and mortality.

We recommend that new studies force ESBL production into the final multivariable model. Moreover, we advise, in agreement with Schwaber and Carmeli's proposal [5], to present the results of multivariable analyses with and without inappropriate therapy. Thereby, both the full effect of ESBL production on mortality and a possible effect apart from inappropriate therapy, e.g. due to increased virulence, can be judged. In analyses including inappropriate therapy it is imperative to adjust for sepsis severity as well, as it is a confounder in that case [47]. However, the severity should be assessed immediately before the administration of empiric therapy, and not afterwards, as it will represent an intermediate variable in these cases. Unfortunately, only 7 of 17 studies in our meta-analysis referring to hypotension, severe sepsis or septic shock mentioned when sepsis severity was assessed.

Our study has several limitations. The included studies were very heterogeneous in their designs and patient populations, although the heterogeneity in outcome (as measured as I^2) was moderate in most analyses. We also focused on only three variables for adjustment, and other potential confounders, such as the source of the bacteremia, the presence of immune suppression, where the infection developed (community or nosocomial) and functional capacity at baseline, were not analysed thoroughly, although some were addressed in the Newcastle-Ottawa scale.

Because of these limitations, our aOR for mortality associated with ESBL+ EB bacteremia should not be interpreted as a precise estimate. We have used the meta-analytical approach to investigate and demonstrate that this estimate is susceptible to adjustment. The finding that even the most conservative adjusted estimate indicates a statistically significant association between ESBL+ EB bacteremia and mortality, and that adjustment for inappropriate empiric therapy reduces the association, supports the hypothesis that this infection indeed increases mortality and that this is mediated through inappropriate empiric therapy.

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CHAPTER 2
SUPPLEMENTARY MATERIAL

Effects of confounders and intermediates on the association of bacteremia caused by extended-spectrum β-lactamase-producing Enterobacterales and patient outcome: a meta-analysis

Modified Newcastle-Ottawa quality assessment scale for cohort studies

Based on: Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. [Internet]. Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp [accessed 2011 Jan 6]

A study can be awarded a maximum of one star (*) for each numbered item within the *selection* and *outcome* categories. A maximum of two stars can be given for *comparability*.

Selection

1. Representativeness of the exposed cohort:
    a. ** all consecutive extended-spectrum β-lactamase (ESBL) producing (ESBL+) cases (or through surveillance system), and >95% of eligible cases actually included*
    b. ** reference to all cases, a database or overview of bacteremia counts, and >95% of potential cases actually included*
    c. no references such as consecutive, surveillance system, all cases, database or overview of bacteremia counts
    d. selected group of ESBL+ cases included
    e. <95% of eligible cases actually included
    f. no description of the derivation of the cohort

    Random samples and restrictions of the domain are allowed
2. Selection of the non-exposed cohort:
    a. ** all non-ESBL-producing (ESBL-) cases included*
    b. ** random sample of ESBL- cases included*
    c. ** consecutive ESBL- case(s) included after ESBL+ case, may be on a per hospital basis*
    d. ESBL- cases matched to ESBL+ cases
    e. no description of the derivation of the non-exposed cohort
3. Ascertainment of exposure:
    a. ** microbiological methods for (ESBL) detection described and involves β-lactamase inhibitor*
    b. description does not involve β-lactamase inhibitor
    c. no description

4. Demonstration that outcome of interest was not present at start of study:
    a. ** only first episode of Enterobacterales bacteremia included*
    b. ** multiple episodes per patient could be included, but only used in descriptives section*
    c. multiple episodes per patient could be included, also used in mortality analysis
    d. ** no clear description of handling multiple episodes in one patient*

Comparability

1. Comparability of cohorts reached by selection, matching or multivariate analysis:
    a. ** study controls for underlying disease*
    b. ** study controls for at least 3 out of: old age, neutropenia, source of infection, length of stay before onset, nosocomial acquisition of infection*

Outcome

1. Assessment of outcome:
    a. ** in-hospital mortality assessed **
    b. ** mortality at fixed point in time assessed with adequate description of follow-up after discharge*
    c. mortality at fixed point in time assessed with inadequate description or without description of follow-up after discharge
    d. infection-related mortality assessed
    e. follow-up period for mortality not defined
2. Was follow-up adequate for outcomes to occur?
    a. ** yes, all-cause mortality with fixed time-point ≥14 days from onset*
    b. no, all-cause mortality at earlier fixed time-point assessed
    c. no, in-hospital mortality assessed
    d. no, infection-related mortality assessed
    e. follow-up period for mortality not defined
3. Adequacy of follow up of cohorts:
    a. ** complete follow up – all subjects accounted for*
    b. ** subjects lost to follow up unlikely to introduce bias – small number lost: >90% follow up, or description provided of those lost*
    c. follow up rate <90% and no description of those lost
    d. no statement

Supplementary Table 1. Meta-regression of uORs

Significant (p <0.05)			
Mean age in adult studies	13 studies	slope 0.03 for each 1 year increase	p = 0.02
% patients with rapidly fatal disease	9 studies	slope 0.03 for each 1% increase	p = 0.05
Trend (0.05 < p < 0.10)			
% urinary tract infection as source	26 studies	slope 0.01 for each 1% increase	p = 0.08
% neutropenic patients	13 studies	slope 0.01 for each 1% increase	p = 0.07
% patients with shock	7 studies	slope -0.09 for each 1% increase	p = 0.07
Not significant (p >0.10)			
Study size excluding largest 2	30 studies		
ESBL prevalence	30 studies		
% nosocomial excluding 0% and 100%	15 studies		
Mean length of stay before bacteremia	12 studies		
% patients in ICU	10 studies		
% polymicrobial bacteremias	11 studies		

Meta-regression results for study characteristics and patient characteristics that may have had an effect on the outcome reported, i.e. the uOR for the association between ESBL production and mortality.
Abbreviations: ESBL, extended-spectrum β-lactamase; ICU, intensive care unit; uOR, unadjusted odds ratio.

Supplementary Table 2. Patient characteristics and scores on the Newcastle-Ottawa scale of included studies

Reference	First author	Year	Characteristics of included patients										Newcastle-Ottawa scale	
			ESBL prevalence	Mean age	% nosocomial bacteremias	% urinary tract as source	% with septic shock	Mean length of stay before bacteremia	% in ICU at onset	% rapidly fatal[a]	% neutropenic	% with polymicrobial infection	Selection-outcome score	Comparability score
10	Ariffin	2000	52	6				13.4			100		4	0
11	Blomberg	2005	15									12	5	0
12	Borer	2002	5	65	0	65							5	0
13	Cordery	2008	18	56		11			100				6	1
14	Daikos	2007	10	68	69	50		10.3	3	16		0	6	2
15	Du	2002	27	49	100	8	(11[b])	(45% >2 wk)	(11[c])	12	12		6	0
16	Endimiani	2005	44	69	39	30	9	9.4		13			7	0
17	Gudiol	2010	13	59	89	24	7				40	18	5	0
18	Ho	2002	7	77	41	52						0	3	0
19	Kang	2010	10	64	0	39	(17[b])				4		5	2
20	Kim BN	2002	27	54	54	12			(6[c])	15			4	0
21	Kim YK	2002	35	6	73	8	(11[b])				61	7	5	0
22	Marchaim	2010		74	0	62	(19[b])			23	1		4	2
23	Marra	2006	51	27	100	11	(20[b])	(med 15.5)	45	16			6	2
24	Melzer	2007	13	(64% >70)	32	68	(29[b])		5		3		6	1
25	Memon	2009	27	60	39	44	(43[b])				15		5	0
26	Menashe	2001	47	(med 65)	100	22	17	(med 13)	27				5	2
27	Mosqueda-Gómez	2008	14	48	53			13.0	(26[c])				4	0

Supplementary Table 2 (continued)

Reference	First author	Year	Characteristics of included patients										Newcastle-Ottawa scale	
			ESBL prevalence	Mean age	% nosocomial bacteremias	% urinary tract as source	% with septic shock	Mean length of stay before bacteremia	% in ICU at onset	% rapidly fatal[a]	% neutropenic	% with polymicrobial infection	Selection-outcome score	Comparability score
28	Ortega	2009	4	65	29	55	14			4	8	9	6	2
29	Panhotra	2004	38	45	100	12		28.0					5	0
41	Paterson	2004	31	(med 58)	100	17		25.0	27		14	20	5	0
30	Peña	2001	53	60	100	8	(13[b])		70	2			5	1
31	Rodríguez-Baño	2010	7[d]	69	0	62	8				6		6	2
32	Schwaber	2006		74	44	37		10.1		20			4	2
33	Superti	2009	35	60	100	23		19.5	(37[c])			81	6	0
34	Szilágyi	2009	40	56	100			14.5					5	1
35	Trecarichi	2009	42	(med 58.5)	73	11	6				68		6	1
36	Tsai	2010	14	64	24	28						21	5	2
37	Tumbarello	2006	33	60	87	21	(7[b])	32.0	23				6	0
38	Tumbarello	2010	28	62	50	49	10		7			0	6	0
39	Tuon	2010	48	52				20.7	(57[c])				6	0
40	Zaoutis	2005	12	5				32.2	39		14	16	5	0

Patient characteristics and scores on the modified Newcastle-Ottawa scale (see the section **Modified Newcastle-Ottawa quality assessment scale for cohort studies** in this **Supplementary Material**) of the 32 studies that were retrieved in the PubMed search and by checking reference lists of included studies. Values between brackets are not used in the analyses presented. For references, see **main text**.

Abbreviations: ICU, intensive care unit; med, median; wk, week.

[a] According to McCabe-Jackson score.

[b] Septic shock not defined, or definition did not include resistance to fluid resuscitation.

[c] Unclear whether defined as stay in ICU at the moment of bacteremia onset, or defined as ICU admission prior to bacteremia.

[d] Median of 13 hospitals.

Supplementary Table 3. Adjustment methods in multivariable analyses

Reference	First author	Year	Adjusted for:			
			Inappropriate empiric treatment	Sepsis severity	Range of separate comorbidities	Underlying disease in a different way
11	Blomberg	2005	**yes**[a]	no	no	no
13	Cordery	2008	**yes**	no	no	**yes**[b]
14	Daikos	2007	yes	**yes**[c]	no	yes[d]
17	Gudiol	2010	yes	yes[e]	no	no
19	Kang	2010	yes	**yes**[c,e]	**yes**	no
22	Marchaim 1	2010	**yes**	**yes**[c,e]	yes	**yes**[d,f]
22	Marchaim 2	2010	no	**yes**[c,e]	yes	**yes**[d,f]
23	Marra	2006	yes	yes[e,g]	yes	**yes**[d,f,h]
24	Melzer 1	2007	**yes**	**yes**[e]	no[i]	no
24	Melzer 2	2007	no	**yes**[e]	no[i]	no
26	Menashe	2001	not clear	yes[e]	**yes**	**yes**[h]
28	Ortega	2009	**yes**	**yes**[e]	**yes**	yes[d]
30	Peña	2001	yes	**yes**[e]	no	yes[d]
31	Rodríguez-Baño 1	2010	**yes**	**yes**[c,e]	no	yes[j]
31	Rodríguez-Baño 2	2010	no	**yes**[c,e]	no	yes[j]
32	Schwaber	2006	no	no	**yes**	**yes**[d,f,h]
34	Szilágyi	2009	no	no	**yes**	yes[f]
35	Trecarichi	2009	**yes**	yes[e]	no[k]	no
36	Tsai	2010	yes	no	**yes**	no

Overview of the variables that are corrected for in the identified multivariable models for mortality. If variables are printed in bold, these variables were actually included in the final multivariate model. If not, these variables were tested on univariable analysis for their effect on mortality, and did not end up in the final model. If a single study presented multiple multivariable analyses, this is indicated by the addition of a number to the name of the first author. For references, see **main text**.

[a] Defined as inappropriate therapy due to other mechanisms than extended-spectrum β-lactamase (ESBL) production.

[b] By means of a variable based on APACHE score at admission or average SOFA score (if intensive care unit stay lasted more than 7 days).

[c] By means of the Pitt bacteremia score.

[d] By means of the McCabe-Jackson score.

[e] By means of a variable *severe sepsis*, *septic shock* or similar.

[f] By means of a variable *>2 comorbidities*.

[g] By means of the SAPS score.

[h] By means of length of stay prior to onset of bacteremia.

[i] Malignancy only.

[j] By means of the Charlson comorbidity index.

[k] Type of underlying hematological malignancy only.

Supplementary Table 4. Study quality assessment

	No. of analyses	No. of patients	Odds ratio (95% CI)	I^2, %	p^a
Subgroup analyses performed on uORs					
Selection-outcome score Newcastle-Ottawa scale[b]					
3–4	6	1,059	2.46 (1.66–3.67)	24	
5	13	2,109	2.17 (1.51–3.13)	42	0.83
6–7	13	6,444	2.52 (1.76–3.59)	51	
Multiple episodes per patient analysed in mortality analysis					
yes	6	819	1.60 (1.09–2.35)	7	0.05
no or no clear description	26	8,793	2.53 (2.00–3.21)	43	
Adequacy of follow-up					
<90% and no description of patients lost	3	1,056	3.22 (2.09–4.98)	0	0.17
>90% or description of lost to follow-up	29	8,556	2.28 (1.81–2.87)	44	
Subgroup analyses performed on avalaible/imputed ORs or, if no multivariable analysis, uORs[c]					
Comparability score Newcastle-Ottawa scale[b]					
0	16	1,881	2.28 (1.57–3.32)	50	
1	5	763	2.01 (1.22–3.31)	26	0.13
2	9	6,784	1.38 (0.98–1.94)	32	
Subgroup analyses performed on available/imputed aORs only					
Comparability score Newcastle-Ottawa scale[b]					
0–1	6	898	1.82 (1.15–2.89)	25	0.34
2	9	6,784	1.38 (0.98–1.93)	32	
Covariate-to-event ratio in final multivariable model					
<10	9	1,496	1.57 (1.04–2.36)	23	0.89
>10	6	6,186	1.50 (1.00–2.25)	48	
Explicit reporting of procedure and variables eligible for inclusion					
ambiguities exist	8	1,963	1.33 (0.95–1.87)	0	0.25
no ambiguities	7	5,719	1.89 (1.16–3.07)	58	

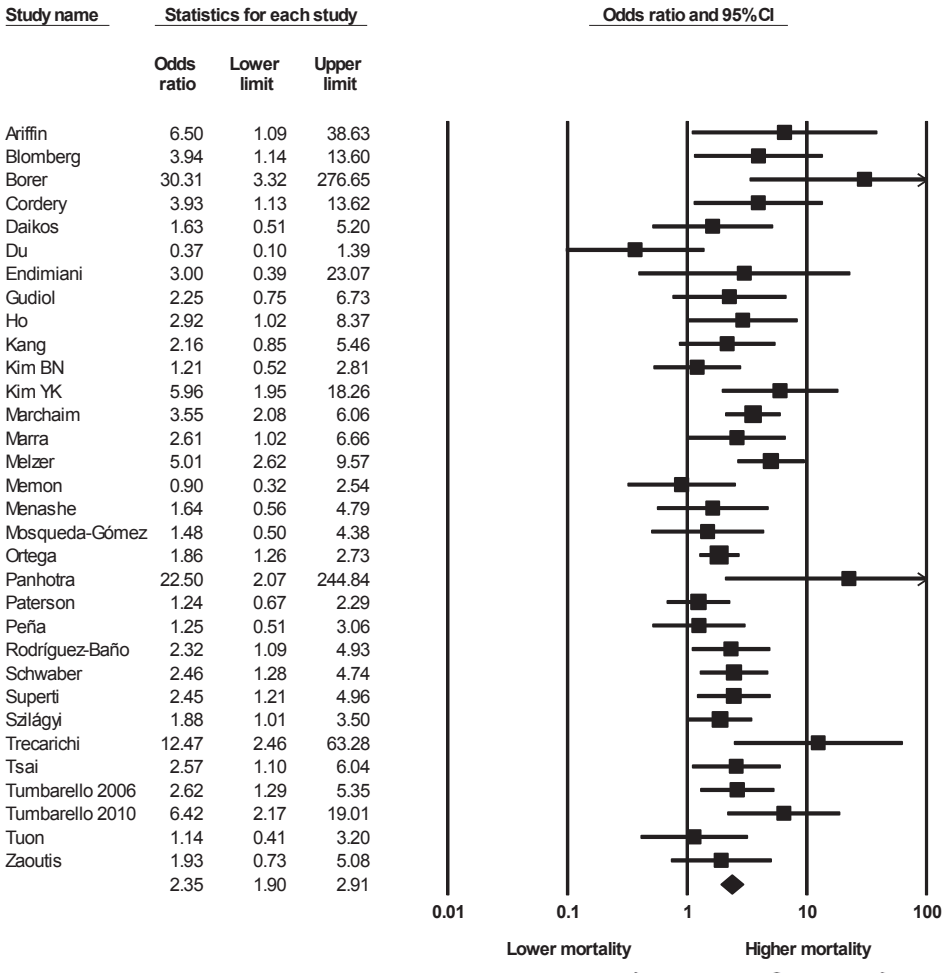
Subgroup analysis of study quality indicators that may have had an effect on the outcome reported, i.e. the uOR or aOR for the association between extended-spectrum β-lactamase (ESBL) production and mortality.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; uOR, unadjusted odds ratio.

[a] *P*-value of mixed-effect analysis.

[b] See the section **Modified Newcastle-Ottawa quality assessment scale for cohort studies** in this **Supplementary Material**.

[c] For this analysis, two studies performing a multivariable analysis for which no aOR could be extracted or imputed, were excluded (references 11 and 15 in the **main text**).



Supplementary Figure 1. Meta-analysis of unadjusted odds ratios (uORs). The mortality data from extended-spectrum β-lactamase (ESBL) producing (ESBL+) and non-ESBL-producing (ESBL-) bacteremias were used to calculate uORs for each study, with uORs higher than 1 indicating a higher mortality in the ESBL+ group. These uORs were then pooled; the Forest plot thereof is shown.

CHAPTER 3

Appropriateness of empiric treatment and outcome in bacteremia caused by extended-spectrum β-lactamase-producing bacteria

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Abstract

We studied clinical characteristics, appropriateness of initial antibiotic treatment, and other factors associated with day 30 mortality in patients with bacteremia caused by extended-spectrum β-lactamase (ESBL)-producing bacteria in eight Dutch hospitals. Retrospectively, information was collected from 232 consecutive patients with ESBL bacteremia (due to *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*) between 2008 and 2010. In this cohort (median age of 65 years; 24 patients were <18 years of age), many had comorbidities, such as malignancy (34%) or recurrent urinary tract infection (UTI) (15%). One hundred forty episodes (60%) were nosocomial, 54 (23%) were otherwise healthcare-associated, and 38 (16%) were community acquired. The most frequent sources of infection were UTI (42%) and intra-abdominal infection (28%). Appropriate therapy within 24 h after bacteremia onset was prescribed to 37% of all patients and to 54% of known ESBL carriers. The day 30 mortality rate was 20%.

In a multivariable analysis, a Charlson comorbidity index of ≥3, an age of ≥75 years, intensive care unit (ICU) stay at bacteremia onset, a non-UTI bacteremia source, and presentation with severe sepsis, but not inappropriate therapy within <24 h (adjusted odds ratio (OR) 1.53, 95% confidence interval (CI) 0.68 to 3.45), were associated with day 30 mortality. Further assessment of confounding and a stratified analysis for patients with UTI and non-UTI origins of infection did not reveal a statistically significant effect of inappropriate therapy on day 30 mortality, and these results were insensitive to the possible misclassification of patients who had received β-lactam/β-lactamase inhibitor combinations or ceftazidime as initial treatment. In conclusion, ESBL bacteremia occurs mostly in patients with comorbidities requiring frequent hospitalization, and 84% of episodes were healthcare-associated. Factors other than inappropriate therapy within <24 h determined day 30 mortality.

Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes that can hydrolyze penicillins, aztreonam, and cephalosporins. Therefore, ESBL-producing Enterobacterales were considered to be resistant to all β-lactam antibiotics except carbapenems. Recently, it has been suggested that cephalosporins [1,2], and β-lactam/β-lactamase inhibitor combinations (BLBLICs) [3,4], may still be used to treat infections with ESBL-positive isolates if minimum inhibitory concentrations (MICs) are below clinical breakpoints. Worldwide, numbers of infections caused by ESBL-producing Enterobacterales are increasing in both the hospital and community settings. It is generally assumed that infections with ESBL-producing pathogens have a worse outcome than their non-ESBL-producing counterparts [5,6].

In the Netherlands, antibiotic resistance levels are low [7], presumably due to the restrictive use of antibiotics [8], and the national infection control policy, including active surveillance and isolation of admitted ESBL carriers [9]. However, the proportion of *Escherichia coli* strains resistant or intermediately resistant to third-generation cephalosporins among invasive isolates increased from 0.2% in 2000 to 5.4% in 2010 [7]. For *Klebsiella pneumoniae*, these percentages were 3.5% in 2005 and 7.2% in 2010. In most hospitals, empiric antibiotic therapy for sepsis with a urinary, abdominal, pulmonary, or unknown source currently consists of second- or third-generation cephalosporins. Increasing rates of infections caused by ESBL-producing bacteria endanger the appropriateness of such regimens and pose the question of whether empiric treatment should also cover ESBL-producing bacteria. However, it is unknown how frequently initial treatment is truly empiric, as previously obtained culture results may guide initial choices. Naturally, this will occur more frequently in patients with previous hospitalizations or other reasons for microbiological testing than in previously healthy subjects with community-onset infections.

In eight Dutch hospitals, we performed a retrospective-cohort study of consecutive patients with bacteremia caused by the three most prevalent ESBL-producing pathogens in the Netherlands, i.e., *E. coli*, *K. pneumoniae*, and *Enterobacter cloacae*. Our aim was to determine the characteristics of patients affected and to study which factors, including appropriate initial antibiotic therapy, predict day 30 mortality.

Methods

Patients

In this retrospective study, all consecutive patients with bacteremia caused by ESBL-producing *E. coli*, *K. pneumoniae*, and *E. cloacae* present in laboratory information system databases in eight Dutch hospitals (three university hospitals and five teaching hospitals) were included. The inclusion period ranged from 1 January 2008 to 31 December 2010 (36 months) in six hospitals and to 1 July 2010 (30 months) in two hospitals. Per patient, only the first episode of bacteremia caused by ESBL-producing Enterobacterales within the study period was included. The study was approved by the institutional review board at the University Medical Center Utrecht.

Medical records were reviewed for the clinical data described in **Table 1**, such as Charlson comorbidity index [10], immunosuppression, urinary tract disease, recent invasive procedures, previous hospital admission abroad, known ESBL carriage at bacteremia onset, and previous use of antibiotics. Data for variables from each bacteremic episode were also collected (most of which are shown in **Table 2**), including origin of bacteremia (nosocomial, healthcare-associated, or community-onset), Pitt bacteremia score [11], presumed bacteremia source, use of antibiotics (including start and stop dates and route of administration), and interval between bacteremia onset and start of appropriate therapy. Outcome data were all-cause mortality 30 days after bacteremia onset (primary outcome), length of hospital stay, and intensive care unit (ICU) admission within 1 week after bacteremia onset (secondary outcomes). If no outpatient visit records were available, patients discharged within 30 days in an apparently healthy state were assumed to have survived the follow-up period.

Microbiological methods

Seven centers used the Vitek 2 system (bioMérieux SA, Marcy l'Etoile, France) and one center used the Phoenix system (BD, Franklin Lakes, NJ, USA) for identification. Susceptibility to antibiotics was determined by Vitek or Phoenix reports in all but one center, which used disk diffusion tests. All centers used CLSI interpretation criteria applicable at that point in time, e.g., CLSI criteria in 2007 [12]. ESBL detection was done according to national guidelines, which have high positive and negative predictive values for detecting ESBLs [13]. In short, screen-positive isolates (MIC of cefotaxime or ceftazidime of ≥1 mg/L or an ESBL warning by an automated system) were subjected to confirmation tests using ESBL Etests (AB Biodisk, Solna, Sweden) or combination disk diffusion tests (BD, MAST, Bootle, United Kingdom, or ROSCO,

Taastrup, Denmark), with cefotaxime and ceftazidime with and without clavulanic acid for *E. coli* and *K. pneumoniae* and with cefepime with and without clavulanic acid for *E. cloacae*.

Definitions

Bacteremia onset was the day on which the first ESBL-positive blood culture was drawn. Bacteremia was considered nosocomial if this culture was taken ≥48 h after hospital admission. Healthcare-associated bacteremia was defined as described previously by Friedman *et al.* [14]. Recurrent urinary tract infections (UTIs) implied at least three UTIs needing antibiotic treatment in the year prior to bacteremia. Severe sepsis and septic shock were defined according to criteria described previously [15]. We defined recurrent UTIs, obstructive urinary tract disease, hospital admission in the previous year, antibiotic use in the year prior to bacteremia, and antibiotic use or hospitalization in a country with high ESBL prevalence as risk factors for ESBL acquisition [16–19].

The time periods between the drawing of the first positive blood culture and the start of appropriate therapy were categorized as <24 h, 24 to 48 h, 48 to 72 h, and >72 h. Initial therapy was defined as therapy given in the first 24 h after blood culture drawing. Appropriateness of non-β-lactam antibiotics and carbapenems was based on susceptibility reports to the clinic, according to CLSI interpretive criteria, which remained unchanged between 2007 [12] and 2010 [2]. We considered oral fluoroquinolones and cotrimoxazole to be appropriate if isolates tested susceptible and if the clinical condition at the time of blood culture was considered nonsevere sepsis. Initially, all β-lactam antibiotics apart from carbapenems were considered inappropriate. In a secondary analysis, appropriateness of BLBLICs and ceftazidime was adjusted according to susceptibility test results, using CLSI interpretive criteria from 2010 [2]. Appropriate therapy also required administration of appropriate agents on ≥7 consecutive days, except if interrupted by the death of a patient. A switch from appropriate to inappropriate therapy within 7 days was classified as inappropriate therapy.

Data analysis

For each study year and within each of the three included species, we calculated the ratio of ESBL-positive isolates to all blood culture isolates for that specific species. Within a single patient, we used a deduplication window of 2 weeks. This analysis could be performed for 5 hospitals only (2 university hospitals and 3 teaching hospitals).

Characteristics of patients receiving appropriate versus inappropriate therapy within <24 h were compared by Pearson's $\chi 2$ tests, Fisher's exact tests, or Mann-Whitney *U* tests.

Determinants associated with inappropriate therapy with p <0.20 were selected for a multivariable logistic regression model using forward stepwise regression based on the Wald statistic. To study the association between inappropriate therapy within <24 h and day 30 mortality, eight covariates that were clinically deemed important confounders or effect modifiers of this association were selected. They were dichotomized or grouped in a manner that best reflected the association between the covariate and mortality. Stratum-specific odds ratios (ORs) for inappropriate therapy within <24 h were calculated, and these were pooled by the Mantel-Haenszel method. Different multivariable logistic regression models explaining day 30 mortality were constructed: a forward stepwise regression with inclusion in the case of p <0.05 for the score test and removal in the case of p <0.10 for the likelihood ratio statistic, incorporating the relevant confounders and with inappropriate therapy initially forced into the model; sensitivity analyses by the above-mentioned reconsideration of appropriateness of BLBLICs and ceftazidime, constructing separate models for urinary tract infection (UTI) and non-UTI bacteremia sources, excluding patients not receiving intravenous therapy <24 h after onset, and assessing appropriateness of therapy for <48 instead of <24 h; and a model starting with inappropriate therapy only, followed by the stepwise addition of variables changing the regression coefficient of inappropriate therapy >10%. The association between inappropriate therapy within <24 h and the secondary outcomes was studied univariably and in forward stepwise regression analyses also incorporating the eight covariates. A p <0.05 was considered statistically significant. All analyses were performed with SPSS Statistics 20.0 (IBM, Armonk, NY, USA).

Results

Prevalence of ESBL bacteremia

During the study period of 276 hospital months, there were 238 patients with an episode of ESBL bacteremia, 6 of whom were excluded due to an absence of clinical data. The total number of included episodes ranged from 9 to 74 per hospital. In the five hospitals with prevalence data available, ESBL prevalences among blood culture isolates were 6.6%, 8.7%, and 10.0% for *E. coli*, *K. pneumoniae*, and *E. cloacae*, respectively. The overall ESBL prevalences among these three species were 7.0%, 7.2%, and 7.6% in 2008, 2009, and 2010, respectively.

Table 1. Characteristics of patients with ESBL bacteremia

	All patients (*N* = 232, 100%), n (%)	Therapy within 24 h that was deemed:		*p*[a]
		Appropriate (*N* = 85, 37%), n (%)	Inappropriate (*N* = 147, 63%), *n* (%)	
Age in years				0.17
1–17	24 (10)	13 (15)	11 (7)	
18–64	87 (38)	29 (34)	58 (39)	
≥65	121 (52)	43 (51)	78 (53)	
Male	140 (60)	51 (60)	89 (61)	1.00
LOS before onset in days, median (IQR)	7.5 (1–21)	6 (1–25)	8 (1–20)	0.83
Comorbidity				
Malignancy	78 (34)	37 (44)	41 (28)	0.02
Obstructive urinary tract disease	43 (19)	18 (21)	25 (17)	0.48
Biliary disease	17 (7)	7 (8)	10 (7)	0.80
Recurrent urinary tract infection				0.71
Yes	35 (15)	14 (17)	21 (14)	
Unknown[b]	41 (18)	15 (18)	26 (18)	
Solid-organ transplant	25 (11)	9 (11)	16 (11)	1.00
Stem cell transplant	14 (6)	6 (7)	8 (5)	0.78
Charlson comorbidity index				0.10
0	44 (19)	9 (11)	35 (24)	
1–2	102 (44)	41 (48)	61 (42)	
3–4	46 (20)	18 (21)	28 (19)	
≥5	40 (17)	17 (20)	23 (16)	
Immune suppression				
Immunosuppressant use	52 (22)	20 (24)	32 (22)	0.87
Neutropenia	25 (11)	14 (17)	11 (8)	0.05
Invasive procedures in last 4 weeks				
Surgical procedure (*n* = 230)	78 (34)	31 (37)	47 (32)	0.57
Urologic procedure (*n* = 218)	48 (22)	23 (28)	25 (19)	0.13
Invasive devices at bacteremia onset				
Mechanical ventilation (*n* = 229)	32 (14)	13 (16)	19 (13)	0.70
CVC/arterial catheter (*n* = 218)	78 (36)	33 (42)	45 (32)	0.19

Table 1 (continued)

	All patients (*N* = 232, 100%), n (%)	**Therapy within 24 h that was deemed:**		***p*[a]**
		Appropriate (*N* = 85, 37%), n (%)	**Inappropriate (*N* = 147, 63%), *n* (%)**	
Previous antibiotic use				
No. of courses previous year:				
≥3	100 (43)	42 (49)	58 (39)	0.21
Unknown	58 (25)	19 (22)	39 (27)	
2/3GCs in previous 2 months (*n* = 227)	77 (34)	30 (37)	47 (33)	0.67
β-Lactams in previous 2 months (*n* = 226)	146 (65)	59 (72)	87 (60)	0.09
Fluoroquinolones in previous 2 months (*n* = 225)	76 (34)	31 (38)	45 (31)	0.31
Known hospitalization abroad previous year	13 (6)	4 (5)	9 (6)	0.77
Known ESBL carrier at bacteremia onset (*n* = 227)	71 (31)	38 (46)	33 (23)	<0.01
Hospital				
University	139 (60)	60 (71)	79 (54)	0.01[c]
1	27 (12)	13 (15)	14 (10)	
2	38 (16)	16 (19)	22 (15)	
3	74 (32)	31 (36)	43 (29)	
Non-university	93 (40)	25 (29)	68 (46)	
1	19 (8)	3 (4)	16 (11)	
2	18 (8)	4 (5)	14 (10)	
3	33 (14)	14 (16)	19 (13)	
4	9 (4)	2 (2)	7 (5)	
5	14 (6)	2 (2)	12 (8)	

Abbreviations: 2/3GC, second- or third-generation cephalosporin; CVC, central venous catheter; IQR, interquartile range; LOS, length of stay.

[a] *P*-value of comparison between patients with appropriate and those with inappropriate therapy, calculated with Pearson's $\chi 2$, Fisher's exact, or Mann-Whitney *U* test when applicable.

[b] Unknown cases were included in the group not having recurrent UTI.

[c] Comparison of university hospital versus non-university hospital patients.

Patient characteristics

Patient characteristics of the 232 included patients are shown in **Table 1**. The median age was 65 years. Only 6% of patients had been hospitalized abroad in the year prior to bacteremia, but 37% patients had a Charlson index of ≥3, and 15% had recurrent UTIs. At least 43% of patients had received more than three antibiotic courses during the last year, and 34% had used second- or third-generation cephalosporins in the two preceding months. In 31% of episodes, prior ESBL-positive culture results were available at bacteremia onset. Most bacteremia episodes were nosocomial (60%) or healthcare-associated (23%) (**Table 2**). Of the community-acquired episodes, 68% originated from the urinary tract. Most patients (at least 71%) with community-onset ESBL bacteremia had one or more of the predefined risk factors for ESBL acquisition. Overall, UTI was the most frequent source of bacteremia (42%) (**Table 2**), and 68% of these patients suffered from obstructive urinary tract disease, had recurrent UTIs, had recently undergone urological procedures, or had a urinary catheter at bacteremia onset.

Antimicrobial susceptibility

For non-β-lactam antibiotics, MICs were available from seven hospitals. According to CLSI interpretive criteria from 2010 [2], rates of coresistance were 75/193 (39%) for gentamicin, 112/200 (56%) for ciprofloxacin, and 156/200 (78%) for trimethoprim-sulfamethoxazole. For β-lactam antibiotics, we analyzed MICs from six hospitals (56% of the total study population). All isolates were susceptible to imipenem and/or meropenem. For amoxicillin/clavulanic acid or piperacillin/tazobactam, MICs below susceptibility breakpoints were demonstrated in 37/127 (29%) and 95/126 (75%) ESBL isolates, respectively. For ceftriaxone and cefotaxime, MICs below breakpoints were measured in 0/79 and 1/33 (3%) cases, respectively, whereas 71/127 (56%) isolates had ceftazidime MICs of ≤4 mg/L.

Antibiotic treatment

Eighty-five patients (37%) received appropriate therapy within <24 h. Of these patients, 67% received carbapenems and 28% received aminoglycoside mono- or combination therapy (**Table 3**). Of the 71 known ESBL carriers, 38 (54%) received appropriate therapy within <24 h. Proportions of patients receiving appropriate therapy after bacteremia onset were 37% (*n* = 85) within <24 h, 59% (*n* = 137) within <48 h, and 74% (*n* = 171) within <72 h. Twenty patients received appropriate therapy within >72 h, 30 received inappropriate treatment only, and 11 patients died before receiving appropriate therapy.

Table 2. Characteristics of ESBL bacteremia episodes

	All patients (*N* = 232, 100%), n (%)	Therapy within 24 h that was deemed:		*p*[a]
		Appropriate (*N* = 85; 37%), n (%)	Inappropriate (*N* = 147; 63%), *n* (%)	
Origin of bacteremia				0.14
Community-onset	38 (16)	10 (12)	28 (19)	
Healthcare-associated	54 (23)	25 (29)	29 (20)	
Nosocomial[b]	140 (60)	50 (59)	90 (61)	0.53
On medical ward	58 (42)	24 (48)	34 (39)	
On surgical ward	45 (33)	14 (28)	31 (35)	
On ICU	35 (25)	14 (24)	21 (26)	
Definitive bacteremia source[c]				0.03
Primary or unknown	24 (10)	7 (8)	17 (12)	
Urinary tract infection	97 (42)	38 (45)	59 (40)	
Pneumonia	11 (5)	0 (0)	11 (7)	
Vascular catheter infection	20 (9)	9 (11)	11 (7)	
Intra-abdominal infection	64 (28)	22 (26)	42 (29)	
Surgical wound infection	5 (2)	3 (4)	2 (1)	
Skin/soft tissue infection	6 (3)	2 (2)	4 (3)	
Other	5 (2)	4 (5)	1 (1)	
Species isolated				0.27
E. coli	163 (70)	65 (76)	98 (67)	
K. pneumoniae	44 (19)	12 (14)	32 (22)	
E. cloacae	25 (11)	8 (9)	17 (12)	
Polymicrobial bacteremia	22 (9)	8 (9)	14 (10)	1.00
Severe sepsis/septic shock (*n* = 226)	75 (33)	31 (37)	44 (31)	0.38
Pitt score				
≥3	81 (35)	29 (34)	52 (35)	0.88
Unknown	42 (18)	19 (22)	23 (16)	
Outcome				
ICU admission				0.47
No ICU admission	168 (72)	58 (68)	110 (75)	
Already in ICU	35 (15)	13 (15)	22 (15)	
Within 2 days after onset	22 (9)	10 (12)	12 (8)	
Within 2–7 days after onset	7 (3)	4 (5)	3 (2)	
In-hospital mortality (*n* = 230)	54 (23)	24 (28)	30 (21)	0.20
Day 30 mortality (*n* = 231)	46 (20)	16 (19)	30 (21)	0.87
LOS after onset in days, median (IQR)	15 (9–30)	16 (10–34)	14 (7–27)	0.09

Abbreviations: ICU, intensive care unit; IQR, interquartile range; LOS, length of stay.

[a] *P*-value of comparison between patients with appropriate and those with inappropriate therapy, calculated with Pearson's $\chi 2$, Fisher's exact, or Mann-Whitney *U* test when applicable.

[b] For two nosocomial cases, the ward type was unknown.

[c] Divided into urinary tract infections, intra-abdominal infections, pneumonias, other sources, and unknown/primary sources for multivariable analysis for prediction of inappropriate therapy.

Table 3. Initial antimicrobial therapy according to appropriateness of therapy within 24 h

	All patients (*N* = 232), *n* (%)	**Therapy within 24 h that was deemed:**	
		Appropriate (*N* = 85; 37%), *n* (%)	**Inappropriate (*N* = 147; 63%), *n* (%)**
Monotherapy			
Amoxicillin	1 (0)	0 (0)	1 (1)
BLBLIC	24 (10)	0 (0)	24 (16)
2GC	14 (6)	0 (0)	14 (10)
3GC	29 (13)	0 (0)	29 (20)
Aminoglycoside	3 (1)	1 (1)	2 (1)
Fluoroquinolone	7 (3)	2 (2)[a]	5 (3)
Cotrimoxazole	2 (1)	0 (0)	2 (1)
Carbapenem	62 (27)	57 (67)	5 (3)
Combination therapy			
Amoxicillin + aminoglycoside	5 (2)	2 (2)	3 (2)
Amoxicillin + fluoroquinolone	4 (2)	0 (0)	4 (3)
BLBLIC + aminoglycoside	12 (5)	4 (5)	8 (5)
BLBLIC + fluoroquinolone	2 (1)	1 (1)[a]	1 (1)
1GC + aminoglycoside	1 (0)	0 (0)	1 (1)
2GC + aminoglycoside	16 (7)	9 (11)	7 (5)
2GC + fluoroquinolone	1 (0)	1 (1)[a]	0 (0)
3GC + aminoglycoside	6 (3)	3 (4)	3 (2)
3GC + fluoroquinolone	3 (1)	0 (0)	3 (2)
β-Lactam + cotrimoxazole	3 (1)	0 (0)	3 (2)
Aminoglycoside + fluoroquinolone	1 (0)	0 (0)	1 (1)
Cotrimoxazole + aminoglycoside + fluoroquinolone	1 (0)	1 (1)	0 (0)
β-Lactam + aminoglycoside + fluoroquinolone	7 (3)	4 (5)	3 (2)
No antimicrobial therapy	5 (2)	0 (0)	5 (3)
Therapy started after 24 h	23 (10)	0 (0)	23 (16)

Appropriateness of therapy was judged according to *in vitro* susceptibility, duration of therapy, and, for oral fluoroquinolones or cotrimoxazole, severity of sepsis (see description in **Methods**).

Abbreviations: 1GC, first-generation cephalosporin; 2GC, second-generation cephalosporin; 3GC, third-generation cephalosporin.

[a] In 3 instances, oral therapy with fluoroquinolones was deemed appropriate: once as monotherapy, once combined with an intravenous BLBLIC, and once combined with an intravenous second-generation cephalosporin.

In **Tables 1 and 2**, predictors of appropriate initial therapy on a univariable level are shown. In the multivariable analysis, known ESBL carriers (OR 4.22, 95% confidence interval (CI), 2.10 to 8.49), patients with neutropenia (OR 2.77, 95% CI 1.04 to 7.37), patients having had a urological procedure (OR 2.55, 95% CI 1.18 to 5.51), and patients admitted to a university hospital (OR 2.41, 95% CI 1.17 to 4.96) received appropriate therapy within <24 h more often.

Day 30 mortality

For patients who received appropriate therapy within <24 h, <48 h, and <72 h, the day 30 mortality rates were 19% (16/85), 18% (24/137), and 16% (30/170), respectively, whereas for people treated inappropriately within these time periods, day 30 mortality rates were 21% (30/146), 23% (22/94), and 31% (19/61), respectively. In the univariable analysis, inappropriate therapy within <24 h was not associated with day 30 mortality (OR 1.12, 95% CI 0.57 to 2.19) (**Table 4**). Based on stratum-specific ORs, the strongest effect modification of the association between inappropriate therapy and mortality was seen for length of stay (LOS) before bacteremia onset, but for none of the strata was inappropriate therapy within <24 h significantly associated with day 30 mortality.

In multivariable analysis, a Charlson index of ≥3, patient age of ≥75 years, staying in the ICU at bacteremia onset, bacteremia source outside the urinary tract, and presence of severe sepsis or septic shock were independent predictors for day 30 mortality. Forcing inappropriate therapy within <24 h into this model failed to reveal a statistically significant association (OR 1.53, 95% CI 0.68 to 3.45) (**Table 4**). When interaction terms between each variable and appropriateness of therapy were added to the latter model, none of them appeared significant. By calculating Mantel-Haenszel pooled ORs, the strongest confounding effect was seen for severe sepsis (**Table 4**). Further analysis of confounding revealed that no other covariate influenced the regression coefficient for appropriateness of therapy by >10% after inclusion of sepsis severity, patient age, and neutropenia, and the association between appropriateness of therapy and mortality remained nonsignificant (OR 1.65, 95% CI 0.76 to 3.59).

Thirty-seven patients did not receive appropriate therapy within <24 h in the primary analysis but received a regimen with a BLBLIC or ceftazidime initially, which, taking into account the criterion for duration of appropriate treatment, could potentially form part of appropriate therapy provided that the isolate was susceptible. Of these patients, 8 (22%) had MICs of the concerned agent below CLSI 2010 clinical breakpoints. Classification of these episodes as receiving appropriate therapy within <24 h, together with the 6 patients (16%) for whom no

Table 4. Association of appropriateness of therapy within 24 h and possible confounders with day 30 mortality

Variables with strata	Mortality, *n/N* within stratum (%)	Unadjusted OR for mortality (95% CI) in univariable analysis	Association model[a]		Adjusted OR for mortality[b] (95% CI) in multivariable analysis
			Stratum-specific OR (95% CI) for appropriate therapy–mortality	MH pooled OR (95% CI)	
Appropriate therapy within <24 h					
Yes	16/85 (19)	1.12 (0.57–2.19)			1.53 (0.68–3.45)
No	30/146 (21)				
Charlson index					
<3	22/145 (15)	2.16 (1.13–4.16)*	1.15 (0.44–3.04)	1.18 (0.59–2.34)	2.80 (1.21–6.51)*
≥3	24/86 (28)		1.20 (0.46–3.17)		
Patient age					
<75 years	29/177 (16)	2.35 (1.17–4.72)*	1.46 (0.61–3.53)	1.21 (0.61–2.42)	3.81 (1.55–9.39)*
≥75 years	17/54 (31)		0.86 (0.27–2.72)		
Length of stay before onset					
<21 days	33/169 (20)	1.09 (0.53–2.25)	0.79 (0.36–1.72)	1.12 (0.57–2.19)	
≥21 days	13/62 (21)		2.95 (0.72–12.04)		
Hospital ward at bacteremia onset					
Other	31/196 (19)	3.99 (1.85–8.64)*	1.26 (0.56–2.86)	1.13 (0.56–2.27)	2.88 (1.05–7.85)*
ICU	15/35 (43)		0.81 (0.20–3.22)		
Bacteremia source					
Urinary tract infection	8/96 (8)	4.31 (1.91–9.71)*	1.10 (0.25–4.90)	1.05 (0.52–2.11)	4.79 (1.74–13.16)*
Other	38/135 (28)		1.04 (0.47–2.29)		
Bacteremia origin					
Community-onset	5/38 (13)	1.78 (0.65–4.85)	1.50 (0.15–15.28)	1.16 (0.59–2.29)	
Nosocomial/healthcare-associated	41/193 (21)		1.13 (0.55–2.31)		
Severe sepsis					
No	14/150 (9)	7.24 (3.53–14.71)*	0.70 (0.23–2.15)	1.34 (0.65–2.74)	5.24 (2.36–11.60)*
Yes	32/75 (43)		2.10 (0.81–5.47)		
Neutropenia					
No	40/206 (19)	1.31 (0.49–3.50)	1.12 (0.54–2.33)	1.15 (0.58–2.27)	
Yes	6/25 (24)		1.38 (0.22–8.67)		

Abbreviations: ICU, intensive care unit; MH, Mantel-Haenszel.

[a] Stratum-specific ORs to study confounding and effect modification of the association between appropriate therapy and mortality by the eight other variables.

[b] Adjusted OR from forward stepwise logistic regression analysis, with appropriate therapy initially forced into the model (including 225 patients).

* p <0.05 determined by Pearson's $\chi 2$ test or Wald test.

MICs were available, did not change the association between inappropriate therapy and day 30 mortality (data not shown). When patients with urinary and nonurinary bacteremia sources were analyzed separately, results for inappropriate therapy in multivariable models did not change appreciably, nor did they change after exclusion of patients not receiving intravenous therapy in the first 24 h (data not shown). Also, inappropriate therapy within <48 h was not associated with day 30 mortality in a multivariable model (OR 1.92, 95% CI 0.89 to 4.16).

Secondary outcomes

Inappropriate therapy within <24 h was associated with neither ICU admission within 1 week of bacteremia onset nor length of hospital stay after bacteremia onset in patients who were discharged alive, by both univariable and multivariable analyses (data not shown).

Discussion

This study demonstrates that, in the Netherlands, 84% of bacteremia episodes caused by ESBL-producing *E. coli*, *K. pneumoniae*, or *E. cloacae* are nosocomial or otherwise healthcare-associated, that ESBL carriage is known at bacteremia onset in 31% of episodes, that 63% of patients still receive inappropriate antimicrobial therapy in the first 24 h after bacteremia onset, and that the day 30 mortality rate of ESBL bacteremia is 20%. Comorbidity, patient age, source of bacteremia, presence of severe sepsis or septic shock, and ICU stay at bacteremia onset, but not appropriateness of antibiotic treatment within <24 h or <48 h after bacteremia onset, were associated with day 30 mortality.

The population of patients with ESBL bacteremia in Dutch hospitals is characterized by high prevalences of malignancies, recurrent UTIs, previous antibiotic use, and long hospital stay before bacteremia onset, which is typical for patients at risk for multiresistant bacterial infections, as reported by others [20]. UTIs and intra-abdominal infections are the major sources of bacteremia. Even most patients with community-onset bacteremia had comorbidities requiring frequent hospital visits or had recently visited a country with a high prevalence of ESBL carriage.

Inappropriate empiric antibiotic therapy is the most feared consequence of the increasing incidences of infections caused by ESBL-producing bacteria. As shown, the prevalence of ESBL-producing bacteria is still low in our country. Prediction rules might be helpful in identifying those patients who should (or should not) be empirically treated with carbapenems or other appropriate combinations of antibiotics. In this study, only half of patients with known ESBL carriage received appropriate therapy within <24 h. Apparently, patient records with

microbiology results were either not consulted or neglected before initiation of empiric therapy. Currently, Dutch national sepsis guidelines recommend prescribing a combination of a second- or third-generation cephalosporin and aminoglycoside or carbapenem monotherapy if a patient is known to be colonized with an ESBL-producing isolate or has used cephalosporins or fluoroquinolones in the past month [21]. In our cohort, 149 patients either had documented ESBL carriage or had used these antibiotics in the past 2 months. Only 65 of them received appropriate initial therapy. Adherence to Dutch national sepsis guidelines, therefore, would increase the proportion of patients receiving appropriate empiric antibiotic treatment.

Inappropriate therapy for sepsis has been shown to increase mortality, especially in critically ill patients [22–24]. Indeed, upon univariable analysis, we observed a trend toward higher mortality in the case of inappropriate therapy in severely septic patients (OR of 2.10 for this stratum, 95% CI 0.81 to 5.47). However, inappropriate therapy within <24 h did not increase day 30 mortality in the multivariable analysis. In this ESBL bacteremia cohort, patients might have died due to underlying diseases and an inability to treat severe sepsis or to control the source of bacteremia, for instance, by surgery. Neither the possible misclassification of cephalosporins and BLBLIs as inappropriate therapy nor the abundance of comparatively benign urinary tract infections appeared to explain the absence of an effect. In other studies of patients with infections caused by ESBL-producing bacteria, conflicting results were obtained with regard to associations between inappropriate treatment and mortality. Strong effects (adjusted ORs of 5.88 to 6.28) were demonstrated in patients with bacteremia caused by ESBL-producing *E. coli* [25] and Enterobacterales [26], whereas no association was reported by others [27–31]. However, the effects of inappropriate empiric therapy on patient outcomes were often evaluated in cohorts combining ESBL and non-ESBL bacteremias. The results of these studies were similarly conflicting [32–35].

Our study has several limitations. First of all, due to its retrospective nature, some data, such as the exact number and duration of the previous use of antibiotics and Pitt scores, could not be retrieved for all patients. Second, the study was performed in eight Dutch hospitals, which may reduce generalizability to other countries. Similarly, inclusion of three out of eight university hospitals in the country may curtail generalizability for the non-university hospitals in the Netherlands. However, the annual proportions of ESBL-producing isolates among Enterobacterales were comparable to those reported for the Netherlands in the EARSS database [7], and characteristics of patients were comparable to those reported in another

Dutch study on ESBL bacteremia [36]. Finally, we did not perform genetic typing of isolates and hence could not assess the role of specific pathogenic clones, such as *E. coli* ST131 and *K. pneumoniae* ST258, as a determinant of mortality in bacteremia.

In conclusion, 84% of ESBL bacteremia episodes in these Dutch patients were nosocomial or otherwise healthcare-associated. Most patients had comorbidities requiring frequent hospital visits. Although inappropriate therapy was not associated with day 30 mortality, appropriateness of initial treatment may be improved in a significant number of patients by consultation of previous culture results.

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CHAPTER 4

Attributable mortality of antibiotic resistance in Gram-negative infections in the Netherlands: a parallel matched cohort study

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Abstract

Introduction: Antibiotic resistance in Gram-negative bacteria, including third-generation cephalosporin (3GC) resistant Enterobacterales, has been associated with increased mortality. This was demonstrated mostly in bacteremias in international studies. Yet, the burden of resistance created by all types of Gram-negative infection and within a single country have not been quantified. We therefore investigated the attributable mortality of antibiotic resistance in Gram-negative infections in the Netherlands.

Methods: In eight Dutch hospitals, a representative sample of Gram-negative infections was identified between 2013 and 2016, and categorized as resistant or not. Both cohorts were matched 1:1 to non-infected control patients on hospital, length of stay on the date of infection onset, and age. In this parallel matched cohort set-up, 30-day mortality was compared between infected and non-infected patients, with analytic control for confounding. The impact of resistance was then assessed by dividing the two separate risk ratios (RRs) for mortality attributable to Gram-negative infection.

Results: We matched 1,954 Gram-negative infections (61% caused by *Escherichia coli*, 39% bacteremia) to 1,941 controls. Resistant Gram-negatives, mostly 3GC-resistant Enterobacterales (78%), caused 243 infections (12% of all infections). There were no infections with carbapenem-resistant Enterobacterales. Mortality for resistant infections was increased compared to their non-infected controls (adjusted RR 1.40 with 95% confidence interval (CI) 0.64–3.05), similarly as was the case for susceptible infections (RR 1.33, 95% CI 1.07–1.65). The RR reflecting attributable mortality of resistance was 1.05 (95% CI 0.46–2.35).

Conclusion: In the Netherlands, antibiotic resistance was not associated with 30-day mortality in Gram-negative infections. The attributable mortality of resistance in infection may not be the same across European countries.

Introduction

The dissemination of resistant Gram-negative bacteria has become a major public health concern over the last decades. In the Netherlands in 2017, levels of third-generation cephalosporin (3GC) resistance among bacteremia isolates amounted to 6.8% and 11.8% for *Escherichia coli* and *Klebsiella pneumoniae*, respectively [1]. Resistance mostly resulted from production of extended-spectrum β-lactamases (ESBLs) [2]. Outbreaks of carbapenemase-producing bacteria occur sporadically, mostly in hospitals after unnoticed introduction from abroad [3]. Dutch infection prevention guidelines define several Gram-negative highly resistant micro-organisms (HRMOs), for which targeted control measures are recommended to limit spread in healthcare settings (**Table 1**) [4].

Controlling spread of resistant Gram-negatives in healthcare settings poses a large burden on resources, personnel, and patients [5]. This is justified by the perceived negative consequences of infections caused by resistant Gram-negatives for patients. Evidence for these negative consequences naturally stems from observational studies, which are hampered by confounding bias. To reduce residual confounding, De Kraker *et al.* proposed the parallel matched cohort design, in which both patients infected with resistant pathogens and patients infected with susceptible pathogens are compared with their own non-infected controls [6,7]. In their study, performed in thirteen European countries but not in the Netherlands, bacteremia caused by *E. coli* resistant to 3GCs yielded an odds ratio (OR) of 2.5 (95% confidence interval (CI) 0.9–6.8) for 30-day mortality when compared to susceptible *E. coli* [7].

Yet, as only patients with bacteremia were studied, it remained unknown how resistance impacts non-bacteremic infections, reflecting the majority of infections, and to what extent these findings reflected the situation in the Netherlands. Therefore, we studied the attributable mortality of HRMO Gram-negative infections in a parallel matched cohort in Dutch hospitals.

Methods

Study design, setting and participants

The aim of the study was to compare clinical outcome in patients with Gram-negative HRMO infections to patients with infections with susceptible Gram-negatives. For this, both groups have their own matched non-infected controls for comparison, as such building two parallel cohorts. Subsequently, the two within-cohort estimates are contrasted (**Figure 1**). The institutional review board of the University Medical Center Utrecht judged that the Dutch Medical Research Involving Human Subjects Act did not apply to this study, and a waiver for

Table 1. Definition of Gram-negative HRMOs

Organism group	HRMO definition based on Dutch HRMO guideline [4]
Enterobacterales[a]	(ceftazidime R *OR* cefotaxime/ceftriaxone R)[b] *OR* meropenem R[c] *OR* (ciprofloxacin R *AND* (gentamicin R *OR* tobramycin R))
Pseudomonas aeruginosa	*3/5 from:* piperacillin/tazobactam[d] R, ceftazidime R, meropenem R[c], (gentamicin R *OR* tobramycin R), ciprofloxacin R
Acinetobacter spp.	meropenem R[c] *OR* (ciprofloxacin R *AND* (gentamicin R *OR* tobramycin R))
Stenotrophomonas maltophilia	co-trimoxazole R

Resistance (R) is defined by applying to EUCAST clinical breakpoints [30] to minimum inhibitory concentrations obtained through automated systems (Vitek 2 (bioMérieux SA, Marcy l'Etoile, France) or Phoenix (BD, Franklin Lakes, NJ, USA)), and includes isolates categorized as intermediate to the antibiotic.

[a] In this study, Enterobacterales include *Citrobacter* spp., *Enterobacter* spp. (including *Enterobacter/Klebsiella aerogenes*, *Enterobacter/Kluyvera intermedia* and *Enterobacter/Cronobacter sakazakii*), *Escherichia* spp., *Hafnia* spp., *Klebsiella* spp. (including *Klebsiella/Calymmatobacterium granulomatis* and *Klebsiella/Raoultella* spp.), *Morganella* spp., *Pantoea* spp., *Proteus* spp., *Providencia* spp., and *Serratia* spp.

[b] Dutch HRMO guideline uses ESBL-positive for this criterion.

[c] Dutch HRMO guideline uses carbapenemase-positive for this criterion.

[d] Dutch HRMO guideline uses piperacillin-resistant for this criterion.

informed consent with regard to the information presented in this manuscript was obtained in all participating hospitals. This study formed part of a more extensive project named GRAND-ABC (registered at clinicaltrials.gov under number NCT02007343; succinctly described in the **Supplementary Material**).

We aimed to enroll a representative sample of 2,000 patients with Gram-negative infection from eight Dutch hospitals, including one university hospital (**Supplementary Table 1**). Gram-negatives included are presented in **Table 1**. We defined Gram-negative infections based on microbiological and clinical criteria as described by Horan *et al.* [8]. Enrolled patients had to be at least 18 years of age, infection episodes had to be associated with admission to a clinical acute care ward, and patients had to be treated with oral or intravenous antibiotics, for some types of infection with antibiotics specifically aimed at the Gram-negatives identified in microbiological cultures. An individual patient could be included with several infection episodes.

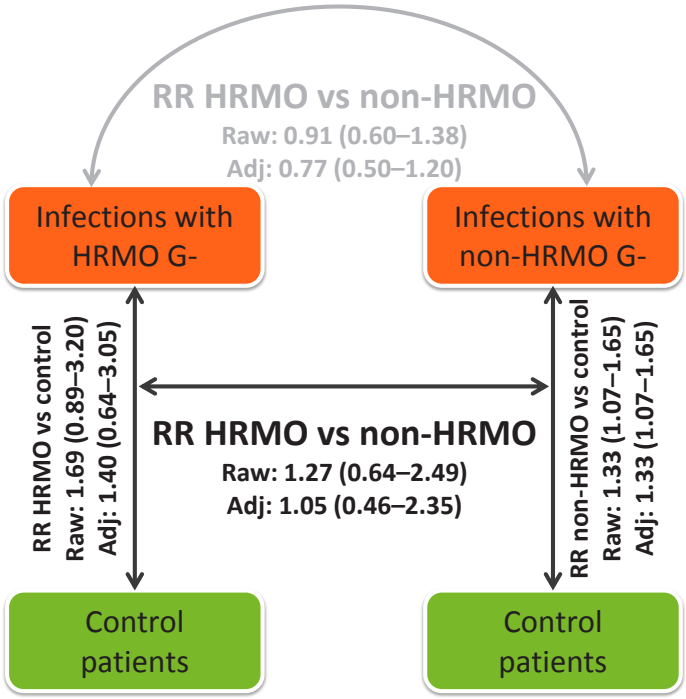

Figure 1. Structure of the parallel matched cohort. This figure depicts the two methods applied to derive a RR comparing HRMO to non-HRMO infections with regard to 30-day mortality. The elements of the parallel-cohorts analysis are shown in black, and the infection-cohort analysis is shown in grey.
Abbreviations: Adj, adjusted; G-, Gram-negative.

Index cultures of an infection episode included all first cultures with Gram-negatives related to the infection episode. Subsequent culture results could only qualify as index cultures if they provided new relevant information on the source of the infection. For example, a blood culture yielding *E. coli* considered an index culture, could be accompanied by a urine culture yielding *E. coli* from the next day, and this would change the categorization from *secondary bacteremia* to *urinary tract infection with bacteremia*.

To obtain an unbiased study sample we used a scheme repeated weekly in each hospital. On a dedicated weekday, all new Gram-negative isolates were grouped by the day that the antibiogram was reported to the clinic, and were put in a computer-generated random order within each day. Starting from the most recently reported isolates, trained research nurses consecutively assessed whether these represented index cultures. This was continued until five Gram-negative infection episodes were identified. Weekly screening sessions continued for 52

weeks with a targeted 260 episodes per study site. Hospital-specific study periods all fell between June 2013 and February 2016 (**Supplementary Table 1**).

For each infection episode, a control patient from the same hospital with no evidence of Gram-negative infection was matched based on a similar length of stay in the same hospital (for the day of the index culture) and similar age. For community-onset infections, only emergency admissions were eligible for matching. A single patient could serve as the control patient for several infection episodes.

Considerations for the sample size, the screening procedure, definitions of infection entities and index cultures, and the procedure for matching control patients are described in detail in the **Supplementary Material**.

Data collection for exposure, outcomes and confounders

All Gram-negatives obtained from index cultures were considered causative pathogens of the infection episodes. Based on antibiotic susceptibility testing, isolates were categorized as HRMO or non-HRMO (**Table 1**). If at least one isolate constituted an HRMO, the infection was considered HRMO infection. All others were categorized as non-HRMO infections.

Infection onset was defined as the moment at which the first index culture was obtained. For each infection episode and control patient, relevant information (such as patient demographics, comorbidity, prior healthcare exposure, and prior medical procedures) was obtained from medical files.

The criteria used to define infection episodes provided information on the source of the infection, the presence of bacteremia, and any association with previous surgery. If according to these criteria, an infection could only be categorized as *secondary bacteremia*, the working diagnosis for the bacteremia source was registered. Furthermore, sepsis severity at infection onset, source control procedures, and complications of infection, including abscess formation, spread to adjacent structures and hematogenous spread, were registered. Intravenous and oral antibiotic therapy provided on the day of infection onset was categorized as appropriate or inappropriate based on the susceptibility of the Gram-negative isolates in index cultures.

The primary outcome was all-cause mortality within 30 days after infection onset or day of matching, based on information in the medical file, or the nationwide Personal Record Database, if needed. Secondary outcomes were length of hospital stay (prespecified) and ICU stay after infection onset, discharge destination and infection resolution at 14 days after

infection onset. Resolution of infection was defined as termination of all treatment, including non-antibiotic treatment related to source control, and disappearance of symptoms (e.g. fever or pain) and findings (e.g. abscesses) related to infection. If patients had been discharged before day 14 after infection onset, resolution of infection was assessed at discharge. More details on definitions of variables are in the **Supplementary Material**.

Statistical analysis

All statistical analyses were performed in R (version 3.4.3) [9], with the use of packages *Hmisc* [10], *rms* [11], *mice* [12] and *xtable* [13]. Missing data was dealt with through multiple imputation (see **Supplementary Material**). Cox proportional hazard models with an arbitrary single follow-up time and Efron approximation for tied survival times were used to obtain risk ratios (RRs) relating independent variables to 30-day mortality [14].

The primary analysis, the parallel-cohorts analysis, started with the creation of two separate models: one comparing non-HRMO infections and one comparing HRMO infections to their respective non-infected controls. Matched sets of one infected and one non-infected patient were accounted for by clustering and robust standard errors. Both models were further adjusted by means of the confounder selection process described in the **Supplementary Material**. Then, a risk ratio (RR) for HRMO status was calculated by dividing the HRMO cohort-specific RR by the non-HRMO cohort-specific RR. CIs for this RR were derived as described by Altman and Bland [15].

A secondary analysis, the infection-cohort analysis, was performed without reference to the matched non-infected patients. It provided an opportunity to study infection-related variables not available for non-infected control patients. Again in a Cox proportional hazard models, but this time without any clustering, RRs directly contrasting HRMO and non-HRMO with regard to 30-day mortality were calculated. These were adjusted using a procedure similar to the parallel-cohorts analysis, but additionally, infection-related mediators (such as source, pathogen and sepsis severity) were added to evaluate their contribution to any relation between HRMO status and mortality (see **Supplementary Material**). An adjusted model including admission-related variables only (i.e. admission type and ward) was also created, because we noted considerable differences in ward distributions between HRMO and non-HRMO infections, and this variable constitutes both a confounder and a mediator of infection-related mortality. Finally, models were created to analyze the mediating potential of

Table 2. Distribution of characteristics among all cultures, screened cultures and index cultures

	All relevant isolates during study period[a], *n* (%)	**Bacterial isolates from screened cultures, *n* (%)**	**Bacterial isolates from index cultures, *n* (%)**
Material			
Blood culture	4,008 (8.38)	1,519 (10.31)	1,155 (32.59)
Urine	24,323 (50.83)	6,845 (46.47)	1,160 (32.73)
Lower respiratory tract	8,079 (16.88)	2,637 (17.90)	251 (7.08)
Fluid, pus, tissue (biopsy)	5,505 (11.50)	1,962 (13.32)	718 (20.26)
Swab	5,186 (10.84)	1,549 (10.52)	243 (6.86)
Other	754 (1.58)	219 (1.49)	17 (0.48)
Bacterial isolate			
Escherichia coli	22,145 (46.28)	6,705 (45.52)	1,904 (53.72)
Pseudomonas aeruginosa	5,835 (12.19)	1,916 (13.01)	337 (9.51)
Klebsiella pneumoniae	4,426 (9.25)	1,389 (9.43)	346 (9.76)
Proteus mirabilis	3,609 (7.54)	1,079 (7.32)	232 (6.55)
Enterobacter cloacae	2,587 (5.41)	801 (5.44)	177 (4.99)
Other	9,253 (19.34)	2,841 (19.29)	548 (15.46)
HRMO isolate	6,323 (13.21)	1,972 (13.39)	390 (11.00)
Total number of isolates	**47,855 (100.00)**	**14,731 (100.00)**	**3,533 (100.00)**

[a] All Gram-negative isolates (defined in **Table 1**) with an antibiogram, from patients ≥18 years of age, from culture potentially indicating infection.

appropriate antibiotic therapy provided on the day of infection onset, with adjustment for patient- and infection-related variables.

Two exploratory subgroup analyses were performed. The first used the matched cohort design restricted to hospital-onset infections and their controls, and calculated the attributable mortality risk of a Gram-negative infection (HRMO or non-HRMO) acquired during hospitalization. The second derived the attributable mortality of HRMO infection specific for the subset of bacteremia episodes, without reference to their non-infected controls. Both analyses were corrected for patient-related variables.

Results

Study patients

During the study periods, microbiology laboratories in the eight participating hospitals reported 47,855 Gram-negative isolates with an antibiogram in a clinical specimen potentially

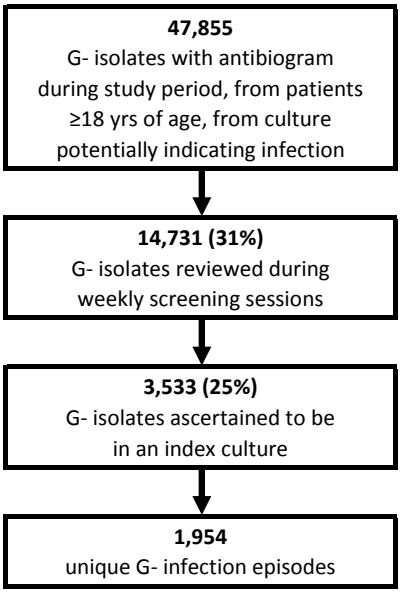

Figure 2. Flow diagram of the screening process. Abbreviations: G-, Gram-negative (defined in **Table 1**).

indicating infection obtained from adult patients. Of these, 14,731 (31%) were reviewed in the weekly screening sessions. The screened subset was comparable to the entire set with regard to microorganism distribution and HRMO proportion, but included more blood cultures and less urine cultures (**Table 2**). Based on protocolized selection, 1,954 Gram-negative infection episodes were included (**Figure 2**).

Most infections involved *E. coli* (*n* = 1,190, 61%), and *P. aeruginosa* (*n* = 210, 11%), and in 293 episodes (15%) more than one Gram-negative species was cultured (**Table 3**). At least one HRMO was identified in 243 (12%) infections, which were mostly caused by 3GC-resistant Enterobacterales (*n* = 189, 78%), followed by Enterobacterales with combined aminoglycoside and fluoroquinolone resistance (*n* = 47, 19%) and multidrug-resistant *P. aeruginosa* (*n* = 9, 4%). Bacteremia was present in 758 (39%) of infections. Most infections had the urinary tract as source (*n* = 1,002, 52%), and less than 5% of infections were complicated by hematogenous spread, infection of prosthetic material, osteomyelitis, and/or endocarditis. Post-operative infections constituted 9% of the cohort.

HRMO infections more frequently had prior treatment restrictions and prior ICU admissions, were less frequently community-onset, had a longer length of stay prior to infection (**Table 4**), and were less frequently associated with bacteremia (**Table 3**). Proportions of patients receiving oral or intravenous therapy on the day of infection onset, and the day before, were comparable for HRMO and non-HRMO infections (**Table 3**). Yet, antibiotic therapy on the day of infection onset was inappropriate in 68% and 39% of HRMO and non-HRMO infections, respectively.

30-day mortality was 10% (*n* = 25) for HRMO and 11% (*n* = 192) for non-HRMO infections (RR for HRMO vs non-HRMO 0.91, 95% CI 0.60–1.38; **Table 5**). Inappropriate antibiotic therapy on the day of infection onset was not associated with higher 30-day mortality (unadjusted RR 0.81, 95% CI 0.61–1.09; adjusted RR 0.76, 95% CI 0.57–1.03).

Table 3. Characteristics of Gram-negative infection episodes

	Patients with non-HRMO infection, n/N with data (%)	Patients with HRMO infection, n/N with data (%)
Type of infection		
Bacteremia	680/1,711 (40)	78/243 (32)
Urinary tract infection	885/1,691 (52)	117/240 (49)
Respiratory tract infection	139/1,691 (8)	19/240 (8)
Intra-abdominal infection (excl biliary tract)	198/1,691 (12)	32/240 (13)
Biliary tract infection	130/1,691 (8)	18/240 (8)
Skin/soft tissue/wound infection (incl mediastinitis)	196/1,691 (12)	38/240 (16)
Other infection source	81/1,691 (5)	11/240 (5)
Postoperative infection	141/1,711 (8)	28/243 (12)
Causative pathogen[a]		
Escherichia coli	881/1,711 (51)	116/243 (48)
Klebsiella pneumoniae	132/1,711 (8)	12/243 (5)
Enterobacter cloacae	47/1,711 (3)	25/243 (10)
Proteus mirabilis	96/1,711 (6)	3/243 (1)
Pseudomonas aeruginosa	135/1,711 (8)	9/243 (4)
Other species	183/1,711 (11)	22/243 (9)
Multiple species	237/1,711 (14)	56/243 (23)
Bacteria other than study pathogens or yeast obtained from index cultures	456/1,708 (27)	72/243 (30)
Sepsis severity at infection onset[a]		
No sepsis	512/1,710 (30)	74/243 (30)
Sepsis	963/1,710 (56)	133/243 (55)
Severe sepsis	115/1,710 (7)	21/243 (9)
Septic shock	120/1,710 (7)	15/243 (6)
Antibiotic treatment during the infection episode		
Receipt of antibiotic therapy prior to hospital admission	167/1,710 (10)	30/243 (12)
Receipt of oral/intravenous antibiotic therapy[b] on the day prior to infection onset	249/1,466[c] (17)	46/210[c] (22)
Receipt of oral/intravenous antibiotic therapy[b] on the day of infection onset	1,176/1,466[c] (80)	164/210[c] (78)
Receipt of inappropriate antibiotic therapy[d] on the day of infection onset	567/1,466[c] (39)	142/210[c] (68)
Source control performed during the admission after infection onset	570/1,711 (33)	94/243 (39)
Status of the infection episode at 14 days after infection onset[a]		
Patient admitted – infection resolved	183/1,711 (11)	38/243 (16)
Patient admitted – mere completion of antibiotic course	60/1,711 (4)	10/243 (4)
Patient admitted – infection ongoing	150/1,711 (9)	30/243 (12)
Patient discharged – infection resolved at discharge	290/1,711 (17)	55/243 (23)
Patient discharged – mere completion of antibiotic course after discharge	810/1,711 (47)	77/243 (32)
Patient discharged – infection ongoing at discharge	103/1,711 (6)	17/243 (7)
Patient deceased	115/1,711 (7)	16/243 (7)

[a] Mutually exclusive categories.

[b] In-hospital prescriptions only.

[c] Available for seven of eight hospitals.

[d] In-hospital and post-discharge prescriptions only. Includes receipt of no oral/intravenous antibiotic therapy.

Table 4. Characteristics and outcomes of patients with Gram-negative infections and non-infected control patients

	non-HRMO cohort		HRMO cohort	
	Non-infected control patients, n/N with data (%)	Patients with Gram-negative infection, n/N with data (%)	Non-infected control patients, n/N with data (%)	Patients with Gram-negative infection, n/N with data (%)
Female	845/1,700 (50)	825/1,711 (48)	116/241 (48)	90/243 (37)
Age, median (IQR)	72 (62–81)	71 (61–80)	70 (60–77)	68 (60–77)
Other bacterial infection at infection onset	361/1,700 (21)	137/1,446 (9)	57/241 (24)	12/204 (6)
Known colonization with an HRMO	35/1,700 (2)	74/1,711 (4)	10/241 (4)	68/243 (28)
Gram-negative bacteremia during the year prior to infection onset	14/1,700 (1)	77/1,711 (5)	6/241 (2)	22/243 (9)
Preceding hospital admission within 3 months prior to infection onset	373/1,695 (22)	553/1,710 (32)	55/241 (23)	90/243 (37)
Admission from long-term care facility	61/1,699 (4)	90/1,710 (5)	7/241 (3)	23/243 (9)
Admission type				
Via emergency ward	1,364/1,700 (80)	1,345/1,711 (79)	185/241 (77)	178/243 (73)
Other form of emergency admission	151/1,700 (9)	159/1,711 (9)	19/241 (8)	20/243 (8)
Elective admission	131/1,700 (8)	176/1,711 (10)	27/241 (11)	36/243 (15)
Transfer from other hospital	54/1,700 (3)	31/1,711 (2)	10/241 (4)	9/243 (4)
Origin of infection				
Community-onset, not healthcare-associated	891/1,687 (53)	660/1,705 (39)	112/240 (47)	62/242 (26)
Community-onset, possibly healthcare-associated	14/1,687 (1)	59/1,705 (3)	0/240 (0)	6/242 (2)
Community-onset, healthcare-associated	319/1,687 (19)	522/1,705 (31)	36/240 (15)	83/242 (34)
Hospital-onset	463/1,687 (27)	464/1,705 (27)	92/240 (38)	91/242 (38)
Length of hospital stay prior to infection onset in case of hospital-onset infection, median (IQR)	8 (5–14)	8 (5–14)	12 (6–21)	12 (7–25)
Hospital ward at infection onset				
Emergency ward	793/1,700 (47)	733/1,711 (43)	100/241 (41)	83/243 (34)
Internal medicine	197/1,700 (12)	217/1,711 (13)	32/241 (13)	35/243 (14)
Surgery or gastro-enterology	280/1,700 (16)	390/1,711 (23)	46/241 (19)	79/243 (33)
Urology	33/1,700 (2)	91/1,711 (5)	5/241 (2)	19/243 (8)
Pulmonary medicine	92/1,700 (5)	83/1,711 (5)	10/241 (4)	11/243 (5)
ICU	43/1,700 (3)	67/1,711 (4)	8/241 (3)	7/243 (3)
Other ward	262/1,700 (15)	130/1,711 (8)	40/241 (17)	9/243 (4)

Table 4 (continued)

	non-HRMO cohort		HRMO cohort	
	Non-infected control patients, n/N with data (%)	**Patients with Gram-negative infection, n/N with data (%)**	**Non-infected control patients, n/N with data (%)**	**Patients with Gram-negative infection, n/N with data (%)**
Charlson comorbidity index, median (IQR)	1 (0–3)	2 (0–3)	1 (0–3)	2 (1–4)
Immunodeficiency	145/1,700 (9)	206/1,710 (12)	23/241 (10)	31/243 (13)
Solid malignancy	335/1,700 (20)	507/1,711 (30)	44/241 (18)	75/243 (31)
Treatment restriction in place prior to infection onset	438/1,699 (26)	423/1,711 (25)	57/241 (24)	79/243 (33)
Surgical procedure during the 30 days prior to infection onset	251/1,700 (15)	325/1,486 (22)	36/241 (15)	57/218 (26)
ICU stay during the 30 days prior to infection onset	113/1,700 (7)	133/1,452 (9)	22/241 (9)	33/209 (16)
Receipt of prophylactic antibiotic therapy at hospital admission	44/1,699 (3)	50/1,711 (3)	5/240 (2)	16/243 (7)
ICU stay during the admission from infection onset onwards				
No	1,579/1,700 (93)	1,475/1,711 (86)	227/241 (94)	205/243 (84)
Already on ICU for > 12 hrs at infection onset	33/1,700 (2)	42/1,711 (2)	5/241 (2)	7/243 (3)
Already on ICU for 0–12 hrs at infection onset	18/1,700 (1)	36/1,711 (2)	2/241 (1)	2/243 (1)
Admission to ICU within 0–12 hrs after infection onset	26/1,700 (2)	90/1,711 (5)	4/241 (2)	18/243 (7)
Admission to ICU >12 hrs after infection onset	44/1,700 (3)	68/1,711 (4)	3/241 (1)	11/243 (5)
Length of hospital stay after infection onset, median (IQR)	5 (3–9)	8 (5–14)	6 (3–11)	9 (6–16)
Discharge destination				
Home	1,156/1,700 (68)	993/1,711 (58)	152/241 (63)	116/243 (48)
Home with home healthcare	115/1,700 (7)	255/1,711 (15)	22/241 (9)	45/243 (19)
Long-term care facility	259/1,700 (15)	263/1,711 (15)	46/241 (19)	50/243 (21)
Terminal care	25/1,700 (1)	36/1,711 (2)	5/241 (2)	5/243 (2)
Deceased during admission	81/1,700 (5)	138/1,711 (8)	6/241 (2)	21/243 (9)
Other hospital	64/1,700 (4)	26/1,711 (2)	10/241 (4)	6/243 (2)
Gram-negative bacteremia within 7 to 90 days after infection onset	20/1,700 (1)	54/1,711 (3)	3/241 (1)	10/243 (4)
All-cause mortality within 30 days after infection onset	145/1,695 (9)	192/1,709 (11)	15/241 (6)	25/243 (10)

In case of non-infected control patients, infection onset refers to the moment at which the matched infected patient has their infection onset.
Abbreviations: ICU, intensive care unit; IQR, interquartile range.

Table 5. All-cause mortality within 30 days after infection onset

	non-HRMO cohort, n/N within stratum (%)	HRMO cohort, n/N within stratum (%)	All episodes, n/N within stratum (%)
	Patients with gram-negative infection		
Community-onset infection	118/1,239 (9.5)	17/151 (11.3)	135/1,390 (9.7)
Hospital-onset infection	73/464 (15.7)	8/91 (8.8)	81/555 (14.6)
All infections	**192/1,709 (11.2)**	**25/243 (10.3)**	**217/1,952 (11.1)**
	Non-infected control patients		
Community-onset control patients	95/1,220 (7.8)	8/148 (5.4)	103/1,368 (7.5)
Hospital-onset control patients	50/462 (10.8)	7/92 (7.6)	57/554 (10.3)
All control patients	**145/1,695 (8.6)**	**15/241 (6.2)**	**160/1,947 (8.2)**

In case of non-infected control patients, the distinction community-onset vs. hospital-onset is based on the moment at which the matched infected patient has their infection onset.

Matched non-infected control patients were found for 1,941 infected patients. Control patients had similar age and prior length of stay, but were admitted to different wards, had less comorbidity, and in general had had less healthcare exposure (**Table 4**). After the day of matching, their hospital stay was shorter than for infected patients (5 vs 8 days), and 30-day mortality was lower (8% vs 11%; **Table 5**).

Attributable mortality

After full adjustment for confounding variables, the relative risks for 30-day mortality were 1.40 (95% CI 0.64–3.05) for HRMO infections compared to their non-infected controls, and 1.33 (95% CI 1.07–1.65) for non-HRMO infections compared to their non-infected controls (**Figure 1**). Based on both RRs, the overall RR for 30-day mortality associated with HRMO status was 1.05 (95% CI 0.46–2.35).

When analyzing infected patients only (i.e. without controls) the RR for 30-day mortality for HRMO infections was 0.77 (95% CI 0.50–1.20; **Figure 1**) after adjustment for patient-related factors, and 0.93 (95% CI 0.59–1.47) after inclusion of infection-related variables in the adjustment procedure. When including admission-related variables only for adjustment, the RR for 30-day mortality for HRMO infections was 0.93 (95% CI 0.61–1.42).

Hospital-acquired Gram-negative infections (both HRMO and non-HRMO; n = 555) were, compared to their non-infected controls, associated with increased 30-day mortality (adjusted RR 1.58 with 95% CI 1.12–2.22). Within the subgroup of infections associated with bacteremia

(*n* = 758), HRMO infections tended to be associated with lower 30-day mortality with an unadjusted RR of 0.61 (95% CI 0.30–1.26) and an adjusted RR of 0.59 (95% CI 0.28–1.23).

Discussion

In this study, we aimed to derive a cohort of patients with Gram-negative infections accurately reflecting patients with Gram-negative infections admitted in Dutch hospitals, as well as a matched cohort of non-infected control patients. The infected cohort was characterized by a 12% prevalence of HRMOs, most notably Enterobacterales being resistant to 3GCs or to both aminoglycosides and fluoroquinolones, and absence of carbapenem-resistant Enterobacterales. Based on different methods for quantifying the association between antibiotic resistance and patient outcome we estimate that the attributable mortality of antibiotic resistance is close to zero, despite a 30% lower proportion of patients with infections caused by resistant strains receiving appropriate antibiotic therapy at the time of infection onset.

Our findings markedly differ from those obtained in two large European multicenter studies, and from meta-analyses on the burden of infections caused by ESBL-producing bacteria [16,17]. De Kraker *et al.* reported a 2.5 (95% CI 0.9–6.8) increase in the odds of 30-day mortality in case of 3GC resistance in *E. coli* bacteremia [7], and Stewardson *et al.* reported a 1.63 (95% CI 1.13–2.35) increase in the daily risk of death during admission when comparing 3GC-resistant to 3GC-susceptible Enterobacterales bacteremia [18].

A delay in achieving appropriate antibiotic therapy is considered the most important reason for increased mortality in patients infected with antibiotic resistant Gram-negatives [19]. Inappropriate empiric antibiotics have been related to mortality in all forms of sepsis [20], and specifically in septic shock, for which associations between increasing mortality for every hour that appropriate antibiotics were delayed have been reported [21]. However, many of these studies are methodologically flawed, as they do not take into consideration the time-varying nature of antibiotic therapy, competition between appropriate therapy and mortality, time-varying confounding and collider bias, or the physiologically expected absence of a clear threshold for sufficiently timely initiation, and the dogma of irreparable damage in case of inappropriate initial antibiotics has been questioned recently [22]. A pragmatic solution to circumvent these methodological challenges is to restrict the analysis to inappropriate therapy on the day of onset of infection. In doing so, British investigators also failed to demonstrate

an impact of inappropriate initial therapy on outcome in a large multicenter study on Gram-negative bacteremia [23].

Other explanations for the discrepancy in attributable mortality between previous studies and our findings may well include local practices of treating hospitalized patients. For instance, turn-around-times for antibiotic susceptibility results and the subsequent adaptation of inappropriate antibiotic therapy may differ between countries. In the current study, only 33% of HRMO infections received appropriate initial antibiotic therapy. In another European study on bacteremia caused by carbapenemase-producing Enterobacterales, 22% of the patients did not receive appropriate antibiotics during the first five days after infection onset [24]. In theory, differences in local bacterial epidemiology may influence attributable mortality, but to the best of our knowledge, the relevance of highly virulent and resistant Gram-negatives has never been convincingly demonstrated.

Finally, in contrast to prior studies, 61% of infections included in our study were non-bacteremic, and different Enterobacterales and non-fermenters with multiple resistance patterns were studied. However, mortality rates were similar for bacteremic and non-bacteremic infections, and in the subgroup of infections accompanied by bacteremia, the lack of attributable mortality due to antibiotic resistance was even more pronounced.

The absence of a discernable increase in mortality for resistant pathogens does not imply that there is no burden imposed by these pathogens. Antibiotic-resistant pathogens may not just replace their antibiotic-susceptible counterparts, but their dissemination may in fact inflate the total number of infections [25,26]. Furthermore, increased morbidity and higher costs associated with antibiotic resistance may still be relevant, for instance in specific subgroups of infected patients, such as those with septic shock, that could not be evaluated in our study.

The parallel matched cohort design applied in this study has been used before to decrease the potential for confounding in observational studies on the impact of antimicrobial resistance [6,7]. This method provides a wealth of information for identifying risk factors for resistant infections, and contrasting the impact of resistance to the impact of nosocomial infection. However, this method also has shortcomings. First, a large proportion of Gram-negative infections (71% in our study) are community-onset infections, and the most appropriate controls would be subjects picked from the open population. Second, we dispute the concept that non-infected patients better resemble patients with resistant infections than patients with susceptible infections, as long as matching on length of stay has been performed.

Infected patients have often been exposed to relevant risk factors, such as disturbance of natural barriers, which are more likely to be similarly present among patients with susceptible and resistant infections than among infected and non-infected patients. Length of stay may just be treated as a confounder when analyzing a cohort of patients with resistant and susceptible infections. Third, the parallel matched cohort design does not allow adjustment for infection-related variables, as these are unavailable for non-infected patients. This hinders establishing whether mortality differences are due to patient-related factors (confounding) or infection-related factors (causal mediation). Finally, non-infected patients may be affected by infections later during hospitalizations, and it is unclear how this should be handled when using a parallel matched cohort deign. We, therefore, think that it is not necessary to rely on the parallel matched cohort design for the specific aim to obtain an unbiased estimate of the impact of antibiotic resistance on patient outcome. For our study, resources might have been used more efficiently by including patients from a larger variety of settings and collecting data to allow for other forms of control for confounding.

Several potential study limitations should be discussed. First, this cohort of infected patients was created through a combination of selection and random sampling among all Gram-negative infections in the participating hospitals. The seven to one ratio of non-academic and academic hospitals does reflect the Dutch situation and within these hospitals, subsets of screened and included culture results were proportionally similar to all culture results (**Table 2**). It should be noted, though, that ICU-acquired pneumonia episodes may have been underrepresented. As respiratory samples from ICU patients were generally qualified as colonization, these infections relied on results of blood cultures or cultures obtained through bronchoscopy. Yet, we do consider our cohort representative of Gram-negative infections occurring in Dutch hospitals.

A second potential study limitation is that screening and selection of episodes may have been subjective and amenable to inter-observer variability [27], and selective inclusion conditional on HRMO status may have occurred. Also, for including infections based on Gram-negatives in sputum and wound cultures, adjustment of antibiotic therapy to the susceptibility results was a prerequisite. This restriction may have hampered inclusion of HRMO infections, as standard empiric antibiotic regimens may not always be tailored to culture results in infections with a benign course. However, bacteremias were not affected by these potential limitations and findings in patients with bacteremia were to those including all infections. The somewhat lower proportion of HRMO in index cultures compared to all screened cultures (**Table 2**), may

have resulted from cultures growing HRMO more often being follow-up cultures during protracted infection episodes.

Third, HRMO infections might represent infections in which diagnostic culturing was performed late, yielding culture results reflecting selection of resistant flora by antibiotic treatment. Moreover, HRMO infections could also reflect patients under increased surveillance for the occurrence of infection because of risk factors for antibiotic resistance, implying that less severe infections may have been ascertained. Both mechanisms would reduce mortality in HRMO infections. Again, bacteremia episodes would not be affected by these sampling issues and findings for bacteremia episodes yielded similar results as for non-bacteremic infections.

Lastly, the definition of HRMO bears particular relevance to Dutch infection control practices, and does not match international consensus definitions of MDR micro-organisms [28]. Our findings, without infections caused by carbapenem-resistant Enterobacterales, are, therefore, not generalizable to countries with a different resistance epidemiology.

Our findings imply that currently in the Netherlands, the attributable mortality due to antibiotic resistance in Gram-negatives is very low. This contradicts the recent estimate of 206 deaths annually due to antibiotic resistance in the Netherlands in a Europe-wide study [29]. Most of these deaths (*n* = 187) reportedly occurred in patients suffering Gram-negative infections. We conclude that this estimate does not accurately reflect reality, and may have resulted from using an unrealistic attributable mortality factor derived from studies, that were not performed in the Netherlands. Our findings emphasize the need of obtaining reliable estimates of attributable mortality per country to quantify the national and international burden of antibiotic resistance.

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CHAPTER 4
SUPPLEMENTARY MATERIAL

Attributable mortality of antibiotic resistance in Gram-negative infections in the Netherlands: a parallel matched cohort study

The GRAND-ABC project

GRAND-ABC (The attributable burden and costs of infections caused by antibiotic-resistant Gram-negative bacteria in Dutch hospitals) is a study funded by Dutch government agency ZonMw (Netherlands Organization for Health Research and Development; project number 205200007) within the *Priority Medicines Antimicrobial Resistance* research program of ZonMw. The project formally ran from 2012 through 2017.

The aims of GRAND-ABC were fivefold:

1. To provide a more accurate estimate than currently available of the incremental disease burden and attributable costs of antibiotic-resistant as compared to antibiotic-susceptible Gram-negative bacteria (i.e. Enterobacterales and non-fermenters; in the current study antibiotic-resistant and antibiotic-susceptible Gram-negative bacteria are referred to as highly resistant micro-organisms (HRMOs) and non-HRMOs, respectively). This analysis was focused on Gram-negative infections for which patients are hospitalized. In a less detailed manner, the same analysis of disease burden and costs should be performed for acquiring a Gram-negative infection during hospitalization.
2. To identify determinants associated with resistance in Gram-negative infections, to the extent that they are confounders of the relation between resistance and outcome.
3. To adapt and optimize existing methodology to measure the burden of resistance, among others by calculating disability-adjusted life years (DALYs) which incorporate not merely mortality, but also morbidity.
4. To apply an innovative research method (latent class model) to better deal with confounding and clustering effects in assessing the burden of resistance.
5. To determine cost-effectiveness of infection prevention methods aimed at resistant gram-negatives by integrating our findings with another ZonMw-funded project.

The current manuscript is the first scientific publication stemming from the GRAND-ABC project and is focused on a specific part of aim 1. Publications on other aspects of the project are forthcoming.

The core of the GRAND-ABC study is the parallel matched cohort study of which the structure is described in the **main text**. This design is based on the studies by De Kraker *et al.* [1,2]. Apart from what is described in this study, additional data was collected with regard to resource use during hospitalization. Also, the cohort of patients with HRMO infections, and a

random 20% of the cohort with non-HRMO infections was asked to participate in extended follow-up until 90 days after infection. This consisted of additional file review to ascertain the occurrence of long-term sequelae of the infection episode. In addition, these patient were asked to participate in two questionnaires (at 30 and 90 days after infection). These questionnaires related to costs generated outside of the participating hospital, quality of life (measured by with EQ-5D-5L), and long-term sequelae modifiable by the general practitioner.

Sample size

The sample size calculation in the original grant request focused on the number of variables that could be included in multivariable models without overfitting. In order to identify determinants associated with HRMO infection and to determine the effect of HRMO compared to non-HRMO infection on patient outcome it was decided that 2000 patients with Gram-negative infections and 2000 matched non-infected control patients had to be included in order to develop a final model of 10–15 variables of significant importance. This was based on an expected 5–10% of Gram-negative infections caused by HRMOs.

Screening procedure

The screening procedure is described in the **main text**, but several additional guidelines for inclusion of infection episodes were adhered to:

- To discern protracted infections with flares from new infection episodes, the instruction was that new infection episodes could only begin if all symptoms related to a previous similar infection episode had subsided and all treatment for this episode (not limited to antimicrobial treatment) had been stopped in between. Yet, Gram-negative infections independently emerging during another Gram-negative infection (i.e. by no means an infectious complication or relapse) were eligible for inclusion as so-called superinfections.
- In general, patients could be included with multiple separate infection episodes during the course of the study. However, patients with infections could not be represented in the study multiple times with overlapping follow-up periods. This meant that patients who had been included with an infection episode during the same hospitalization or within the past 30 (standard follow-up) or 90 days (if eligible for extended follow-up, although this extension was not used in the current study). As only a sample of Gram-negative infections was included in the study, inclusion was seldom hampered by this specific criterion.

- If Gram-negatives were cultured late during the course of an infection episode, these isolates could still serve as index cultures, as long as the Gram-negatives were assumed to have played a role at the beginning of the infection and had influenced antibiotic therapy for the infection episode. This could even be the case if earlier cultures relevant for the infection episode yielded micro-organisms other than Gram-negatives. Alternatively, the Gram-negatives represented a superinfection eligible for inclusion, but in that case, new symptoms should be apparent. If earlier cultures also yielded Gram-negatives, then the more recent Gram-negatives could only be considered to form part of index cultures in case of a superinfection. Otherwise these Gram-negatives, irrespective of alterations in species or phenotype, were considered *later cultures* and not eligible for inclusion.

Definitions of Gram-negative infections

With some exceptions indicated in **Supplementary Table 3**, definitions of infection entities were copied from the Centers for Disease Control and Prevention (CDC) criteria described by Horan *et al.* [3]. Naturally, for each entity, only those criteria incorporating a clinical culture through which a causative pathogen could be established were applied. Furthermore, only infection entities with septic potential relevant for adults were included in the study, implying that e.g. ear-nose-throat infections except mastoiditis, infections of the eye or oral cavity, gastroenteritis, and asymptomatic bacteriuria were excluded.

Matching control patients

For each infection episode, an overview was created of patients admitted to the same hospital on the day of infection onset (matching day), including patients admitted or discharged on that specific calendar day. These potential control patients had to be at least 18 years of age, and had to be admitted to an acute care ward on the matching day. In case of an infection onset occurring before hospital admission or during the first two days of hospital admission, patients admitted electively were excluded as potential control. Further, all patients were removed who fulfilled the criteria of a Gram-negative infection episode on the matching day. Notably, developing a Gram-negative infection later during the admission was not an exclusion criterion. If the patient had a Gram-negative infection shortly before the matching day, symptoms had to have disappeared and treatment (antibiotics or other modes of treatment) had to be withdrawn on the matching day to be eligible as a potential control.

From all potential controls, a further selection was made of those patients having a length of hospital stay (counted in days) equal to the length of hospital stay of the patient with the infection episode on the matching day. If no such patients were available, all patients were selected with a length of hospital stay within a one day margin (lower or higher) of the length of hospital stay of the infected patient. If still no potential controls were available, this margin was increased to two days, etc. If the infected patient had their infection onset during the days prior to hospital admission, all patients entering the hospital on the day of infection onset were selected (i.e. length of stay equal to 1).

For all patients in this selection, the absolute age difference in days with the infected patient was calculated. The non-infected patient with the smallest difference was then selected as the control patient.

Variable definitions

Definitions for Gram-negatives, HRMO, infection episode, index cultures, causative pathogens, infection onset, and most outcomes are provided in the **main text**. Infection entities are defined in **Supplementary Table 3**. In **Supplementary Table 4**, definitions are provided for patient-related confounders, and some additional infection-related intermediates and outcomes.

Antibiotic therapy on a specific day referred to all oral and intravenous antibiotics provided on that day, including prescriptions stopped or started on that day. Thus, combination therapy may not always have been given concurrently, and may indicate a switch in antibiotic regimen on that day. Appropriateness of antibiotic therapy on the day of infection onset was based on minimum inhibitory concentrations (MICs) from automated systems (Vitek 2 (bioMérieux SA, Marcy l'Etoile, France) or Phoenix (BD, Franklin Lakes, NJ, USA)), although some laboratory systems overwrote these results if an alternative method for MIC determination was applied (e.g. E-test). MICs were interpreted according to the breakpoints set by the he European Committee on Antimicrobial Susceptibility Testing (EUCAST) [4]. For non-fermenters (*Pseudomonas aeruginosa*, *Acinetobacter* spp., *Stenotrophomonas maltophilia*), intrinsic resistance as indicated by EUCAST was additionally incorporated [5]. For Enterobacterales, no further expert rules were applied, and resistance was solely based on interpretation of raw MIC's according to EUCAST criteria, also in case of β-lactam MICs for Enterobacterales species with chromosomal β-lactamases.

Missing data

For most variables, only sporadic missings occurred (less than 0.1% of data points). However, more notably, some variables were not registered for all included patients, because early during the course of the study, the time period to which the variable applied was changed from *during the hospital stay prior to infection onset* to *within the prior 30 days*. This affected the variables for 304 subjects (7.8% of all infection episodes and non-infected control patients). Also, the variable *other bacterial infection at infection onset* was introduced later during the course of the study, and again was not registered for 304 subjects (7.8%). Furthermore, in some cases of secondary bacteremia, the bacteremia source was not registered (*n* = 23; 1.1% of all infections). Finally, at one study site, and in some sporadic cases, antibiotic therapy on the day of onset of infection was not available (*n* = 278; 14.2% of all infections).

Assuming a missing completely at random (MCAR) pattern of missingness, these variables were imputed to increase precision [6]. Imputation was performed separately for the infection-cohort analysis, and for the parallel-cohorts analysis, as for the first analysis, the dataset consisted of the infection episodes only, and allowed the use of infection-related intermediates, and variables related to the provision and appropriateness of antibiotic therapy. Using the *multivariate imputation by chained equations* procedure as incorporated in the *mice* package (version 2.46.0) for R [7], 25 imputed datasets were created for both datasets. Variables used in the imputation process were all other recorded variables (confounders, intermediates, outcomes) with a Pearson's correlation coefficient ≥0.1 for the variable to be imputed. No interactions were included. Rubin's rules were used for pooling estimates from models developed on the imputed datasets.

Adjustment for confounding

Many different adjusted models were created (**Supplementary Table 5**). They made use of different sets of variables included for adjustment (**Supplementary Table 6**), were applied to different subsets of the study subjects (parallel-cohorts analysis, infection-cohort analysis; bacteremia and hospital-onset subgroup analyses) and evaluated different exposures of interest (Gram-negative infection, HRMO infection, appropriateness of antibiotic therapy on the day of infection onset). In addition, two different statistical techniques were used to achieve adjustment for confounders or intermediates. The results from the first technique are

presented in the **main text**. The second technique should be considered a sensitivity analysis and results are presented in **Supplementary Table 4**.

The first technique involved backward elimination of variables. A set of variables deemed potential confounders or intermediates (**Supplementary Table 6**) was included in the so-called *full model*, together with the exposure evaluated. It was then evaluated in a stepwise procedure whether variables could be removed from the model while retaining approximately the same β coefficient for the exposure. This was done to increase precision of the effect estimate, reflected by a narrowing of its confidence interval [8]. Removal of variables started with removing the variable that would result in a new model with the smallest deviance in β coefficient for the exposure compared to the *full model*. Subsequently, all variables were evaluated again, and the variable impacting the β coefficient the least in this round, was removed, always with reference to the β coefficient of the exposure in the *full model*. This iterative process was halted if the β coefficient would deviate >10% from the β coefficient in the *full model* if one of the remaining variables were to be removed.

For the primary technique, we made a selection of potential patient-related confounders on which data were collected (the small set in **Supplementary Table 6**). This was done to prevent overfitting when starting off with the *full model*. In order to establish if we missed any important confounders with this *a priori* selection, a forward sensitivity analysis was performed in which all potential patient-related confounders were available for inclusion (the large set in **Supplementary Table 6**). The model started with the exposure only, and subsequently, for all potential confounders, it was evaluated how much the β coefficient for the exposure would be changed in case of incorporation into the model. The potential confounder with the largest resulting change in β coefficient was selected for inclusion. Taking this new model as the starting point, all remaining potential confounders were evaluated again for their effect on the β coefficient of the exposure. In each round, one variable could be incorporated into the model, as long as it would change the β coefficient >10%. To prevent overfitting, after inclusion of a new confounder, it was also evaluated whether any confounders already included could be removed again from the model. Variables were removed if the β coefficient of the exposure in the current model differed <10% from a model without the variable, starting with the variable with the smallest change in β coefficient. These cycles were repeated until no excluded variable could be found for which inclusion would change the β coefficient >10%, and no included variable had an impact <10% on the β coefficient. When cycles of exclusions

and inclusions involving the same variables were detected by the algorithm, all cycling variables were included in the model.

Supplementary Table 1. Characteristics of study sites

Hospital and location	Hospital characteristics		Study period		Screening results		
	Hospital type	No. of hospital beds in 2013[a]	Date of first included infection episode	Date of last included infection episode	Total No. of G- isolates[b]	No. of screened G- isolates (%)	No. of included infection episodes
Amphia Ziekenhuis - Breda	General	854	4 Jun 2013	14 Jul 2014	5,691	1,578 (27.7)	254
St. Antonius Ziekenhuis - Nieuwegein	General	798	25 Sep 2014	16 Nov 2015	10,087	2,583 (25.6)	244
Catharina Ziekenhuis - Eindhoven	General	696	1 Aug 2014	2 Sep 2015	4,013	1,219 (30.4)	252
Diakonessenhuis - Utrecht	General	536	17 Oct 2013	4 Dec 2014	4,945	1,529 (30.9)	258
St. Elisabeth Ziekenhuis[c] - Tilburg	General	555	18 Apr 2014	22 Jun 2015	5,888	2,628 (44.6)	236
Meander Medisch Centrum - Amersfoort	General	543	21 Feb 2014	20 May 2015	3,520	1,057 (30.0)	236
Tergooi - Hilversum/Blaricum	General	633	29 Dec 2014	17 Feb 2016	5,111	1,993 (39.0)	236
Universitair Medisch Centrum Utrecht - Utrecht	University	1,042	26 Oct 2013	14 Nov 2014	8,600	2,144 (24.9)	236

Abbreviations: G-, Gram-negative (restricted to species indicated in **Table 1** in the **main text**).

[a] Source: https://nl.wikipedia.org/wiki/Lijst_van_Nederlandse_ziekenhuizen.

[b] With antibiogram, during study period, from patients ≥18 years of age, from culture potentially indicating infection.

[c] Now part of Elisabeth-TweeSteden Ziekenhuis.

Supplementary Table 2. Characteristics of infection episodes and control patients per site

Hospital (anonymized)	Characteristics of infection episodes				% 30-day mortality among:	
	% HRMO	% hospital-onset infection	% bacteremia	% urinary tract infection	Infection episodes	Non-infected control patients
Hospital A	8	18	36	67	9	11
Hospital B	11	27	29	51	7	7
Hospital C	12	25	47	49	10	5
Hospital D	12	27	43	53	12	10
Hospital E	13	31	42	53	14	6
Hospital F	13	27	52	49	13	10
Hospital G	14	29	33	56	11	10
Hospital H	16	44	26	37	13	8

Abbreviations: HRMO, with highly resistant micro-organism (defined in **Table 1** in the **main text**) among causative pathogens.

Supplementary Table 3. Infection entities

Infection entity	Cultures on which entity can be based	Modifications of original criteria by Horan *et al.* [3] and other comments
Urinary tract infection (SUTI)	Urine	$\geq 10^5$ microorganisms per cc of urine was not used as a criterion; decisions by the laboratory whether or not to report an isolate were followed. Whether an appropriate technique to obtain the culture was used, was not verified.
Pneumonia (PNEU)	Blood, pleural fluid, culture from lower respiratory tract (BAL, suction catheter), sputum	Combines criteria from *Pneumonia with specific laboratory findings* and *Pneumonia in immunocompromised patient*. Sputum is an addition, but could only be used if any Gram-negative isolate was taken into account in definitive treatment. Sputum cultures from the intensive care unit could not be used.
Meningitis, ventriculitis (MENI)	CSF, blood culture	
Arterial or venous infection (VASC)	Surgically removed artery/vein, catheter tip (blood culture negative)	
Endocarditis (ENDO)	Valve, vegetation, 2 blood cultures	
Catheter-associated bacteremia (CABI) **Secondary bacteremia (LCBI)** **Primary bacteremia (PRBI)**	Blood culture	Modification of *Laboratory-confirmed bloodstream infection* by dropping criterion on no relation to an infection at another site and thereby including all bacteremias. Based on treating physician's interpretation, the bacteremia is categorized as *catheter-associated* (whether or not confirmed by catheter tip culture), *secondary* (related to any other infection at another site, which may or may not be recorded as a separate entity), or *primary* (not related to any infection at another site). LCBI is an entity that can be attached to all other infections as marker of severity (e.g. meningosepsis can be MENI + LCBI, even if MENI is based on blood culture).

Supplementary Table 3 (continued)

Infection entity	Cultures on which entity can be based	Modifications of original criteria by Horan *et al.* [3] and other comments
Superficial incisional surgical site infection (SISI)	Wound fluid/tissue, wound swab after opening	No differentiation between primary and secondary incisions. Opening of the wound to obtain a swab was not verified. Taking Gram-negative isolate into account in definitive treatment was a prerequisite.
Deep incisional surgical site infection (DISI)	Wound swab after opening/spontaneous dehiscence	No differentiation between primary and secondary incisions.
Post-operative organ/space infection (OSSI)	Fluid/tissue from organ/space	Always combined with another entity referring to infected organ or space. Not used in e.g. appendicitis with culturing of intraperitoneal pus during surgery.
Other intra-abdominal infection (IABI) **Cholangitis/cholecystitis (CHOL)** **Spontaneous bacterial peritonitis/primary peritonitis (PERI)**	Purulent material/tissue from operation/needle aspiration/endoscopy, fluid from surgical drain, blood	Merged with *gastrointestinal tract infection* by adding tissue and endoscopy. Based on treating physician's interpretation, the intra-abdominal infection is categorized as *cholangitis/cholecystitis*, *spontaneous bacterial peritonitis/primary peritonitis*, or *any other infection*.
Skin infection (SKIN)	Skin swab, blood	Skin swab is a modification, but could only be used if any Gram-negative isolate was taken into account in definitive treatment.
Soft tissue infection (SOTI)	Tissue/drainage from affected site, blood	
Decubitus ulcer (DECU)	Needle aspiration of fluid, biopsy ulcer margin, blood (no wound swab)	
Burn infection (BURN)	Blood	
Osteomyelitis (BONE)	Bone, blood	In accordance with Horan *et al.* [3]: not reported if also *mediastinitis*.
Joint or bursa infection (JNTI)	Joint fluid, synovia	
Discitis (DISC)	Disc space tissue from operation/needle aspiration	
Other infections of the urinary tract (OUTI)	Fluid/tissue from affected site (not urine), blood	
Intracranial infection (ICRI)	Brain tissue, dura	
Spinal abscess without meningitis (SPAB)	Abscess in spinal epidural/subdural space, blood	In accordance with Horan *et al.* [3]: not reported if also *meningitis*.

Supplementary Table 3 (continued)

Infection entity	Cultures on which entity can be based	Modifications of original criteria by Horan *et al.* [3] and other comments
Myocarditis/ pericarditis (CARD)	Pericardial tissue/fluid from operation/needle aspiration	
Mediastinitis (MEDI)	Mediastinal tissue/fluid from operation/needle aspiration	
Mastoiditis (MAST)	Purulent drainage from mastoid	
(Tracheo)bronchitis/ tracheitis without evidence of pneumonia (BRON)	Culture from lower respiratory tract (BAL, deep tracheal aspirate), sputum	Sputum is an addition, but could only be used if any Gram-negative isolate was taken into account in definitive treatment. Sputum cultures from the intensive care unit could not be used.
Other infections of the lower respiratory tract (LUNG)	Lung tissue/fluid (including pleural fluid)	In accordance with Horan *et al.* [3]: not reported if also *pneumonia*.
Other infections of the reproductive tract (OREP)	Tissue/fluid from affected site (including fluid/tissue from endometrium from operation/needle aspiration), blood	Merged with *endometritis*, and *vaginal cuff infection*.
Breast abscess or mastitis (BRST)	Affected breast tissue/fluid from operation/incision and drainage	

Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

Supplementary Table 4. Variable definitions

Variable	Definition
Admission type	Classified as either *elective admission*, *emergency admission via emergency ward*, *other form of emergency admission* (e.g. from outpatient or daycare clinic), or *transfer from other hospital* (direct transfer from emergency ward excluded).
Admission ward	Treating specialty for which the patient is admitted to the hospital.
Hospital ward at infection onset	Treating specialty at infection onset. If the patient is in the emergency ward at infection onset, the treating specialty is always *emergency ward*. If infection onset occurs in the operating room, the treating specialty directly before the operation was registered.
Hospital-onset/ community-onset infection[a]	Infection onset at least 48 h after hospital admission (including any preceding hospital transfer). All other infections are classified as *community-onset infection*.
Healthcare-associated infection[a]	Any *community-onset infection* (see above) fulfilling ≥1 of the following criteria: • Intravenous therapy at home or in a daycare clinic within one month prior to infection • Nursing at home within one month prior to infection onset • Wound care at home or at an outpatient clinic within one month prior to infection onset • Hemodialysis within one month prior to infection onset • *Preceding hospital admission within 3 months prior to infection onset* (see below) • *Admission from long-term care facility* (see below) Adapted from Friedman *et al.* [9]
Preceding hospital admission within 3 months prior to infection onset	Hospital admission of ≥2 nights during the three months prior to infection onset. The current admission is excluded, just like any directly preceding stay in another hospital in case of a hospital transfer.
Admission from long-term care facility	Admission from a nursing home or rehabilitation center. If the patient has been transferred from another hospital, the initial hospital admission should be evaluated.
Known colonization with Gram-negatives	Any culture positive for any Gram-negative included in the study (defined in **Table 1** in the **main text**) obtained between 365 and 4 days prior to infection onset. Only colonization detected in the hospital in which the infection or control episode occurred, is included. Any colonization is further specified as *colonization with an HRMO*, *carbapenem-resistant Enterobacterales*, *3GC-resistant Enterobacterales*, *non-fermenters*, and/or *Pseudomonas aeruginosa*.
Gram-negative bacteremia during the year prior to infection onset	Blood cultures positive for any Gram-negative included in the study (defined in **Table 1** in the **main text**) obtained between 365 and 7 days prior to infection onset. Only bacteremias detected in the hospital in which the infection or control episode occurred, are included. Any bacteremia is further specified as *Enterobacterales*, *Pseudomonas* and/or *HRMO bacteremia*.

Supplementary Table 4 (continued)

Variable	Definition
Other bacterial infection at infection onset	Any co-occurring bacterial infections at infection onset, without a relation to the included infection episode. In case of non-infected control patients, any bacterial infection at infection onset is registered.
Myocardial infarction	Patients with one or more definitive or probable myocardial infarctions; diagnosed by a physician in a hospital. Adapted from Charlson *et al.* [10]
Congestive heart failure	Patients with congestive heart failure who are at least in NYHA class II. Left-sided, right-sided and biventricular heart failure, and systolic and diastolic heart failure are all included. Also, new-onset acute heart failure or acute decompensated heart failure accompanied by cardiac asthma is included. Adapted from Charlson *et al.* [10]
Peripheral vascular disease	Patients with intermittent claudication or those who had a bypass for arterial insufficiency, those with gangrene or acute arterial insufficiency, and those with an untreated thoracic or abdominal aortic aneurysm (5 cm or more). Adapted from Charlson *et al.* [10]
Chronic pulmonary disease	Patients who are dyspnoeic at rest, with light/moderate activity, or with attacks (e.g. COPD from GOLD grade 2 onwards, asthma, cystic fibrosis, pulmonary fibrosis, pulmonary metastases/lymphangitis carcinomatosa). Adapted from Charlson *et al.* [10]
Hemiplegia	Patients with complete hemiplegia or paraplegia. Adapted from Charlson *et al.* [10]
ICU-acquired weakness or similar	Patients who are bedridden or only mobilize with help of a wheelchair.
Cerebrovascular disease	Patients with a history of a cerebrovascular accident or transient ischemic attack. Adapted from Charlson *et al.* [10]
Connective tissue disease	Patients with systemic lupus erythematosus, scleroderma/systemic sclerosis/CREST syndrome, Sjögren syndrome, dermatomyositis, polymyositis, mixed connective tissue disease, polymyalgia rheumatica, and moderate to severe rheumatoid arthritis. Vasculitis and sarcoidosis are excluded. Adapted from Charlson *et al.* [10]
Renal disease	Patients on dialysis, those who had a transplant, and those with serum creatinines of >265 µmol/L (documented as chronic renal disease). Specified as on *hemodialysis*, on *peritoneal dialysis*, and/or *post renal transplant*. Adapted from Charlson *et al.* [10]
Diabetes mellitus	Patients treated with insulin or oral hypoglycemic agents. All types of diabetes mellitus are included. Specified as *with* or *without end organ damage* (microvascular complications, e.g. retinopathy, neuropathy or nephropathy). Adapted from Charlson *et al.* [10]
Ulcer disease	Patients who have been diagnosed with a gastric or duodenal ulcer by means of gastroscopy, and those who were surgically treated for a (perforated) ulcus. Adapted from Charlson *et al.* [10]

Supplementary Table 4 (continued)

Variable	Definition
Liver disease	Patients with cirrhosis or chronic hepatitis. Specified as *mild* (no signs of portal hypertension) or *moderate/severe* (signs of portal hypertension: oesophageal/gastric/rectal varices with or without bleeding, splenomegaly, caput medusae, or ascites diagnosed by imaging). Adapted from Charlson *et al.* [10]
(Par)enteral feeding	Patients who receive enteral feeding (via a nasogastric feeding tube or PEG tube) or total parenteral nutrition.
Solid malignancy without metastases[b]	Patients with solid malignancies (carcinomas, sarcomas; hematological malignancies and benign tumors such as adenomas, lipomas and myomas are excluded, with the exception of brain tumors such gliomas, meningiomas, and pituitary adenomas) without documented metastases, but initially treated in the last five years. Among others breast, colon, and lung tumors are included. Adapted from Charlson *et al.* [10]
Metastasized solid malignancy[b]	Patients with solid malignancies that have metastasized at any point in time, independent of when treatment has occurred, even if metastasectomy was performed. Metastasization is based on staging as M1; lymphatic spread is not included. Adapted from Charlson *et al.* [10]
Hematological malignancy	Patients with all forms of lymphomas (including Waldenström's macroglobulinemia), leukemias, and multiple myeloma (not M-GUS). Many lymphoproliferative and myeloproliferative syndromes (a.o. polycythemia vera and myelofibrosis) are excluded. Acute malignancies are always included, chronic ones only if treated. Adapted from Charlson *et al.* [10]
Dementia	Patients with a diagnosis of a dementia syndrome (e.g. Alzheimer's disease). Adapted from Charlson *et al.* [10]
Intellectual disability	Patients with a diagnosis of this neurodevelopmental disorder.
Alcohol abuse	Patients for whom alcohol abuse is documented by a physician, i.e. not based on reported alcohol use during medical history taking.
Solid organ transplant	Patients having had any solid organ transplant, including liver, lung, heart, and renal transplants.
Neutropenia at infection onset[c]	Neutrophils $\leq 0{,}5 \times 10^9$ or leukocytes $\leq 1{,}0 \times 10^9$ on the day of infection onset.
Preceding corticosteroid use[c]	Use of a daily high dose or oral/intravenous corticosteroids (≥20mg prednisone or equivalent) during for ≥14 consecutive days during the 30 days prior to infection onset. Substitution therapy for adrenal insufficiency is excluded. Adapted from CDC Yellow Book [11].

Supplementary Table 4 (continued)

Variable	Definition
Preceding immunosuppressive therapy[c]	Use of other forms of systemic immunosuppression during the 30 days prior to infection onset. Alkylating agents, antimetabolites (including weekly methotrexate), transplant-related immunosuppressants, chemotherapeutics for cancer, and immunomodulating antibodies are included. Excluded are hormonal therapy for cancer, and disease-modifying antirheumatic drugs from other categories, such as mesalazine, sulfasalazine, hydroxychloroquine, and gold salts. Adapted from CDC Yellow Book [11].
Congenital immunodeficiency[c]	Includes severe combined immunodeficiency, common variable immunodeficiency, X-linked agammaglobulinemia, chronic granulomatous disease, hyper-IgM syndrome, selective IgA deficiency, Wiskott-Aldrich syndrome, DiGeorge syndrome. Functional asplenia, splenectomy and complement deficiencies are excluded.
Treatment restriction in place prior to infection onset	Any treatment restriction in place before the day of infection onset, including *do not resuscitate* orders.
Surgical procedure during the 30 days prior to infection onset	All open and endoscopic procedures (e.g. thoracoscopy, transurethral resection of the prostate, arthroscopy) and excisions in the operating room, during the 30 days prior to infection onset (if performed on the day of onset: only scored if finished before obtainment of the first index culture). Insertion of epidural catheters, and peripheral or central venous catheters in the operating room are excluded. The *number of procedures* is specified.
ICU or MCU stay during the 30 days prior to infection onset	Stay of any duration in an MCU or ICU during the 30 days prior to the day of onset of infection. Stays extending before or after this 30 day window are also included. Any stay is further specified as *ICU* or *MCU stay*.
Sepsis severity at infection onset	Categorized as *sepsis*, *severe sepsis* or *septic shock*, based on evaluation of the patient from 24 h before infection onset until 3 h after within the current hospital. *Sepsis* was defined by the presence of ≥2 of the SIRS criteria: • Temperature >38°C or <36°C • Heart rate >90/min • Respiratory rate >20/min or PaCO2 <32 mmHg • Leukocyte count >12x10^9/L or <4 x10^9/L, or >10% immature (band) forms *Severe sepsis* was defined by the presence of sepsis together with signs of organ dysfunction and/or hypoperfusion (e.g. oliguria, alteration in mental status, acute respiratory distress syndrome, coagulopathy, hyperbilirubinemia, heart failure, lactic acidosis), and/or hypotension (decrease in systolic blood pressure >40 mmHg compared to previously, with the most probable cause being the infection). *Septic shock* was defined as the persistence of sepsis-induced hypotension despite adequate fluid resuscitation, and/or the provision of vasopressor agents (excluding those provided during an operation only). Adapted from Bone *et al.* [12]

Supplementary Table 4 (continued)

Variable	Definition
Infectious complications	Any processes besides inflammation occurring at the original site of infection (abscess formation, necrosis), spread to difficult-to-treat structures (osteomyelitis, arthritis), or the occurrence of hematogenous spread (metastatic infection, endocarditis, other forms of endovascular infection, spondylodiscitis).
Source control performed during the admission after infection onset	Any treatment of the infection (including its complications) not involving drug administration, including surgery or interventional radiology (e.g. incision and drainage), insertion or replacement of a biliary stent, removal or replacement of a urinary catheter, and removal or replacement of a central line. Any procedures during which the first index culture was obtained may be included. Procedures before obtainment of the first index culture are excluded, except removal of a central line right before obtainment of the first index culture.
Discharge destination	Classified as *deceased during admission*, *home without additional healthcare*, *home with home healthcare* (excluding activities of daily living assistance), *long-term care facility* (nursing home or rehabilitation center), *terminal care* (at home or in a hospice), and *other hospital*.
Gram-negative bacteremia within 7 to 90 days after infection onset	Blood cultures positive for any Gram-negative included in the study (defined in **Table 1** in the **main text**) obtained between 7 days and 90 days after infection onset. Only bacteremias detected in the hospital in which the infection or control episode occurred, are included. Any bacteremia is further specified as *Enterobacterales*, *Pseudomonas* and/or *HRMO bacteremia*.

Abbreviations: 3GC, third-generation cephalosporin; HRMO, highly resistant micro-organism; ICU, intensive care unit; MCU, medium care unit; NYHA, New York Heart Association; SIRS, systemic inflammatory response syndrome.

[a] Referred to as *origin of infection*.

[b] Combined into *solid malignancy*.

[c] Combined with *human immunodeficiency virus (HIV) infection* (irrespective of CD4 count) into *immunodeficiency*.

Supplementary Table 5. Overview of adjusted models

Analysis type	Exposure evaluated	Model technique	Adjustment variables	RR (95% CI)	Variables included in final model
ICA	HMO Infection	Unadjusted	-	0.91 (0.60–1.38)	-
		Backward elimination	Patient-related (small)	0.77 (0.50–1.20)	Age Known colonization with an HRMO HCA/HO infection Preceding hospital admission Other bacterial infection at infection onset Metastasized solid malignancy Immunodeficiency Preceding treatment restriction
		Forward addition	Patient-related (large)	0.76 (0.49–1.17)	Known colonization with a G- HRMO HO infection Other bacterial infection at infection onset Metastasized solid malignancy Preceding treatment restriction
		Backward elimination	Patient-related[a] Infection-related	0.93 (0.59–1.47)	Age Known colonization with an HRMO HCA/HO infection Preceding hospital admission Other bacterial infection at infection onset Metastasized solid malignancy Preceding treatment restriction Bacteremia Urinary tract infection Pneumonia Infection with *Escherichia coli* Infection with *Enterobacter cloacae* Infection with other G- species Infection with *Enterococcus* spp. Infection with CNS Severe sepsis at infection onset Septic shock at infection onset Antibiotic therapy prior to admission
		Plus one[b]	Patient-related[c] Infection-related[c] Therapy-related	0.99 (0.62–1.59)	As previous model and: Inappropriate antibiotic therapy on the day of infection onset
		Backward elimination	Admission-related	0.93 (0.61–1.42)	Admission ward: surgery Admission ward: urology Admission ward: ICU Preceding length of hospital stay

Supplementary Table 5 (continued)

Analysis type	Exposure evaluated	Model technique	Adjustment variables	RR (95% CI)	Variables included in final model
ICA	Inappropriate antibiotic therapy on the day of infection onset	Unadjusted	-	0.81 (0.61–1.09)	-
		Backward elimination	HRMO infection Patient-related (small) Infection-related	0.76 (0.57–1.03)	Bacteremia Urinary tract infection Infection with *Pseudomonas aeruginosa*
		Forward addition	HRMO infection Patient-related (large) Infection-related	0.76 (0.56–1.03)	HCA/HO infection Bacteremia Urinary tract infection Lower respiratory tract infection Infection with *Pseudomonas aeruginosa*
PCA: non-HRMO	Gram-negative infection	Unadjusted		1.33 (1.07–1.65)	-
		Backward elimination	Patient-related (small)	1.33 (1.07–1.65)	None
		Forward addition	Patient-related (large)	1.25 (1.00–1.56)	Other bacterial infection at infection onset (Par)enteral feeding Preceding treatment restriction Charlson comorbidity index ≥3
PCA: HRMO		Unadjusted		1.69 (0.89–3.20)	-
		Backward elimination	Patient-related (small)	1.40 (0.64–3.05)	Known colonization with an HRMO Preceding G- bacteremia Hospital-onset infection Admission from long-term care facility Other bacterial infection at infection onset Renal disease Preceding surgical procedure Preceding treatment restriction
		Forward addition	Patient-related (large)	1.19 (0.53–2.64)	Known colonization with an HRMO HO infection Admission from long-term care facility Other bacterial infection at infection onset Solid malignancy Preceding surgical procedure Preceding treatment restriction Peripheral vascular disease
PCA: HO		Unadjusted		1.45 (1.04–2.04)	-
		Backward elimination	Patient-related (small)	1.58 (1.12–2.22)	Preceding treatment restriction

Supplementary Table 5 (continued)

Analysis type	Exposure evaluated	Model technique	Adjustment variables	RR (95% CI)	Variables included in final model
ICA: bacteremia subgroup	HRMO infection	Unadjusted	-	0.61 (0.30–1.26)	-
		Backward elimination	Patient-related (small)	0.59 (0.28–1.23)	Age Known colonization with an HRMO Admission from long-term care facility Metastasized solid malignancy
		Backward elimination	Patient-related[a] Infection-related	0.81 (0.38–1.71)	Age Known colonization with an HRMO Metastasized solid malignancy Urinary tract infection Intra-abdominal infection (excl biliary) Postoperative infection Infection with *Escherichia coli* Infection with *Klebsiella pneumoniae* Infection with *Enterobacter cloacae* Infection with other G- species Severe sepsis at infection onset Septic shock at infection onset
		Plus one[b]	Patient-related[c] Infection-related[c] Therapy-related	0.90 (0.41–1.97)	As previous model and: Inappropriate antibiotic therapy on the day of infection onset

Abbreviations: CI, confidence interval; CNS, coagulase-negative *Staphylococcus* spp.; G-, Gram-negative; HRMO, highly resistant micro-organism; HCA, healthcare-associated; HO, hospital-onset; ICA, infection-cohort analysis; ICU, intensive care unit; PCA, parallel cohorts analysis; RR, risk ratio.

[a] Variables remaining after backward elimination of *patient-related confounders*.

[b] No further elimination of adjustment variables was performed.

[c] Variables remaining after backward elimination of *patient-related confounders*, and subsequent backward elimination of *infection-related mediators*.

Supplementary Table 6. Sets for confounding and mediating variables

Set	Variables
Patient-related confounders (small set)	• Sex, age • Known colonization with an HRMO, preceding G- bacteremia • HO infection, HCA infection, preceding hospital admission, admission from long-term care facility • Other bacterial infection at infection onset • Solid malignancy, metastasized solid malignancy, hematological malignancy • Diabetes mellitus, renal disease, liver disease • (Par)enteral feeding, immunodeficiency • Preceding surgical procedure, preceding ICU stay • Preceding treatment restriction
Patient-related confounders (large set)	Variables from the small set, supplemented by: • Known colonization with carbapenem-resistant Enterobacterales, with 3GC-resistant Enterobacterales, with G- non-fermenters, or with *Pseudomonas* spp. • Preceding bacteremia with Enterobacterales, with *Pseudomonas* spp., or with an HRMO • Preceding length of hospital stay • Myocardial infarction, congestive heart failure, peripheral vascular disease, chronic pulmonary disease, ICU-acquired weakness or similar, cerebrovascular disease, connective tissue disease, ulcer disease, hemiplegia, dementia, intellectual disability, alcohol abuse • Charlson comorbidity index ≥3, or ≥5 • Solid organ transplantation, neutropenia at infection onset, preceding corticosteroid use, preceding immunosuppressive therapy • ≥2 preceding surgical procedures, preceding MCU or ICU stay • Receipt of prophylactic antibiotic therapy
Infection-related intermediates	• Causative pathogens, including Gram-negatives and others (16 variables) • Type of infection (10 variables) • Sepsis severity at infection onset (3 variables) • Antibiotic therapy prior to admission
Therapy-related intermediates	• Inappropriate antibiotic therapy on the day of infection onset
Admission-related confounders	• Admission ward (6 variables) • Admission type (3 variables) • Preceding length of hospital stay • Hospital-onset infection

Abbreviations: 3GC, third-generation cephalosporin; G-, Gram-negative; HCA, healthcare-associated; HO, hospital-onset; ICU, intensive care unit; MCU, medium care unit.

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CHAPTER 5

Attributable mortality of vancomycin resistance in ampicillin-resistant *Enterococcus faecium* bacteremia in Denmark and the Netherlands: a matched cohort study

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Abstract

Introduction: In many European hospitals, ampicillin-resistant *Enterococcus faecium* (ARE) is endemic, while outbreaks of vancomycin-resistant *E. faecium* (VRE), belonging to the same genetic lineage, are increasingly reported. We studied the attributable mortality due to vancomycin resistance in patients with *E. faecium* bacteremia and evaluated whether this is mediated by a delay in appropriate antibiotic therapy.

Methods: In a retrospective matched cohort study, patients with VRE bacteremia occurring between 2009 and 2014 in 20 Dutch and Danish hospitals were matched to patients with ARE bacteremia, on hospital, ward, length of hospital stay prior to bacteremia, and age. The risk ratio (RR) for 30-day mortality contrasting VRE with ARE was estimated with further analytic control for confounding factors.

Results: In all, 63 VRE and 234 ARE episodes were matched (36 and 130 for the Netherlands and 27 and 104 for Denmark). Crude 30-day mortality was 27% and 38% for ARE in the Netherlands and Denmark, respectively, and 33% and 48% for VRE in the respective countries. The adjusted RR for 30-day mortality for VRE was 1.54 (95% confidence interval (CI) 1.06–2.25). Although appropriate therapy was initiated later for VRE than for ARE bacteremia, this did not appear to be the reason for the increased mortality risk.

Conclusion: Compared to ARE bacteremia, VRE bacteremia was associated with higher 30-day mortality. One explanation for this association is unmeasured confounding. Alternatively, increased virulence in VRE may be the cause, although both phenotypes belong to the same well-characterized core genomic lineage.

Introduction

As many other countries, the Netherlands and Denmark have faced increasingly frequent polyclonal hospital outbreaks of *Enterococcus faecium* with combined resistance to ampicillin and vancomycin (VRE) during the past years [1,2]. In these countries, ampicillin-resistant, vancomycin-susceptible *E. faecium* (ARE) has become the dominant hospital phenotype of *E. faecium* in recent decades [2,3]. Since hospital-acquired VRE and ARE are genetically indistinguishable at the core genome level, VRE is assumed to have originated from the omnipresent ARE through acquisition of *vanA* or *vanB* genes [4–7]. In both countries, infection control policies have been implemented to prevent nosocomial transmission of VRE (contact precautions for VRE carriers, supplemented by contact tracing and augmented general hygiene measures in case of outbreaks [8,9]), but not for ARE. Failure to control VRE transmission will most likely result in VRE endemicity, because the nosocomial ARE populations will in part be supplanted by VRE [3,10].

Controlling VRE outbreaks imposes a great burden on finances and hospital personnel [11]. To make an appropriate cost-benefit analysis of containing VRE spread in a healthcare system, it is essential to quantify the benefits of such a strategy. The most important threat for individual patients is the adversity patients will experience due to VRE infection as compared to ARE infection. A meta-analysis reported increased mortality after VRE bacteremia compared to ARE bacteremia [12], but most studies included had been performed before effective antibiotics for VRE were available. Since then, few have attempted to quantify the effects of VRE infection compared to ARE infection, and those available suffered from methodological drawbacks, such as combining *E. faecium* and *Enterococcus faecalis* infection and incomplete control for confounding [13].

We, therefore, sought to investigate the fraction of mortality in VRE bacteremia superimposed by vancomycin resistance, in both the Netherlands and Denmark. We also analyzed whether any such increase is the result of a delay in appropriate antibiotic therapy, as this is *a priori* the most likely mediating mechanism.

Methods

Study design, setting and participants

We addressed a causal research question with an observational study in which confounding bias was dealt with in a two-stepped approach. First, by means of matching, patients with ARE

bacteremia and with underlying disease severity similar to the VRE bacteremia patients were chosen as comparison group. Second, we controlled for remaining imbalances in confounding factors after matching by means of adjustment in multivariable models.

This resulted in a retrospective matched cohort study in which episodes of bacteremia caused by *E. faecium* with co-resistance to ampicillin and vancomycin (designated as VRE) were compared to control episodes of bacteremia caused by *E. faecium* with resistance to ampicillin and susceptibility to vancomycin (designated as ARE). Episodes with ampicillin-susceptible VRE bacteremias were excluded. Depending on the availability, a maximum of 4 ARE bacteremias were matched to each VRE bacteremia, using the variables hospital, hospital ward at bacteremia onset, age and length of stay prior to bacteremia (see **Supplementary Material** for a complete description).

No formal sample size calculation was performed, as in both involved countries, VRE bacteremias are rare occurrences, and we had to rely on the willingness of hospitals country-wide to participate. In the Netherlands, VRE bacteremia episodes were identified in 13 hospitals through the national surveillance system ISIS-AR [14], and eleven participated in this study, as did five hospitals not linked to ISIS-AR (see **Supplementary Table 1** for details of participating hospitals). In Denmark, the DACOBAN database was used to identify patients with VRE bacteremia. DACOBAN is a registry of all positive blood cultures from 10 of 11 hospitals in the Capital Region of Denmark (the exception being the tertiary referral center Rigshospitalet) [15]. Patients with VRE bacteremia were identified in five hospitals of which four participated in this study.

In the Netherlands, we included patients with VRE bacteremia that occurred between 1 January 2009 and 1 January 2013, with deviations in some hospitals (**Supplementary Table 1**). In Denmark, we included patients with VRE bacteremia that occurred between 1 January 2012 and 1 January 2015.

Minimum inhibitory concentrations (MICs) for ampicillin and vancomycin were used as reported by local laboratories. All Danish laboratories interpreted antimicrobial susceptibility according to EUCAST standards, but most Dutch laboratories switched from CLSI to EUCAST standards in recent years [14]. Vancomycin resistance had to be confirmed by E-test or demonstration of the presence of *vanA* or *vanB*. The specific VRE genotype was based on PCR-testing or on teicoplanin susceptibility (resistant categorized as *vanA*, susceptible as *vanB*) if PCR testing had not been performed.

The Institutional Review Board of the coordinating center judged the study to be exempt from the Dutch Medical Research Involving Human Subjects Law due to its retrospective nature. Informed consent was not necessary, as data were provided anonymized by treating physicians. In all participating study sites, local regulations for such studies were followed. In Denmark, the study was approved by the Danish Data Protection Agency (registered under 2012-58-0004) and the Danish Health and Medicines Authority (registered under 3-3013-1118/1).

Data collection

After selection of cases and controls, charts were manually reviewed with the date of the index blood culture (bacteremia onset) as reference date. A description of the potential confounding variables and infection-related variables for which data were collected is provided in the **Supplementary Material**.

Additionally, antibiotic use was registered from 30 days prior to bacteremia onset until 14 days after onset, including type of antibiotic, route of administration, and starting and stopping dates. Antibiotic use prior to bacteremia was considered a potential confounder, whereas treatment provided for the *E. faecium* bacteremia episode was considered the main intermediate variable on the causal pathway leading from vancomycin resistance to increased mortality. To analyze this variable, on each calendar day from bacteremia onset onwards (considered day 0), antibiotic treatment was categorized as (a) either *E. faecium*-covering (i.e. including vancomycin, linezolid, daptomycin, teicoplanin, quinupristin/dalfopristin and/or tigecyclin, regardless of vancomycin resistance phenotype) or not, and (b) appropriate (i.e. all of the aforementioned antibiotics for ARE infection, all except vancomycin for *vanB* VRE infection, and all except vancomycin and teicoplanin for *vanA* VRE infection) or inappropriate.

The primary outcome of the study was mortality within 30 days of bacteremia onset, and secondary outcomes were mortality within 1 year, in-hospital mortality, length of hospital stay after bacteremia onset, and intensive care unit admission within 7 days of bacteremia onset. For all bacteremia cases, follow-up data (censoring date or date of death) for at least 30 days after bacteremia onset, but preferably up to 1 year after bacteremia onset were collected.

Statistical analysis

The relation between ARE/VRE and 30-day mortality was estimated using Cox regression models, unadjusted as well as adjusted for potential confounding variables. All models used Cox regression, with stratification on matched sets, robust standard errors, and correlation

between individuals that were included multiple times. For models without censoring, all episodes were given the same arbitrary follow-up time and the Efron approximation for tied survival times was used, so that hazard ratios (HR) could be interpreted as risk ratios (RR) [16]. The standard adjusted models involved inclusion of all potential confounders *a priori* deemed relevant by us to achieve optimal correction, followed by removal of redundant variables to increase precision [17]. As a sensitivity analysis, stepwise addition and removal of potential confounders was performed, starting from a model including only the exposure of interest. In the **Supplementary Material**, exact procedures are described.

Several additional models were created to evaluate mediation of the effect of VRE on mortality through appropriateness of therapy. For this, an interaction between vancomycin resistance and appropriateness of therapy was included. As appropriateness of therapy is a time-varying variable, three models were created in which the baseline was moved to the end of day 0 (day of the index blood culture), +1, and +2, respectively. Patients having died or censored before or on the day of the baseline were removed from the analysis. Appropriateness of therapy in each model reflected the state at baseline. Finally, in some models, the continuous variables age and prior length of stay were included as restricted cubic splines with three knots, to allow for non-linear effects. All statistical analyses were performed in R (version 3.4.3) [18], with the use of packages *survival* [19], *cmprsk* [20], *rms* [21], *mice* [22] and *xtable* [23].

Results

Patient characteristics

In all, 63 VRE episodes were matched to 234 ARE episodes (36 and 130 for the Netherlands and 27, and 104 for Denmark). VRE and matched ARE bacteremia episodes had largely similar characteristics (**Table 1** and **Supplementary Table 2**). Differences between both countries were also present, most prominently involving treatment restriction prior to bacteremia. The latter variable is generally registered on a dedicated location in Dutch health records, but had to be abstracted from written notes in Denmark. Also, comorbidities were retrieved from the DACOBAN registry in Denmark, whereas they were abstracted from medical notes in the Netherlands.

Most VRE were *vanA* (*n* = 41, 65%), 19 were *vanB* (30%), one isolate carried both *vanA* and *vanB* and two isolates could not be categorized. All VRE isolates from Denmark (*n* = 27) were *vanA*. Seventeen isolates were categorized based on teicoplanin susceptibility (3 *vanA* and 14 *vanB*, all from the Netherlands).

Table 1. Characteristics and outcomes of VRE and matched ARE bacteremias

	Netherlands		Denmark	
	ARE bacteremia, n/N with data (%)	**VRE bacteremia, n/N with data (%)**	**ARE bacteremia, n/N with data (%)**	**VRE bacteremia, n/N with data (%)**
Potential confounding variables				
Female	47/130 (36)	19/36 (53)	51/104 (49)	10/27 (37)
Age, median (IQR)	70 (62–76)	69 (62–76)	69 (63–77)	71 (58–76)
Hospital ward at bacteremia onset				
Internal medicine	47/130 (36)	15/36 (42)	31/104 (30)	8/27 (30)
ICU	48/130 (37)	11/36 (31)	25/104 (24)	6/27 (22)
Gastro-enterology/surgery	34/130 (26)	9/36 (25)	31/104 (30)	8/27 (30)
Other	1/130 (1)	1/36 (3)	17/104 (16)	5/27 (19)
Bacteremia origin				
Hospital-onset	113/130 (87)	29/36 (81)	93/104 (89)	24/27 (89)
Healthcare-associated	15/130 (12)	4/36 (11)	10/104 (10)	1/27 (4)
Community-onset	2/130 (2)	3/36 (8)	1/104 (1)	1/27 (4)
Length of hospital stay prior to bacteremia, median (IQR)	17 (11–24)	20 (14–36)	18 (6–24)	21 (10–29)
Preceding hospital admission within 3 months prior to bacteremia	58/130 (45)	15/36 (42)	47/103 (46)	13/26 (50)
Charlson index, median (IQR)	2 (2–3)	2 (1–4)	3 (1–4)	3 (2–6)
Hematological malignancy – under treatment	31/130 (24)	10/36 (28)	9/104 (9)	6/27 (22)
Metastasized solid malignancy	13/130 (10)	1/36 (3)	13/104 (12)	6/27 (22)
Neutropenia at bacteremia onset	32/130 (25)	11/36 (31)	8/104 (8)	6/27 (22)
Treatment restriction in place at bacteremia onset	26/130 (20)	10/36 (28)	5/102 (5)	2/27 (7)
Surgical procedure within 30 days prior to bacteremia	46/130 (35)	9/36 (25)	33/103 (32)	12/27 (44)
Known colonization with *E. faecium*				
No	90/130 (69)	18/36 (50)	88/104 (85)	16/27 (59)
Yes: – ARE	38/130 (29)	7/36 (19)	16/104 (15)	2/27 (7)
Yes – VRE	2/130 (2)	11/36 (31)	0/104 (0)	9/27 (33)
Antibiotic use within 30 days prior to bacteremia	122/130 (94)	33/36 (92)	95/104 (91)	25/27 (93)
Vancomycin use within 30 days prior to bacteremia	13/130 (10)	15/36 (42)	9/104 (9)	6/27 (22)
Infection-related variables				
Polymicrobial bacteremia	35/130 (27)	8/36 (22)	31/103 (30)	11/27 (41)
Severe sepsis at bacteremia onset	30/129 (23)	10/36 (28)	20/104 (19)	4/27 (15)

Table 1 (continued)

	Netherlands		Denmark	
	ARE bacteremia, n/N with data (%)	**VRE bacteremia, n/N with data (%)**	**ARE bacteremia, n/N with data (%)**	**VRE bacteremia, n/N with data (%)**
Bacteremia source				
Primary bacteremia/central line infection/not identifiable from medical file	60/130 (46)	19/36 (53)	59/104 (57)	11/27 (41)
Biliary tract infection	15/130 (12)	4/36 (11)	10/104 (10)	2/27 (7)
Other intra-abdominal infection	35/130 (27)	9/36 (25)	12/104 (12)	7/27 (26)
Other	20/130 (15)	4/36 (11)	23/104 (22)	7/27 (26)
Source control performed before day +7[a] (if applicable to source)	38/79 (48)	8/19 (42)	25/47 (53)	4/13 (31)
Outcome variables				
ICU admission before day +7[a] (if not yet in ICU)	4/82 (5)	4/25 (16)	9/79 (11)	1/21 (5)
Length of hospital stay after bacteremia onset (median, IQR)	22 (10–38)	13 (8–24)	16 (8–36)	14 (6–30)
In-hospital mortality	34/130 (26)	10/36 (28)	38/104 (37)	16/27 (59)
Mortality before day +30[a]	35/130 (27)	12/36 (33)	40/104 (38)	13/27 (48)

This table presents a selection of recorded variables. A full overview is available in **Supplementary Table 2**.
Abbreviations: ICU, intensive care unit; IQR, interquartile range.
[a] Day 0 is the day of the index blood culture of the ARE/VRE bacteremia episode.

Table 2. Regression models for 30-day mortality

	Unadjusted models			Combined adjusted models		
	Combined	**Netherlands**	**Denmark**	**Main analysis**	**Sensitivity analysis**	**Therapy added**
No. of observations	*297*	*166*	*131*	*297*	*295*	*297*
No. of events	*100*	*47*	*53*	*100*	*99*	*100*
No. of variables	*1*	*1*	*1*	*5*	*8*	*6*
Events to variables	*100*	*47*	*53*	*20*	*12.4*	*16.7*
Vancomycin resistance	1.27 (0.87–1.84)	1.16 (0.65–2.06)	1.37 (0.84–2.25)	1.54 (1.06–2.25)	1.49 (0.99–2.22)	1.55 (1.07–2.26)
Inappropriate therapy on day of index blood culture						2.79 (0.99–7.86)

This table presents RRs (95% CI) for 30-day mortality for the exposure of interest (*vancomycin resistance*), with and without adjustment for confounding variables, and with and without the intermediate variable inappropriate therapy on day of index blood culture. In the **Supplementary Material**, the adjustment procedure is described. Combined models include data from both the Netherlands and Denmark.

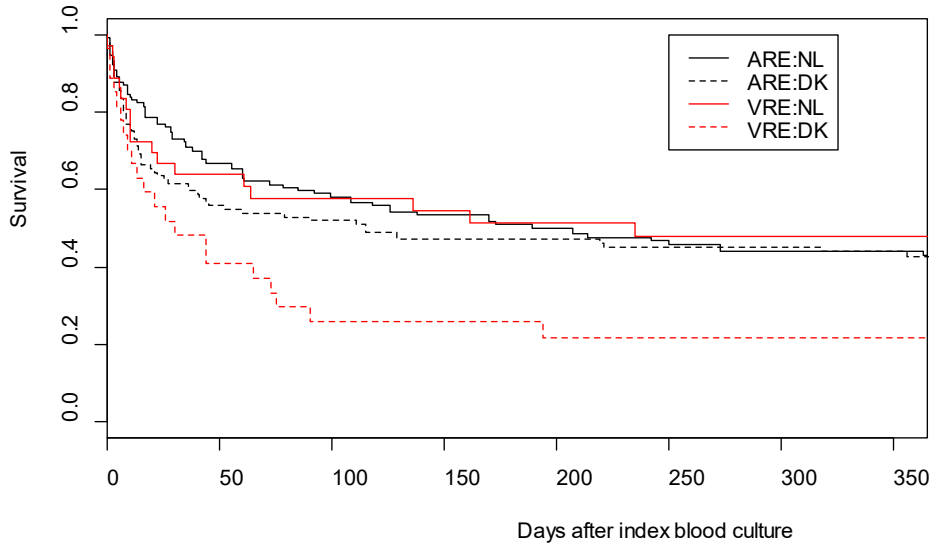

Figure 1. Kaplan-Meier plot indicating one-year survival after ARE/VRE bacteremia, stratified on vancomycin resistance and country.
Abbreviations: NL, Netherlands, DK, Denmark.

Mortality

All patients could be assessed for 30-day mortality, and 76% of censored patients had a follow-up time of at least one year. Crude 30-day mortality was 40% for VRE and 32% for ARE: 33% and 27% for VRE and ARE, respectively, in the Netherlands and 48% and 38% in Denmark. In the Netherlands, 30-day mortality per VRE phenotype was 29% for *vanA* and 37% for *vanB*. The unadjusted RR for 30-day mortality of VRE (compared to ARE) was 1.27 (95% confidence interval (CI) 0.87–1.84; 1.16 (95% CI 0.65–2.06) for the Netherlands, 1.37 (95% CI 0.84–2.25) for Denmark; **Table 2**). Adjustment for confounding increased the RR to 1.54 (95% CI 1.06–2.25). Within the Dutch subgroup, addition of the confounder *Acute Physiology Score before bacteremia onset* to an otherwise optimally adjusted model reduced the RR of vancomycin resistance from 1.62 to 1.17 (see **Supplementary Material**).

In a Kaplan-Meier plot with one year follow-up for mortality, Danish patients with VRE had worse survival compared to patients with ARE bacteremia or Dutch patients with VRE or ARE bacteremia (**Figure 1**). In the multivariable Cox model for mortality up to one year, the HR for VRE amounted to 1.25 (95% CI 0.80–1.98; **Table 3**).

Table 3. Regression models for one-year follow-up for mortality

	Unadjusted models			Combined adjusted models	
	Combined	**Netherlands**	**Denmark**	**Main analysis**	**Sensitivity analysis**
No. of observations	*297*	*166*	*131*	*294*	*297*
No. of events	*170*	*90*	*80*	*170*	*170*
No. of variables	*1*	*1*	*1*	*12*	*7*
Events to variables	*170*	*90*	*80*	*14.2*	*24.3*
Vancomycin resistance	1.18 (0.84–1.65)	0.91 (0.53–1.55)	1.51 (0.97–2.36)	1.25 (0.80–1.98)	1.46 (0.95–2.25)

This table presents HRs (95% CI) for mortality (follow-up of 1 year with censoring) for the exposure of interest (*vancomycin resistance*), with and without adjustment for confounding variables. In the **Supplementary Material**, the adjustment procedure is described. Combined models include data from both the Netherlands and Denmark.

Antibiotic therapy

Visual inspection of cumulative incidence plots revealed that initiation of *E. faecium*-covering antibiotic therapy occurred faster in VRE than in ARE episodes (**Figure 2**), but that initiation of appropriate antibiotic therapy occurred faster for ARE compared to VRE bacteremia (**Figure 3**). In Denmark, appropriate antibiotic therapy for both ARE and VRE bacteremia was started earlier than in the Netherlands, and often consisted of linezolid daptomycin combination treatment (**Table 4**).

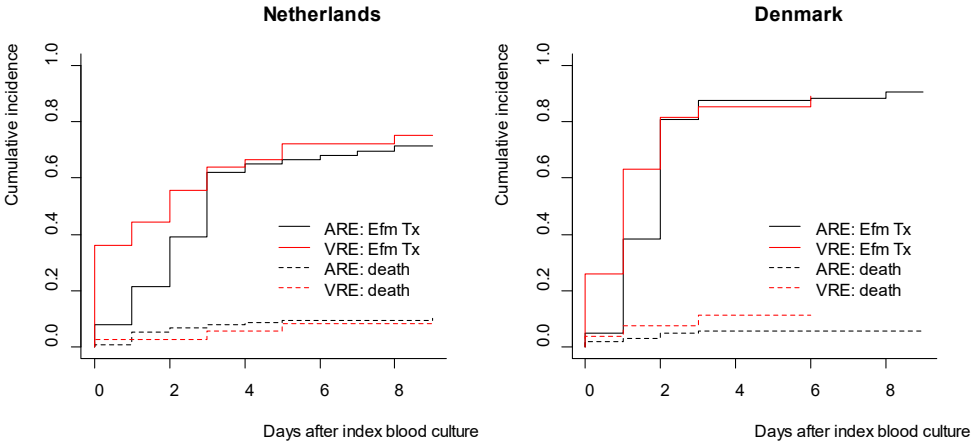

Figure 2. Cumulative incidence plots of *initiation of E. faecium-covering antibiotic therapy after onset of bacteremia* and its competing risk *mortality before onset of E. faecium-covering antibiotic therapy*, stratified on *vancomycin resistance*.
Abbreviations: Efm Tx, Enterococcus faecium-covering therapy.

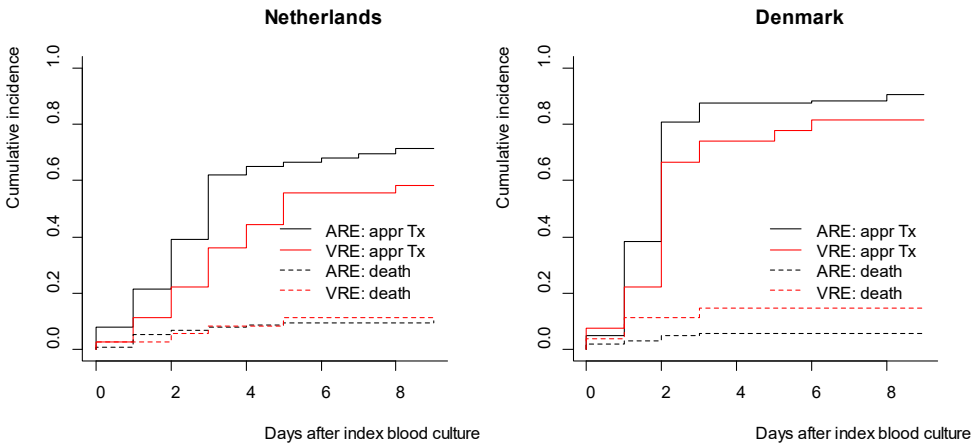

Figure 3. Cumulative incidence plots of initiation of *appropriate antibiotic therapy* after onset of bacteremia and its competing risk *mortality before onset of appropriate antibiotic therapy*, stratified on *vancomycin resistance*. Abbreviations: appr Tx, appropriate therapy.

Inclusion of inappropriate antibiotic therapy on the day of the index blood culture (day 0), in itself associated with mortality (RR 2.79 (95% CI 0.99–7.86)), did not alter the effect of VRE on 30-day mortality (**Table 2**). In models with an interaction between vancomycin resistance and appropriateness of therapy, VRE patients on inappropriate therapy increasingly fared worse over time compared to ARE patients on inappropriate therapy (**Table 5**). ARE patients on appropriate therapy had better survival than those on inappropriate therapy, but this protective effect seemed to diminish over time. The effect estimates for VRE patients on appropriate therapy were uncertain.

Discussion

This study reveals that, after matching on ward type, length of stay prior to bacteremia and age, and further analytic control for confounders, VRE bacteremia was, compared to ARE bacteremia, associated with 54% higher risk for mortality after 30 days (RR 1.54, 95% CI 1.06–2.25). Yet, this increased risk of death must be explained by other factors than a delay in appropriate antibiotic therapy.

Our estimate for the effect of VRE on mortality is similar to the reported pooled OR of 2.52 (95% CI 1.9–3.4) in a meta-analysis from 2005 [12], which translates to a RR of 1.70 in case of 32% death rate in the non-exposed group [24]. This seems remarkably identical, as the studies included in that meta-analysis had been performed before the availability of effective antibiotics for VRE, such as linezolid and daptomycin. Yet, a more recent but relatively small

Table 4. Overview of antibiotic therapy for VRE and matched ARE bacteremias

	End of day 0[a]				End of day +3[a]			
	ARE		VRE		ARE		VRE	
	NL, n/N with data (%)	DK, n/N with data (%)	NL, n/N with data (%)	DK, n/N with data (%)	NL, n/N with data (%)	DK, n/N with data (%)	NL, n/N with data (%)	DK, n/N with data (%)
Deceased	1/130 (1)	2/104 (2)	1/36 (3)	1/27 (4)	12/130 (9)	13/104 (12)	4/36 (11)	4/27 (15)
Censored[b]					1/130 (1)			
Discharged	1/130 (1)			1/27 (4)	5/130 (4)	1/104 (1)	1/36 (3)	2/27 (7)
E. faecium-covering therapy	10/129 (8)	5/102 (5)	13/35 (37)	7/26 (27)	76/117 (65)	82/91 (90)	20/32 (62)	22/23 (96)
vancomycin iv	9/129 (7)	5/102 (5)	12/35 (34)	5/26 (19)	72/117 (62)	79/91 (87)	8/32 (25)	2/23 (9)
linezolid iv			1/35 (3)	1/26 (4)	1/117 (1)	1/91 (1)	6/32 (19)	7/23 (30)
daptomycin iv + linezolid iv								10/23 (43)
teicoplanin iv	1/129 (1)				2/117 (2)		4/32 (12)	
linezolid po				1/26 (4)			2/32 (6)	2/23 (9)
daptomycin iv						2/91 (2)		
vancomycin intrathecally					1/117 (1)			
linezolid iv + tigecyclin iv								1/23 (4)
Appropriate therapy	10/129 (8)	5/102 (5)	1/35 (3)	2/26 (8)	76/117 (65)	82/91 (90)	12/32 (38)	20/23 (87)
Appropriate therapy or central line removed	12/129 (9)	5/102 (5)	2/35 (6)	2/26 (8)	76/117 (65)	82/91 (90)	12/32 (38)	20/23 (87)

Abbreviations: DK, Denmark; iv, intravenously; NL, Netherlands; po, orally.

[a] Day 0 is the day of the index blood culture of the ARE/VRE bacteremia episode.

[b] One Dutch patient with ARE bacteremia was censored for the assessment of antibiotic therapy (not for mortality) due to transfer to another hospital.

Table 5. Regression models for 30-day mortality evaluating appropriateness of therapy

	Baseline after day:		
	0	**+1**	**+2**
	Unadjusted models		
No. of observations	*289*	*283*	*274*
No. of events	*95*	*88*	*80*
ARE – on inappropriate therapy	Reference	Reference	Reference
ARE – on appropriate therapy	0.41 (0.10–1.74)	0.82 (0.45–1.51)	1.01 (0.57–1.79)
VRE – on inappropriate therapy	1.24 (0.82–1.85)	1.15 (0.71–1.88)	1.40 (0.73–2.70)
VRE – on appropriate therapy	NA	1.53 (0.68–3.46)	1.32 (0.64–2.71)
	Adjusted models – main analysis		
No. of observations	*289*	*276*	*268*
No. of events	*95*	*87*	*80*
No. of variables	*9*	*14*	*14*
Events to variables	*10.6*	*6.2*	*5.7*
ARE – on inappropriate therapy	Reference	Reference	Reference
ARE – on appropriate therapy	0.33 (0.08–1.40)	0.79 (0.43–1.45)	0.82 (0.47–1.43)
VRE – on inappropriate therapy	1.69 (1.09–2.61)	2.01 (1.13–3.57)	2.43 (0.94–6.33)
VRE – on appropriate therapy	NA	5.79 (1.43–23.40)	1.73 (0.83–3.61)
	Adjusted models – sensitivity analysis		
No. of observations	*285*	*278*	*270*
No. of events	*94*	*87*	*80*
No. of variables	*16*	*17*	*15*
Events to variables	*5.9*	*5.1*	*5.3*
ARE – on inappropriate therapy	Reference	Reference	Reference
ARE – on appropriate therapy	0.31 (0.08–1.13)	0.69 (0.38–1.28)	0.88 (0.42–1.84)
VRE – on inappropriate therapy	1.42 (0.79–2.55)	1.81 (0.99–3.31)	2.38 (0.99–5.74)
VRE – on appropriate therapy	NA	2.60 (0.55–12.32)	2.12 (0.88–5.09)

This table presents RRs (95% CI) for 30-day mortality for the interaction between *vancomycin resistance* and *appropriateness of therapy*. The baseline for these models is positioned at three different moments, namely the end of the day of the index blood culture (day 0), the end of the day after (day +1), and the end of the day thereafter (day +2). This implies that separately for each baseline, patients having died (or censored, as antibiotic therapy could not be fully assessed) before this moment were removed from the dataset. *Appropriateness of therapy* refers to the antibiotic therapy provided at baseline. RRs are presented with and without adjustment for confounding variables. In the **Supplementary Material**, the adjustment procedure is described. All models include data from both the Netherlands and Denmark.

study focusing on the effects of these newer antibiotics on the outcome of VRE bacteremia in 113 patients concluded that newer antibiotics had not brought discernable benefits to patient outcome [25]. A more recent meta-analysis on the effect of VRE on mortality in the era of effective antibiotic therapy could only present an unadjusted estimate, and hence cannot be compared to our study [13]. In a recent Australian study, *vanB* VRE bacteremia, when compared to vancomycin-susceptible *Enterococcus* spp. bacteremia, had an adjusted OR of 1.21 (95% 0.53–2.79) for in-hospital mortality [26]. This effect seems smaller than that observed in the current study, but may have been influenced by the simultaneous inclusion of the intermediate variable *days to appropriate antibiotic* in the model for the Australian study.

There are three causal pathways along which vancomycin resistance could lead to increased mortality: (i) increased virulence of VRE compared to ARE, (ii) less effective antibiotics for VRE than for ARE, and (iii) a delay in initiation of appropriate antibiotic therapy for VRE bacteremia. We cannot fully exclude a systematic difference in pathogenicity between ARE and VRE, as for example Bender *et al.* have shown that acquisition of *vanB* by *E. faecium* is accompanied by the transfer of larger genetic fragments [27]. However, most studies conclude that both phenotypes belong to the same, well-characterized, core genomic lineage of *E. faecium* [4,7]. Furthermore, to the best of our knowledge, there is no evidence that the appropriate antibiotic options for *E. faecium*, most prominently vancomycin, linezolid or daptomycin, have different efficacy for susceptible strains. In this study, vancomycin was mostly used for ARE, and linezolid and daptomycin for VRE.

The observed increased mortality in case of VRE bacteremia, therefore, could be expected to result from a delay in appropriate therapy, which has been implicated previously in worse outcomes in case of enterococcal bacteremia [28]. However, our models that include appropriateness of therapy do not offer support for this hypothesis. VRE patients on inappropriate therapy continuously fare worse than ARE patients on inappropriate therapy. Therapy over time may be reflective of the evolving disease severity of the patient, and collider bias may be induced by conditioning on appropriateness of therapy [29]. This means that effect estimates of appropriateness of therapy after baseline may not reflect true causal associations. However, seeing that this trend is discernable from the day of the index blood culture onwards increases our confidence that the difference in duration until appropriate therapy is unable to explain the increased mortality in case of VRE bacteremia. A final indication for this stems from the comparison between countries in this study. Overall mortality in Denmark for both ARE and VRE bacteremia is higher than in Netherlands, although

appropriate therapy for both types of bacteremia is initiated considerably faster in Denmark than in the Netherlands.

As these biologically plausible mediators cannot explain increased mortality due to vancomycin resistance, the possibility remains that these observed effects are due to unmeasured confounding. This possibility is supported by two additional observations. First, a measure for clinical disease severity immediately before bacteremia onset was not available for the Danish patients. For the Dutch patients, we could calculate the Acute Physiology score, and when included in our country-specific analyses, it substantially reduced the effect estimate for mortality (see **Supplementary Material**). Second, the association between vancomycin resistance and mortality persisted over the course of a full year. Infections may have long-term sequelae [30], but it seems unlikely that sustained mortality differences will emerge that can be causally related to vancomycin resistance. A recent population-based study reported that the incidence of recurrent bacteremia, an example of a long-term consequence, only marginally differed between ARE and VRE bacteremia [31]. An alternative explanation is that underlying prognostic factors at the time of onset of enterococcal bacteremia were dissimilar.

Several limitations of this study should be discussed. First, results of this study may not apply to *E. faecium* bacteremia in general, as a non-random subset of ARE bacteremias was included. The matched design does not allow for direct comparisons of raw proportions other than for VRE vs. ARE. Second, some loss in precision may be expected in stratified analyses, as not all matched sets can be used for parameter estimation. Third, measurements of comorbidities and treatment restrictions differed between both countries, whereas these differences were not included in models. Fourth, duration until initiation of appropriate therapy could not be reliably measured in hours, and was reflected instead by calendar days.

Finally, some studies suggest that the incidence of infections with VRE occur on top of the existing incidence of infections caused by vancomycin-susceptible enterococci [32,33]. In that case, a comparison between VRE bacteremia and an uninfected control group would be more appropriate, as described by Chiang *et al.* [34]. Yet, it is important to note that these incidence rates may have been confounded by the fact that *E. faecalis* was not separated from *E. faecium*, and that results from molecular epidemiological studies provided strong evidence that ARE and VRE occupy the same niche within the bacterial hospital ecology [4].

In conclusion, VRE bacteremia was, when compared to ARE bacteremia, associated with higher mortality. This could not be explained by delays in initiation of appropriate antibiotic therapy,

although the relevant models are possibly underpowered and should be interpreted with caution. Because of the large heterogeneity among infected patients and the multiple determinants that mediate the outcome for patients developing *E. faecium* bacteremia, unmeasured confounding is a likely explanation. In that case, replacement of ARE infections by VRE infections would not lead to higher 30-day mortality. The alternative explanation is that VRE is more virulent than ARE. Given the resemblance of the core genomes of ARE and VRE, the genetic basis for hypervirulence would then be most likely encoded in the accessory genome, the mobilome. In that case, emergence of VRE could not only replace ARE infections but also increase the total burden of infection. Further studies are warranted to explore this possibility.

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CHAPTER 5
SUPPLEMENTARY MATERIAL

Attributable mortality of vancomycin resistance in ampicillin-resistant Enterococcus faecium bacteremia in Denmark and the Netherlands: a matched cohort study

Data collection on potential confounders and infection-related variables

Potential confounders for which data were collected, included age, gender; ward; preceding length of hospital stay and – if applicable – intensive care unit stay; bacteremia origin; admission from long-term care facility, earlier hospital admission of ≥2 nights during the preceding 3 months; comorbidity; immunodeficiency; treatment restriction agreed upon; any surgical procedure during the preceding 30 days; mechanical ventilation and/or central venous catheter present at bacteremia onset; cultures with ampicillin-resistant *Enterococcus faecium* (ARE), vancomycin-resistant *E. faecium* (VRE), or methicillin-resistant *Staphylococcus aureus* (MRSA) during the preceding year (with result known at bacteremia onset); and the Acute Physiology Score before bacteremia onset (see **Analysis with Acute Physiology Score** in this **Supplementary Material**) [1].

Bacteremia origin was defined as hospital-onset if bacteremia onset was ≥48 h after admission. Other episodes were categorized as either community-onset or healthcare-associated (in case of admission from long-term care facility; earlier hospital admission of ≥2 nights during the preceding 3 months; or intravenous therapy, nursing at home, hemodialysis, or wound care during the preceding month) [2]. Immunodeficiencies recorded included neutropenia at bacteremia onset ($<500x10^6$/L), high daily dose corticosteroid therapy (equivalence of ≥20mg prednisone) of ≥14 days' duration during the preceding month, and other forms of immunosuppressive therapy. The Charlson index, with additional information recorded on the type of hematological or solid malignancy [3], was used to quantify comorbidity.

Infection-related variables were bacteremia source, source control procedures, sepsis severity at bacteremia onset, and isolation of pathogens other than *E. faecium* from the index blood culture. Bacteremia source was based on the final interpretation by treating physicians and consulting medical microbiologists or infectious disease specialists. If patients died before consultation, the clinical working diagnosis at onset was registered. If no clear source was registered, the source was classified as primary, and when no or conflicting information was available in the medical file, the source was classified as not identifiable. Any source control procedure (including but not restricted to removal of vascular catheters, surgical procedures, percutaneous abscess drainage, and insertion of biliary stents) up to 7 days after bacteremia onset was registered, including the date of the procedure. Sepsis severity on the calendar day on which the index blood culture was obtained was categorized as either severe sepsis (including septic shock) or not [4].

Matching procedure

Study periods in hospitals in the Netherlands generally extended from 1 January 2009 to 31 December 2012, with a few exceptions due to inclusion of hospitals with VRE outbreaks occurring in 2013, or availability of data when the Dutch antimicrobial resistance surveillance database ISIS-AR was queried (**Supplementary Table 1**). Since 2011, the number of VRE outbreaks has substantially increased in the Netherlands. We decided however, to extend the study period further backwards to 1 January 2009, firstly to increase sample size by including several sporadic VRE bacteremias occurring before 2012, and secondly to enlarge the pool of potential ARE controls, especially in smaller hospitals and on hospital wards with sporadic *E. faecium* bacteremias. 1 January 2009 was chosen as the study start date because of data availability for relevant hospitals in ISIS-AR.

In Denmark, VRE outbreaks increased in number from 2012 onwards, and the study period was set to 1 January 2012 through 31 December 2014. An extension back in time was not considered necessary to improve selection of ARE controls.

For VRE, only the first episode of bacteremia was included, and any ARE bacteremia before or after was not eligible as control episode. As VRE bacteremia could have been preceded by ARE bacteremia, all episodes of ARE bacteremia were eligible for selection as control episode, unless preceded by ARE bacteremia in the prior 30 days, in order to prevent inclusion of bacteremia relapses. This, however, was not an exclusion criterion for VRE bacteremias

Matching variables were *hospital*, *hospital ward at bacteremia onset*, *age* and *length of hospital stay prior to bacteremia*. *Wards* were defined as internal medicine, intensive care unit (ICU), gastro-enterology, surgery, cardiology, pulmonary medicine, urology, and orthopedics. In one Danish hospital, gastro-enterology and surgery could not be separated during the matching process and were treated as a single ward (4 VRE bacteremias affected).

The matching protocol consisted of three steps. First, for each VRE bacteremia, all episodes of ARE bacteremia occurring on the same *ward*, in the same *hospital*, during the entire hospital-specific study period were selected (*potential match pool*). Second, *length of hospital stay prior to bacteremia* was log-transformed (referred to as ln(LOS)) for all VRE and ARE bacteremia episodes, and for each VRE bacteremia episode, the *definitive match pool* was created by selecting controls with a ln(LOS) that fell within a 2.5% absolute difference margin of the ln(LOS) of the VRE bacteremia. If the *definitive match pool* did not contain at least five ARE

bacteremia episodes, the absolute difference margin for ln(LOS) was increased by steps of 2.5%, until the minimum of five was reached.

Third, from the *definitive match pool*, the four ARE bacteremia patients with the smallest absolute difference in *age* were selected as controls. In case of identical absolute age differences, the elder ARE bacteremia patient was preferred, and otherwise a random selection was made. If the *definitive match pool* contained fewer than five ARE bacteremia episodes, all were selected as control, and a matched set of size below five emerged (2 sets of four, 4 sets of two, and 1 set of VRE only).

As analyses were always performed within sets, a single ARE bacteremia episode could serve as a control for different VRE bacteremia episodes. In fact, 26 ARE bacteremias were included twice, and 4 thrice.

Variable selection in multivariable analyses

For the adjusted analysis, the first step involved *a priori* selection of a set of confounders and including them all in the so-called *full model*, together with the exposure evaluated (generally *vancomycin resistance*). It was then evaluated in a stepwise procedure whether variables could be removed from the model while retaining approximately the same β coefficient for the exposure. This was done to increase precision of the effect estimate, reflected by a narrowing of its confidence interval. Removal of variables started with removing the variable that would result in a new model with the smallest deviance in β coefficient for the exposure compared to the *full model*. Subsequently, all variables were evaluated again, and the confounder impacting the β coefficient the least in this round, was removed, always with reference to the β coefficient of the exposure in the *full model*. This iterative process was halted if the β coefficient would deviate >10% from the β coefficient in the *full model* if one of the remaining confounding were to be removed. If the exposure consisted of multiple levels (in case of treatment variables), all β coefficient reflecting the different levels were evaluated, and if any would change >10%, the process was halted. If the resulting *reduced model* would be extremely overfitted (<5 events per variable), the cut-off of 10% could be increased.

As we made a selection of potential confounders on which data were collected, to include in the *full model* in order to prevent overfitting, a stepwise *sensitivity analysis* was performed in which all potential confounders were available for inclusion. The model started with the exposure only, and subsequently, for all potential confounders, it was evaluated how much the β coefficient for the exposure would be changed in case of incorporation into the model.

The potential confounder with the largest resulting change in β coefficient was selected for inclusion. Taking this new model as the starting point, all remaining potential confounders were evaluated again for their effect on the β coefficient of the exposure. In each round, one variable could be incorporated into the model, as long as it would change the β coefficient >10%. To prevent overfitting, after inclusion of a new confounder, it was also evaluated whether any confounders already included could be removed again from the model. Variables were removed if the β coefficient of the exposure in the current model differed <10% from a model without the variable, starting with the variable with the smallest change in β coefficient. These cycles were repeated until no excluded variable could be found for which inclusion would change the β coefficient >10%, and no included variable had an impact <10% on the β coefficient. When cycles of exclusions and inclusions involving the same variables were detected by the algorithm, all cycling variables were included in the model. If the result of the *sensitivity analysis* would be extremely overfitted (<5 events per variable), the cut-off of 10% could be increased.

All model variants for 30-day mortality with the baseline on the day of the index blood culture are presented in **Supplementary Table 2**. In the **main text**, only the *reduced model* presented (referred to as *main analysis*) and the *sensitivity analysis* are shown, and only the effect estimate of the exposure evaluated is presented. Confounders are left out for clarity. If an intermediate variable related to treatment was included in a model, its effect estimates are also presented.

Analysis with Acute Physiology Score

In the Netherlands, Acute Physiology Scores (APS) as described for APACHE III were collected for included patients (n = 166) [1]. This confounding variable was supposed to represent underlying disease severity before the onset of bacteremia. In principle, all parameters were recorded on the second day before onset of bacteremia (day -2). In the case of laboratory parameters, other days prior to bacteremia could be used if unavailable on this day. In order of preference these days were -3, -1, -4, and in the case of albumin, day -5 through -9. If parameters were measured several times on the same calendar day, both the highest and lowest value were recorded, and the resulting most extreme score was used to calculate the APS.

Due to unavailability of records, in a considerable proportion of cases (n = 41, 25%) only laboratory values could be recorded, and in some cases (n = 4), there was a total absence of

data on the APS. In order to perform valid analyses, an imputation procedure was used, assuming a missing at random (MAR) mechanism. Using the *multivariate imputation by chained equations* procedure as incorporated in the *mice* package (version 2.46.0) for R, 50 imputed datasets were created for the Dutch dataset.

Variables used in the imputation process were all other recorded potential confounders (indicated in **Table 1**), *hospital*, *ward*, infection-related variables (*vancomycin resistance*, *polymicrobial bacteremia*, *severe sepsis at bacteremia onset*, *bacteremia source*), treatment-related variables (*source control performed before day +7*, *intravenous antibiotics on day 0*, *inappropriate therapy on day 0*, *day of initiation of appropriate therapy*), outcome-related variables (*length of hospital stay after bacteremia onset*, *in-hospital mortality*, *30-day mortality*, *1-year mortality*), and the *APS for laboratory parameters only*. *Age*, *length of hospital/ICU stay before/after bacteremia onset*, and *laboratory and total APS* were included as continuous predictors, while *hospital* (16 categories), *ward* (internal medicine, ICU, gastro-enterology/surgery, other), *bacteremia origin* (hospital-onset, healthcare-associated, community-onset), *bacteremia source* (primary/central line/unknown, biliary, intra-abdominal, other), *Charlson index* (0–1, 2, 3–4, 5+), *number of comorbidities* (0, 1–2, 3+), and *known colonization with E. faecium* (no, ARE, VRE) were included as categorical predictors. The remainder of variables were binary predictors. No interactions were included.

Apart from imputing APS values, some other missings were imputed, namely for central venous catheter at bacteremia onset (n = 1), severe sepsis at bacteremia onset (n = 1), 1-year mortality (n = 19), and day of initiation of appropriate therapy (n = 1).

With these 50 imputed datasets, several models were constructed, using Rubin's rules for pooling of estimates. First, using the regularly available confounders, an optimally corrected (specifically for the Dutch dataset) model was created by combining results from the *main* and *sensitivity analyses*. The steps during creation of this model are indicated in **Supplementary Table 2**. Subsequently, the additional confounder APS was added to this model, while applying a restricted cubic spline function with three knots to allow for non-linearity. The effect estimates for the exposure *vancomycin resistance* could then be contrasted between models (**Supplementary Table 2**).

Supplementary Table 1. Hospital characteristics

Hospital	Country	Location	Hospital type	Start date	Stop date	No. of VRE episodes	No. of ARE episodes
Bispebjerg Hospital	DK	Copenhagen	General	1 Jan 2012	1 Jan 2015	10	36
Herlev Hospital	DK	Herlev	General	1 Jan 2012	1 Jan 2015	10	40
Hvidovre Hospital	DK	Hvidovre	General	1 Jan 2012	1 Jan 2015	6	24
Rigshospitalet Glostrup	DK	Glostrup	General	1 Jan 2012	1 Jan 2015	1	4
St. Antonius Ziekenhuis	NL	Utrecht/Nieuwegein	General	1 Jan 2009	1 Oct 2012[a]	3	12
Canisius-Wilhelmina Ziekenhuis	NL	Nijmegen	General	1 Jan 2009	1 Jan 2013	7	28
Catharina Ziekenhuis	NL	Eindhoven	General	1 Jan 2009	1 Jan 2013	1	4
Deventer Ziekenhuis	NL	Deventer	General	1 Jan 2009	1 Jan 2013	1	4
Flevoziekenhuis	NL	Almere	General	1 Jan 2009	1 Jan 2013	2	1
Isala	NL	Zwolle	General	1 Jan 2009	1 Jan 2013	4	14
Jeroen Bosch Ziekenhuis	NL	's-Hertogenbosch	General	1 Jan 2009	1 Jan 2013	1	4
Maasstad Ziekenhuis	NL	Rotterdam	General	1 Jan 2009	1 Jan 2013	3	9
Martini Ziekenhuis	NL	Groningen	General	1 Jan 2009	1 Jan 2013	1	4
Máxima Medisch Centrum	NL	Eindhoven/Veldhoven	General	1 Jan 2009	1 Jan 2014[b]	6	23
Noordwest Ziekenhuisgroep	NL	Alkmaar	General	1 Jan 2009	1 Jan 2013	1	4
Onze Lieve Vrouwe Gasthuis	NL	Amsterdam	General	1 Jan 2009	1 Jan 2013	2	8
Radboudumc	NL	Nijmegen	University	1 Jan 2009	1 Jan 2013	1	4
VU medisch centrum	NL	Amsterdam	University	1 Jan 2012[c]	1 Jul 2013[c]	1	4
Zuwe Hofpoort Ziekenhuis[d]	NL	Woerden	General	1 Jan 2009	1 Jan 2013	1	1
Zuyderland	NL	Heerlen/Sittard-Geleen	General	1 Jan 2009	1 Jan 2013	1	4

Abbreviations: DK, Denmark; NL, Netherlands.

[a] Earlier stop date due to data availability at the moment of querying ISIS-AR.

[b] Later stop date due to outbreak occurring in 2013.

[c] Earlier start date and later stop date due to outbreak occurring in 2013.

[d] Hospital now merged with Antonius Ziekenhuis in Utrecht/Nieuwegein.

Supplementary Table 2. Full characteristics and outcomes of VRE and matched ARE bacteremias

	Netherlands		Denmark	
	ARE bacteremia, n/N with data (%)	**VRE bacteremia, n/N with data (%)**	**ARE bacteremia, n/N with data (%)**	**VRE bacteremia, n/N with data (%)**
Potential confounding variables				
Female	47/130 (36)	19/36 (53)	51/104 (49)	10/27 (37)
Age, median (IQR)	70 (62–76)	69 (62–76)	69 (63–77)	71 (58–76)
Hospital ward at bacteremia onset				
Internal medicine: hematology	19/130 (15)	7/36 (19)	10/104 (10)	6/27 (22)
Internal medicine: oncology	19/130 (15)	4/36 (11)	5/104 (5)	0/27 (0)
Internal medicine: nephrology	4/130 (3)	1/36 (3)	2/104 (2)	0/27 (0)
Internal medicine: other subspecialism	3/130 (2)	3/36 (8)	11/104 (11)	2/27 (7)
Internal medicine: subspecialism unknown	2/130 (2)	0/36 (0)	3/104 (3)	0/27 (0)
ICU	48/130 (37)	11/36 (31)	25/104 (24)	6/27 (22)
Gastro-enterology	22/130 (17)	7/36 (19)	9/104 (9)	3/27 (11)
Surgery	12/130 (9)	2/36 (6)	22/104 (21)	5/27 (19)
Cardiology	1/130 (1)	1/36 (3)	4/104 (4)	1/27 (4)
Pulmonary medicine	0/130 (0)	0/36 (0)	5/104 (5)	1/27 (4)
Urology	0/130 (0)	0/36 (0)	1/104 (1)	1/27 (4)
Other surgical specialism	0/130 (0)	0/36 (0)	7/104 (7)	2/27 (7)
Bacteremia origin				
Hospital-onset	113/130 (87)	29/36 (81)	93/104 (89)	24/27 (89)
Healthcare-associated	15/130 (12)	4/36 (11)	10/104 (10)	1/27 (4)
Community-onset	2/130 (2)	3/36 (8)	1/104 (1)	1/27 (4)
Community-onset, unknown if healthcare-associated	0/130 (0)	0/36 (0)	0/104 (0)	1/27 (4)
Length of hospital stay prior to bacteremia, median (IQR)	17 (11–24)	20 (14–36)	18 (6–24)	21 (10–29)
Length of ICU stay prior to bacteremia, median (IQR)	8 (2–17)	10 (2–13)	7 (1–9)	11 (4–13)
Preceding hospital admission within 3 months prior to bacteremia	58/130 (45)	15/36 (42)	47/103 (46)	13/26 (50)
Admitted from long-term care facility	2/130 (2)	4/36 (11)	8/103 (8)	1/26 (4)
Charlson index				
0–1	27/130 (21)	12/36 (33)	27/104 (26)	5/27 (19)
2	45/130 (35)	8/36 (22)	20/104 (19)	7/27 (26)
3–4	39/130 (30)	12/36 (33)	36/104 (35)	7/27 (26)
5+	19/130 (15)	4/36 (11)	21/104 (20)	8/27 (30)
Number of comorbidities				
0	13/130 (10)	4/36 (11)	13/104 (12)	1/27 (4)
1–2	101/130 (78)	24/36 (67)	69/104 (66)	21/27 (78)
3+	16/130 (12)	8/36 (22)	22/104 (21)	5/27 (19)

Supplementary Table 2 (continued)

	Netherlands		Denmark	
	ARE bacteremia, n/N with data (%)	**VRE bacteremia, n/N with data (%)**	**ARE bacteremia, n/N with data (%)**	**VRE bacteremia, n/N with data (%)**
Myocardial infarction	27/130 (21)	1/36 (3)	4/104 (4)	0/27 (0)
Chronic pulmonary diseae	19/130 (15)	4/36 (11)	23/104 (22)	5/27 (19)
Diabetes mellitus	23/130 (18)	9/36 (25)	16/104 (15)	6/27 (22)
Cerebrovascular disease	10/130 (8)	3/36 (8)	20/104 (19)	5/27 (19)
Chronic renal disease	9/130 (7)	4/36 (11)	23/104 (22)	2/27 (7)
Hematological malignancy	42/130 (32)	11/36 (31)	9/104 (9)	6/27 (22)
Solid malignancy	41/130 (32)	8/36 (22)	35/104 (34)	10/27 (37)
Metastasized solid malignancy	13/130 (10)	1/36 (3)	13/104 (12)	6/27 (22)
Immunodeficiency	46/130 (35)	11/36 (31)	20/104 (19)	11/27 (41)
Neutropenia at bacteremia onset	32/130 (25)	11/36 (31)	8/104 (8)	6/27 (22)
Treatment restriction in place at bacteremia onset	26/130 (20)	10/36 (28)	5/102 (5)	2/27 (7)
Surgical procedure within 30 days prior to bacteremia	46/130 (35)	9/36 (25)	33/103 (32)	12/27 (44)
Mechanical ventilation at bacteremia onset	34/130 (26)	4/36 (11)	16/104 (15)	4/27 (15)
Central venous catheter at bacteremia onset	65/129 (50)	19/36 (53)	61/103 (59)	13/27 (48)
Known colonization with *E. faecium*				
No	90/130 (69)	18/36 (50)	88/104 (85)	16/27 (59)
Yes – ARE	38/130 (29)	7/36 (19)	16/104 (15)	2/27 (7)
Yes – VRE	2/130 (2)	11/36 (31)	0/104 (0)	9/27 (33)
Known colonization with MRSA	0/130 (0)	0/36 (0)	4/104 (4)	0/27 (0)
Antibiotic use within 30 days prior to bacteremia	122/130 (94)	33/36 (92)	95/104 (91)	25/27 (93)
Prior use of SOD/SDD	31/130 (24)	6/36 (17)	0/104 (0)	0/27 (0)
Prior use of β-lactams	113/130 (87)	32/36 (89)	94/104 (90)	24/27 (89)
Prior use of penicillins	77/130 (59)	18/36 (50)	80/104 (77)	20/27 (74)
Prior use of cephalosporins	77/130 (59)	24/36 (67)	38/104 (37)	7/27 (26)
Prior use of carbapenems	17/130 (13)	6/36 (17)	25/104 (24)	11/27 (41)
Prior use of fluoroquinolones	77/130 (59)	18/36 (50)	50/104 (48)	14/27 (52)
Prior use of aminoglycosides	27/130 (21)	7/36 (19)	18/104 (17)	6/27 (22)
Prior use of vancomycin	13/130 (10)	15/36 (42)	9/104 (9)	6/27 (22)
Infection-related variables				
Polymicrobial bacteremia	35/130 (27)	8/36 (22)	31/103 (30)	11/27 (41)
Severe sepsis at bacteremia onset	30/129 (23)	10/36 (28)	20/104 (19)	4/27 (15)

Supplementary Table 2 (continued)

	Netherlands		Denmark	
	ARE bacteremia, n/N with data (%)	**VRE bacteremia, n/N with data (%)**	**ARE bacteremia, n/N with data (%)**	**VRE bacteremia, n/N with data (%)**
Bacteremia source				
Primary bacteremia	21/130 (16)	9/36 (25)	23/104 (22)	6/27 (22)
Central line-associated bacteremia	18/130 (14)	5/36 (14)	15/104 (14)	2/27 (7)
Not identifiable from medical file	21/130 (16)	5/36 (14)	21/104 (20)	3/27 (11)
Biliary tract infection	15/130 (12)	4/36 (11)	10/104 (10)	2/27 (7)
Spontanous/primary peritonitis	1/130 (1)	0/36 (0)	0/104 (0)	0/27 (0)
Other intra-abdominal infection	34/130 (26)	9/36 (25)	12/104 (12)	7/27 (26)
Urinary tract infection	5/130 (4)	1/36 (3)	11/104 (11)	4/27 (15)
Pneumonia	3/130 (2)	1/36 (3)	2/104 (2)	1/27 (4)
Skin/soft tissue infection	4/130 (3)	1/36 (3)	2/104 (2)	0/27 (0)
Wound infection	3/130 (2)	0/36 (0)	2/104 (2)	0/27 (0)
Endocarditis	0/130 (0)	0/36 (0)	1/104 (1)	0/27 (0)
Other	5/130 (4)	1/36 (3)	5/104 (5)	2/27 (7)
Treatment-related variables				
Intravenous antibiotics on day 0[a]	95/130 (73)	27/36 (75)	89/104 (86)	23/27 (85)
Inappropriate therapy on day 0[a]	120/130 (92)	35/36 (97)	99/104 (95)	25/27 (93)
Day of initiation of appropriate therapy				
Deceased/censored[b] before day +4[a]	10/129 (8)	3/36 (8)	6/104 (6)	4/27 (15)
Day 0[a]	10/129 (8)	1/36 (3)	5/104 (5)	2/27 (7)
Day +1[a]	18/129 (14)	3/36 (8)	35/104 (34)	4/27 (15)
Day +2[a]	23/129 (18)	4/36 (11)	44/104 (42)	12/27 (44)
Day +3[a]	29/129 (22)	5/36 (14)	7/104 (7)	2/27 (7)
No appropriate therapy before day +4[a]	39/129 (30)	20/36 (56)	7/104 (7)	3/27 (11)
≥7 days of appropriate therapy (if initiated before day +4[a] and no death during)	64/75 (85)	10/11 (91)	52/75 (69)	15/17 (88)
Source control performed before day +7[a] (if applicable to source)	38/79 (48)	8/19 (42)	25/47 (53)	4/13 (31)
Outcome variables				
ICU admission before day +7[a] (if not yet in ICU)	4/82 (5)	4/25 (16)	9/79 (11)	1/21 (5)
Length of hospital stay after bacteremia onset (median, IQR)	22 (10–38)	13 (8–24)	16 (8–36)	14 (6–30)
In-hospital mortality	34/130 (26)	10/36 (28)	38/104 (37)	16/27 (59)
Mortality before day +30[a]	35/130 (27)	12/36 (33)	40/104 (38)	13/27 (48)

Abbreviations: ARE, ampicillin-resistant *Enterococcus faecium*; ICU, intensive care unit; IQR, interquartile range; MRSA, methicillin-resistant *Staphylococcus aureus*; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination; VRE, vancomycin-resistant *Enterococcus faecium*.

[a] Day 0 is the day of the index blood culture of the ARE/VRE bacteremia episode.

[b] One Dutch patient with ARE bacteremia was censored for the assessment of antibiotic therapy (not for mortality) due to transfer to another hospital.

Supplementary Table 3. Full overview of different multivariable regression analyses for 30-day mortality

	Netherlands and Denmark combined			Netherlands only					
	Main analysis - full	**Main analysis - reduced**	**Sensitivity analysis**	**Main analysis - full**	**Main analysis - reduced**	**Sensitivity analysis - full**	**Sensitivity analysis - reduced[a]**	**Combined analysis[b]**	**With APS[c]**
No. of observations	*292*	*297*	*29*	*165*	*166*	*166*	*166*	*166*	*166*
No. of events	*100*	*100*	*99*	*47*	*47*	*47*	*47*	*47*	*47*
No. of variables	*16*	*5*	*8*	*16*	*3*	*20*	*5*	*7*	*9*
Events to variables	*6.2*	*20*	*12.4*	*2.9*	*15.7*	*2.4*	*9.4*	*6.7*	*5.2*
Vancomycin resistance	1.49 (0.97–2.31)	1.54 (1.06–2.25)	1.49 (0.99–2.22)	1.82 (0.81–4.09)	1.81 (0.98–3.36)	1.05 (0.46–2.43)	1.10 (0.60–2.04)	1.62 (0.85–3.12)	1.17 (0.55–2.52)
Age[d]			1.02 (0.98–1.07)			1.03 (0.96–1.12)			
Age'[d]			0.99 (0.94–1.04)			1.02 (0.94–1.11)			
Number of comorbidities: 1–2						0.32 (0.17–0.60)	0.23 (0.11–0.50)	0.29 (0.14–0.60)	0.19 (0.08–0.48)
Number of comorbidities: 3+						6.48 (2.43–17.27)	0.64 (0.23–1.78)	0.59 (0.20–1.75)	0.43 (0.14–1.37)
Solid malignancy	1.16 (0.62–2.18)	2.28 (1.47–3.53)		1.25 (0.46–3.41)	2.20 (1.17–4.13)	0.90 (0.44–1.82)		1.72 (0.77–3.81)	2.15 (0.86–5.39)
Metastasized solid malignancy	2.76 (1.39–5.46)		3.45 (2.03–5.85)	3.80 (1.18–12.24)		9.48 (2.88–31.21)	3.61 (1.54–8.51)	2.43 (0.96–6.13)	2.50 (0.98–6.39)
Myocardial infarction	0.65 (0.30–1.38)			0.33 (0.11–0.96)		0.11 (0.04–0.34)			
Diabetes mellitus	1.87 (1.12–3.12)	1.81 (1.11–2.96)		3.05 (1.45–6.39)					
Chronic renal disease	1.57 (0.86–2.87)			0.37 (0.13–1.06)					
Chronic pulmonary disease	1.17 (0.70–1.93)			1.62 (0.82–3.20)		0.30 (0.15–0.60)			
Cerebrovascular disease	0.54 (0.26–1.10)	0.37 (0.16–0.83)		0.07 (0.02–0.27)		0.00 (0.00–0.03)			
Immunodeficiency						0.20 (0.03–1.34)			
Neutropenia at bacteremia onset	0.73 (0.32–1.68)			0.15 (0.04–0.54)					
Treatment restriction in place at bacteremia onset	1.89 (1.12–3.21)			2.59 (1.32–5.07)		2.80 (1.42–5.52)			

Supplementary Table 3 (continued)

	Netherlands and Denmark combined			Netherlands only					
	Main analysis - full	**Main analysis - reduced**	**Sensitivity analysis**	**Main analysis - full**	**Main analysis - reduced**	**Sensitivity analysis - full**	**Sensitivity analysis - reduced[a]**	**Combined analysis[b]**	**With APS[c]**
Preceding hospital admission within 3 months prior to bacteremia			1.28 (0.86–1.92)			0.54 (0.30–0.97)			
Surgical procedure within 30 days prior to bacteremia	1.68 (0.93–3.06)			2.24 (0.94–5.35)		0.98 (0.39–2.44)			
Central venous catheter at bacteremia onset	1.12 (0.60–2.11)			0.42 (0.13–1.31)					
Mechanical ventilation at bacteremia onset	2.68 (1.15–6.25)	3.44 (1.46–8.12)	2.34 (0.96–5.69)	23.80 (5.28–107.28)	12.63 (3.76–42.38)	20.82 (2.01–215.99)		8.28 (2.15–31.83)	5.66 (1.55–20.63)
Known colonization with ARE			1.07 (0.61–1.87)			0.54 (0.23–1.28)			
Known colonization with VRE	0.85 (0.36–2.00)		0.93 (0.40–2.15)	2.19 (0.81–5.96)		1.83 (0.56–6.02)			
β-lactam use within 30 days prior to bacteremia						0.26 (0.07–0.99)			
Penicillin use within 30 days prior to bacteremia						1.79 (0.63–5.08)			
Carbapenem use within 30 days prior to bacteremia	0.78 (0.42–1.44)			0.47 (0.17–1.28)					
Vancomycin use within 30 days prior to bacteremia	1.12 (0.59–2.15)			2.29 (1.11–4.70)		3.79 (1.80–7.98)	1.79 (0.77–4.16)	1.27 (0.51–3.14)	1.74 (0.70–4.30)
APS[d]									0.98 (0.94–1.04)
APS'[d]									1.05 (0.99–1.11)

This table presents risk ratios (95% confidence interval) for 30-day mortality for the exposure of interest (*vancomycin resistance*) and confounders,
Abbreviations: APS, Acute Physiology Score; ARE, ampicillin-resistant *Enterococcus faecium*; VRE, vancomycin-resistant *Enterococcus faecium*.
[a] As the point estimate for vancomycin resistance in the *sensitivity analysis* fluctuated near 1, it became overfitted using the standard 10% inclusion criterion. Therefore, an extra variable reduction round was added, in which the β coefficient for *vancomycin resistance* had to remain within a 200% margin of the β coefficient in the full *sensitivity analysis*.
[b] As the point estimates for *vancomycin resistance* differed considerably between the *main analysis (reduced)* and the *sensitivity analysis (reduced)*, an extra model was created that combined confounding variables retained in both. This model was considered optimally corrected for confounding and was used as reference when including the extra confounder *APS*.
[c] See description in **Analysis with Acute Physiology Score** in this **Supplementary Material**.
[d] Continuous variable included in the model as a restricted cube spline with three knots.

Supplementary Table 4. Incidence density of mortality stratified by time to initiation of appropriate therapy

		Netherlands			Denmark		
		Patient days observed	**Deaths**	**Incidence density (per 1,000 patient days)**	**Patient days observed**	**Deaths**	**Incidence density (per 1,000 patient days)**
Appropriate therapy	from day 0[a] onwards	276	2	7	183	1	6
	from day +1[a] onwards	470	8	17	873	13	15
	from day +2[a] onwards	680	5	7	1,144	20	18
	from day +3[a] onwards	877	3	3	158	6	38
Inappropriate therapy on days 0 through +3[a]		436	14	32	233	10	43
Inappropriate therapy at the end of day +3[a]		1,303	15	12	228	3	13
Total		**4,042**	**47**	**12**	**2,818**	**53**	**19**

This table depicts incidence densities for each level of the time-varying variable reflecting *appropriateness of therapy*. Patients not receiving appropriate therapy on the day of the index blood culture (day 0), start off within *inappropriate therapy on days 0 through +3*. If patients within the latter group started to receive appropriate therapy on day +1, +2 or +3, they transition to the relevant appropriate therapy level on that day, and stay within that level for the remainder of follow-up. Patients not starting appropriate therapy during those days, transition to *inappropriate therapy at the end of day +3* on day +4, and remain there throughout follow-up. Follow-up lasts until day +30 or death if occurring before day +30.

[a] Day 0 is the day of the index blood culture of the ampicillin-/vancomycin-resistant *Enterococcus faecium* (ARE/VRE) bacteremia episode.

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CHAPTER 6

Predictive value of prior colonization and antibiotic use for third-generation cephalosporin-resistant Enterobacterales bacteremia in patients with sepsis

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Abstract

Background: To prevent inappropriate empiric antibiotic treatment in patients with bacteremia caused by third-generation cephalosporin (3GC)-resistant Enterobacterales (3GC-R EB), Dutch guidelines recommend β-lactam and aminoglycoside combination therapy or carbapenem monotherapy in patients with prior 3GC-R EB colonization and/or recent cephalosporin or fluoroquinolone usage. Positive predictive values (PPVs) of these determinants are unknown.

Methods: We retrospectively studied patients with a clinical infection in whom blood cultures were obtained and empiric therapy with broad-spectrum β-lactams and/or aminoglycosides and/or fluoroquinolones was started. We determined the PPVs of prior colonization and antibiotic use for 3GC-R EB bacteremia, and the consequences of guideline adherence on appropriateness of empiric treatment.

Results: Of 9,422 episodes, 773 (8.2%) were EB bacteremias and 64 (0.7%) were caused by 3GC-R EB. For bacteremia caused by 3GC-R EB, PPVs of prior colonization with 3GC-R EB (90-day window) and prior usage of cephalosporins or fluoroquinolones (30-day window) were 7.4% and 1.3%, respectively, and PPV was 1.8% for the presence of any of these predictors. Adherence to Dutch sepsis guideline recommendations was 27%. Of bacteremia episodes caused by 3GC-R and 3GC-susceptible EB, 56% and 94%, respectively, were initially treated with appropriate antibiotics. Full adherence to guideline recommendations would hardly augment proportions of appropriate therapy, but could considerably increase carbapenem use.

Conclusions: In patients receiving empiric treatment for sepsis, prior colonization with 3GC-R EB and prior antibiotic use have low PPV for infections caused by 3GC-R EB. Strict guideline adherence would unnecessarily stimulate broad-spectrum antibiotic use.

Background

Infections caused by Enterobacterales resistant to second- and third-generation cephalosporins (2GCs and 3GCs, respectively) – due to production of extended-spectrum β-lactamases (ESBLs), AmpCs, or other mechanisms – are emerging worldwide [1,2]. Because of their resistance to most β-lactam antibiotics, the risk of inappropriate empiric antibiotic therapy for septic patients has increased. This has stimulated the use of antibiotics that are not affected by these β-lactamases, such as carbapenems [3], thereby enhancing the risk of carbapenem resistance among Gram-negative bacteria.

Physicians are, therefore, challenged to empirically treat those patients with infections caused by 3GC-resistant (3GC-R) Enterobacterales with appropriate antibiotics, and at the same time minimize unnecessary use of last-resort antibiotics, such as carbapenems, in patients with infections caused by susceptible bacteria. Risk stratification based on a combination of suspected source of infection, local pathogen epidemiology, and patient characteristics, such as prior antibiotic use and prior microbiologic culture results, can be used to select empiric antibiotics [4], and in a sample of international guidelines, most advise to do so in general terms (**Supplementary Table 1**). However, Dutch guidelines, issued by the Dutch Working Party on Antibiotic Policy, specifically recommend the use of carbapenem or β-lactam aminoglycoside combination therapy (BLACT) in patients with sepsis of unknown origin with documented ESBL colonization, and also in those that have used cephalosporins or fluoroquinolones in the prior 30 days [5]. It is, however, unknown how well these criteria predict the presence of ESBL-producing Enterobacterales as a cause of infection, to what extent these recommendations are adhered to, and whether they improve empiric antibiotic therapy.

In this retrospective study we determined, in patients with clinical sepsis receiving empiric parenteral broad-spectrum β-lactam, fluoroquinolone, or aminoglycoside antibiotics, the predictive value of prior colonization with 3GC-R Enterobacterales and prior antibiotic use for infections caused by 3GC-R Enterobacterales. In addition, we estimated the consequences of full adherence to guideline recommendations for antibiotic use.

Methods

Definitions

Suspected Gram-negative sepsis (hereafter referred to as sepsis) was defined as an episode of clinical infection in an adult patient (≥18 years), in which blood cultures were obtained and in

which a β-lactam antibiotic and/or a fluoroquinolone and/or an aminoglycoside was started (intravenously or intramuscularly) on the same day or the day after blood culture obtainment. Excluded were episodes (i) in which any of these antibiotics had been initiated before the day of blood culture obtainment and were either continued or switched to any other of the selected antibiotics on the day of blood culture obtainment, (ii) in which penicillin or flucloxacillin monotherapy was started for empiric treatment, and (iii) in which antibiotics were started within 1 day after previous antibiotic use (with any of the selected antibiotics) ended. Episodes were considered either community-onset (if sepsis occurred before the fourth day of hospitalization) or hospital-onset.

3GC-R Enterobacterales were defined as isolates being resistant to ceftriaxone, cefotaxime, and/or ceftazidime. Antibiotic susceptibility was based on minimal inhibitory concentration determination in automated systems (Phoenix (BD, Franklin Lakes, New Jersey) or Vitek 2 (bioMérieux SA, Marcy l'Etoile, France)) using 2012 Clinical and Laboratory Standards Institute criteria [6], with minor modifications to adjust for changes in breakpoints for β-lactam antibiotics that occurred during the study period (**Supplementary Material**) [7].

For each case of sepsis, we determined the occurrence of bacteremia, defined as growth of bacteria or fungi from any of the blood cultures obtained on the day of onset. The onset period involved 2 days if antibiotics were started on the day after the first blood culture. For potential skin contaminants (ie, *Corynebacterium* spp., *Bacillus* spp., *Propionibacterium* spp., coagulase-negative staphylococci, viridans group streptococci, *Aerococcus* spp., Micrococcus spp. [8]), 2 separate sets of blood cultures with bacteria belonging to the same genus were required. In addition, we determined for each episode of sepsis the presence of 3GC-R Enterobacterales in any diagnostic culture other than blood that was obtained within 3 days before or after the day(s) of sepsis onset. The presence of 3GC-R Enterobacterales in blood and/or any diagnostic culture was defined as *any 3GC-R Enterobacterales infection*. Cultures from feces, rectal/perineal swabs, skin swabs, and cultures or swabs from the upper respiratory tract (eg, throat swabs, sinusoidal secretions, but not sputum) were not considered as indicative for infection with 3GC-R Enterobacterales.

Prior colonization was defined as isolation of 3GC-R Enterobacterales from any site within a designated period (90 days and 1 year), until 3 days before the day of sepsis. Prior antibiotic use was defined as use of at least 1 dose of a 2GC, 3GC, or any fluoroquinolone in a designated period (30 days and 90 days) until the day before sepsis.

Appropriate treatment for Enterobacterales bacteremia was defined as treatment that included at least 1 antibiotic for which the causative pathogen was susceptible in vitro. Overtreatment was defined as treatment with a carbapenem or addition of an aminoglycoside or fluoroquinolone to an appropriately covering β-lactam antibiotic in case of infection with a 3GC-susceptible (3GC-S) Enterobacterales.

Data collection and analysis

The study was performed in a 1,042-bed tertiary hospital (UMCU) and in a 605-bed regional teaching hospital (TGH). The Medical Ethics Review Committee of UMCU determined that this study was exempted from evaluation with regard to the Dutch Medical Research Involving Subjects Act. In both hospitals, all blood cultures obtained between 1 January 2008 and 31 December 2010 were taken as the starting point for identifying sepsis episodes. These were subsequently linked to other relevant microbiological and pharmaceutical datasets (the latter retrieved from Utrecht Patient Oriented Database for UMCU; see **Supplementary Material**). Calculations of prevalence, sensitivity, specificity, positive predictive value (PPV), negative predictive value, and positive and negative likelihood ratios were performed using Excel 2010 software (Microsoft Corporation, Redmond, Washington). **Figure 4** was created in R version 3.0.2 using the *ggplot2* package (The R Foundation for Statistical Computing, Vienna, Austria).

In one hospital (TGH), antibiotic prescriptions from outpatient clinics were not available, and in the other hospital (UMCU) 16.6% of the antibiotics prescribed in outpatient clinics lacked stopping dates. Therefore, sensitivity analyses were performed in which outpatient antibiotics were included and excluded in the definition of prior antibiotic use, and in which antibiotics with missing stopping dates were assumed to have been prescribed for 1 day.

In one hospital (UMCU), a random sample of 5% of all sepsis episodes occurring before or on the first day of hospital admission, was subjected to manual chart review to determine the diagnosis and differential diagnosis of the sepsis episode and recorded prior antibiotic use, to estimate the prevalence of community-acquired pneumonia and accuracy of electronic data capture of prior antibiotic use.

Results

Sepsis episodes and outcomes

There were 9,422 sepsis episodes (4,959 in UMCU and 4,463 in TGH) in 7,365 unique patients (**Table 1**). Most patients (*n* = 6,004, 81.5%) experienced a single episode, and 159 (2.2%) had

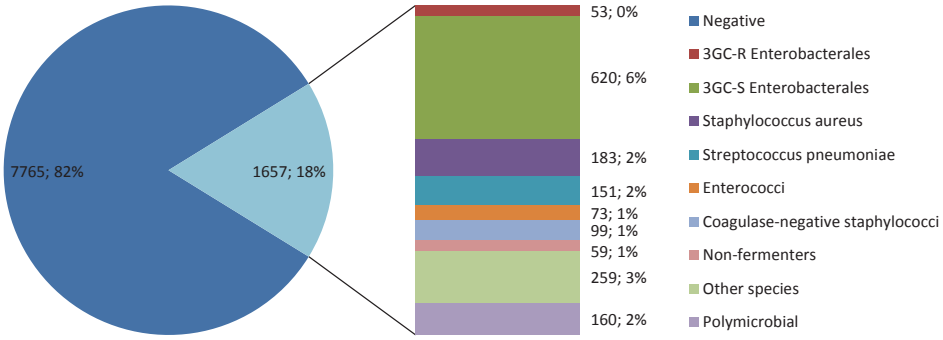

Figure 1. Species isolated from blood cultures in suspected Gram-negative sepsis.

≥4 episodes. Antibiotics were started on the day of sepsis in 7,236 episodes (77%) and on the day after in the remaining 2,186 episodes (23%).

In 1,657 of these 9,422 episodes, 1 or more blood cultures became positive (17.6%), of which 773 were caused by Enterobacterales (8.2%; in 100 episodes in combination with non-Enterobacterales isolates) and 64 by 3GC-R Enterobacterales (0.7%; of which 11 were polymicrobial) (**Figure 1**). *Any 3GC-R Enterobacterales* infection was present in 3.5% (*n* = 331; 64 with bacteremia) of the episodes (**Figure 2**).

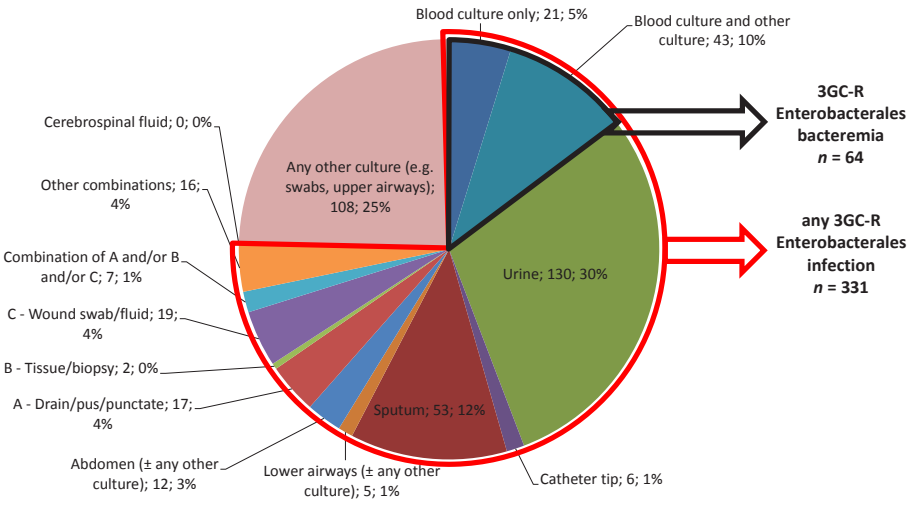

Figure 2. Origin of 3GC-R Enterobacterales cultures at onset of suspected Gram-negative sepsis.

Table 1. Prior colonization and antibiotic use as predictors for 3GC-R Enterobacterales infection in suspected Gram-negative sepsis

Predictor	Sensitivity for:			Prevalence among:	Positive predictive value for:		Positive likelihood ratio for:	
	3GC-R EB bacteremia (*N* = 64, 0.7%), n (%)	Any 3GC-R EB infection[a] (*N* = 331, 3.5%), n (%)	3GC-S EB bacteremia (*N* = 709, 7.5%), n (%)	Suspected Gram-negative sepsis (*N* = 9,422, 100%), n (%)	3GC-R EB bacteremia, %	Any 3GC-R EB infection[a], %	3GC-R EB bacteremia	Any 3GC-R EB infection[a]
Prior colonization[b] with 3GC-R EB: 90 days	27 (42)	125 (38)	30 (4)	363 (4)	7.4	34.4	11.7	14.4
Prior colonization[b] with 3GC-R EB: 1 year	31 (48)	144 (44)	41 (6)	510 (5)	6.1	28.2	9.5	10.8
Prior 2GC or 3GC use: 30 days	15 (23)	85 (26)	61 (9)	997 (11)	1.5	8.5	2.2	2.6
Prior FQ use: 30 days	10 (16)	47 (14)	41 (6)	865 (9)	1.2	5.4	1.7	1.6
Prior 2GC, 3GC or FQ use: 30 days	20 (31)	111 (34)	88 (12)	1,598 (17)	1.3	6.9	1.9	2.1
Prior 2GC, 3GC or FQ use: 90 days	33 (52)	162 (49)	158 (22)	2,211 (23)	1.5	7.3	2.2	2.2
Prior colonization[b] with 3GC-R EB (90 days), or prior 2GC, 3GC or FQ use (30 days)	32 (50)	172 (52)	107 (15)	1,766 (19)	1.8	9.7	2.7	3.0
Prior colonization[b] with 3GC-R EB (1 year), or prior 2GC, 3GC or FQ use (90 days)	42 (66)	210 (63)	176 (25)	2,400 (25)	1.8	8.8	2.6	2.6

Abbreviations: EB, Enterobacterales; FQ, fluoroquinolone.

[a] Implying that the sepsis episode could either be classified as a 3GC-R Enterobacterales bacteremia or that a 3GC-R member of the Enterobacterales was cultured from any other culture (except swabs from the digestive tract or skin, and cultures from feces or the upper respiratory tract) obtained within a 3-day margin around sepsis onset.

[b] Implying that a 3GC-R member of the Enterobacterales was isolated from a culture obtained between *sepsis onset minus 90 days/1 year* and *sepsis onset minus 3 days*.

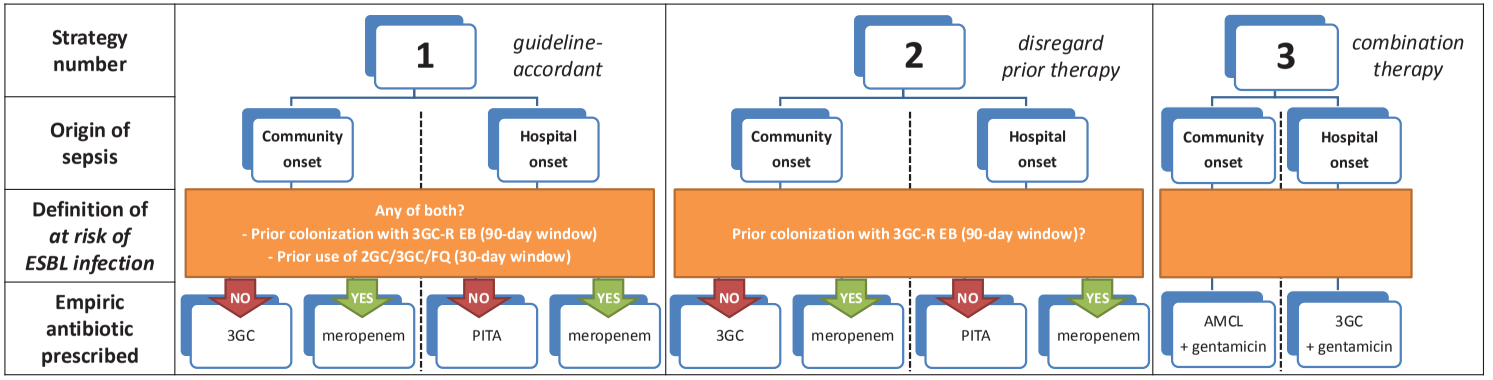

Figure 3. Flow diagrams depicting three hypothetical treatment scenarios for adjusting empiric antibiotic regimens for suspected Gram-negative sepsis on the basis of the origin of the sepsis (community-onset vs hospital-onset) and the definition of risk factors for ESBL infection.
Abbreviations: AMCL, amoxicillin/clavulanic acid; EB, Enterobacterales; FQ, fluoroquinolone; PITA, piperacillin/tazobactam.

Presence of risk factors

Colonization with 3GC-R Enterobacterales within 90 days prior to sepsis and prior use of 2GCs/3GCs or fluoroquinolones within 30 days before sepsis, or any of both, achieved sensitivities for 3GC-R Enterobacterales bacteremia of 31%-50% (**Table 1**; full overview of predictive properties in **Supplementary Table 2**). The PPV of these risks factors ranged from 1.3% for prior antibiotic use alone to 7.4% for prior colonization. The PPV was 1.8% for the presence of any of both risk factors. Maximum sensitivity (66%) was achieved by combining the risk factors and extending the interval for prior colonization to 1 year and for prior antibiotic use to 90 days, while the PPV remained unchanged. Prior 3GC-R Enterobacterales bacteremia had the highest PPV for 3GC-R Enterobacterales bacteremia (28.1%), but a sensitivity of 14%. Sensitivity analyses including cultures requested by general practitioners and outpatient antibiotic prescription did not change interpretation (**Supplementary Table 3**). Furthermore, results obtained for *any 3GC-R Enterobacterales infection* were very similar to those for 3GC-R Enterobacterales bacteremia with regard to sensitivity (**Table 1**). Finally, analyses restricted to sepsis episodes with positive blood cultures only resulted in positive likelihood ratios comparable to those obtained for all sepsis episodes (**Supplementary Table 4**).

Antibiotic therapy prescribed and potential treatment strategies

Carbapenem or BLACT were prescribed in 1,144 episodes of sepsis (12%). More than half of these episodes involved carbapenems (n = 661, 7%), mostly in the UMCU (629 episodes). Of all patients considered at risk of ESBL infection (prior colonization within 90 days or use of 2GCs/3GCs or fluoroquinolones within 30 days; n = 1,766), 474 (27%) received guideline-adherent therapy (ie, a carbapenem or BLACT).

Initial antibiotic therapy was considered appropriate in 653 of 698 episodes of bacteremia caused by 3GC-S Enterobacterales (94%; 11 were excluded due to absence of an antibiogram) and in 36 of 64 episodes (56%) caused by 3GC-R Enterobacterales (p <0.001, Pearson's χ^2 test). In contrast, BLACT or carbapenems were prescribed empirically in 133 of 698 (19%) bacteremia episodes caused by 3GC-S Enterobacterales.

We defined three hypothetical treatment scenarios that differed with regard to the definition of being at risk of ESBL infection and evaluated their effect on appropriateness and overtreatment for all Enterobacterales bacteremias in our cohort (**Figure 3**). Full adherence to any of these recommendations would have resulted in a >50% reduction of inappropriate

Table 2. Appropriate treatment and overtreatment of Enterobacterales bacteremia for observed situation and three hypothetical treatment scenarios

	Strategy	Rate of appropriate treatment[a] for:		Rate of inappropriate treatment[a] for:	Rate of overtreatment[b] for:	Rate of carbapenem use for:
		3GC-R EB bacteremia (*N* = 64), %	3GC-S EB bacteremia (*N* = 698), %	All EB bacteremias (*N* = 762), %		
0	Observed	56%	94%	9.6%	18%	8.3%
1	Hypothetical: Guideline-accordant	59%	100%	3.5%	14%	18%
2	Hypothetical: Disregard prior therapy	56%	99%	4.2%	4%	7.4%
3	Hypothetical: Combination therapy	69%	99%	3.5%	77%	0%

Abbreviations: EB, Enterobacterales.

[a] Appropriate treatment was defined as treatment that included at least one antibiotic for which the causative pathogen had *in vitro* susceptibility.

[b] Overtreatment was defined as treatment with a carbapenem or addition of an aminoglycoside or fluoroquinolone to a β-lactam antibiotic in case of infection with 3GC-S Enterobacterales.

treatment for Enterobacterales bacteremia as compared to the observed situation: from 9.6% to 3.5%, 4.2%, and 3.5% (scenario 1, 2, and 3, respectively; **Table 2** and **Figure 4**). This benefit almost exclusively results from improvement of coverage for 3GC-S Enterobacterales bacteremia. Strategies 1 and 2 would result in a similar amount of appropriateness (56%-59%) for bacteremia caused by 3GC-R Enterobacterales as in the observed setting (56%), but in scenario 1, which represents full adherence to the Dutch guideline, this would be at the cost of increasing carbapenem use by 117%. Only universal BLACT (scenario 3) would improve appropriateness (to 69%), but at the cost of increasing overtreatment by approximately 325%.

Sample results

Medical records review of 123 sepsis episodes upon hospital admission in UMCU (5%) revealed misclassification of origin of infection in 4 patients (3.3%; not community-onset) and of use of 2GCs/3GCs or fluoroquinolones in the 30 days prior to sepsis in 5 patients (increasing the prevalence from 12% based on electronic identification to 15%). The respiratory tract was considered the most likely source of infection in 36 episodes (29%).

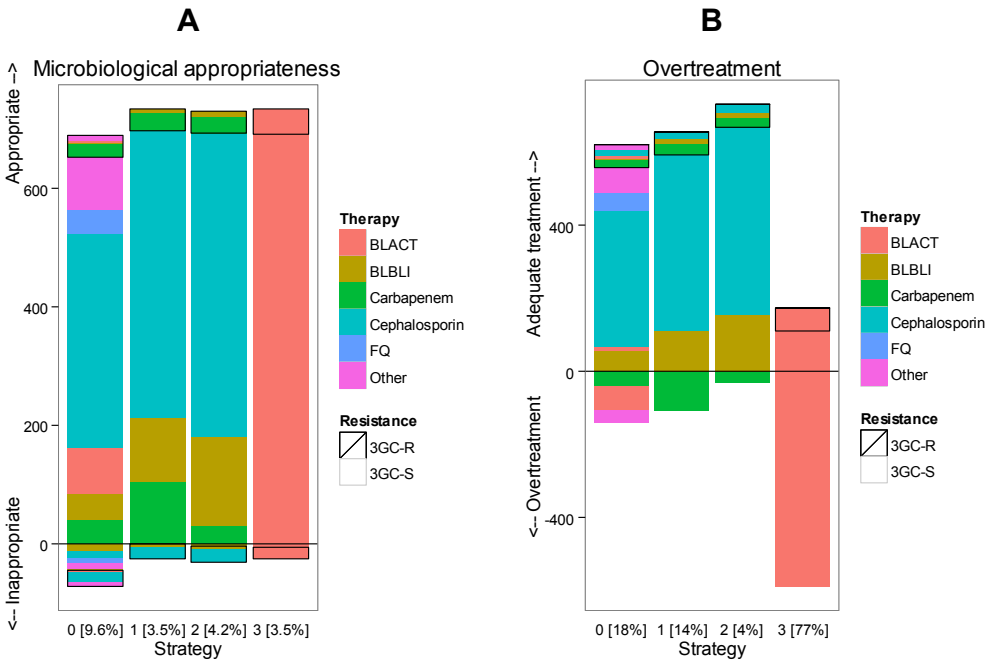

Figure 4. Microbiological appropriateness of treatment (A) and overtreatment (B) of 762 episodes of Enterobacterales bacteremia for the observed real-life setting (strategy 0) and the three hypothetical treatment strategies presented in **Figure 3**. Values between square brackets reflect inappropriateness and overtreatment proportions in the respective figures. Appropriate treatment was defined as treatment that included at least one antibiotic for which the causative pathogen had in vitro susceptibility. Overtreatment was defined as treatment with a carbapenem or addition of an aminoglycoside or fluoroquinolone to a β-lactam antibiotic in case of infection with 3GC-S Enterobacterales. Relevant rates are also presented in **Table 2**.
Abbreviations: BLBLI, β-lactam/β-lactamase inhibitor combination; FQ, fluoroquinolone.

Discussion

This study reveals that in the Netherlands, among patients with a clinical infection in which blood cultures were obtained and empiric antibiotics were started, the likelihood of any infection caused by 3GC-R Enterobacterales was 3.5%, and the likelihood of bacteremia caused by these pathogens was 0.7%. The PPVs of broadly recognized risk factors for 3GC-R Enterobacterales bacteremia, such as prior colonization with 3GC-R Enterobacterales or recent usage of cephalosporins or fluoroquinolones, were 7.4% and 1.3%, respectively. With an observed 27% adherence to Dutch guideline recommendations, 94% and 56% of bacteremias caused by 3GC-S and 3GC-R Enterobacterales, respectively, received appropriate empiric therapy. Yet, 100% adherence to such recommendations would hardly increase appropriateness of empiric therapy for 3GC-R Enterobacterales bacteremia, but has the

potential to substantially increase carbapenem use. If these guidelines are adopted, we propose to omit prior antibiotic use as a risk factor. Better coverage of 3GC-R Enterobacterales bacteremia can only be achieved with combination treatment for all septic patients, but at the expense of massive unnecessary prescription of aminoglycosides. These findings underscore the need for better prediction rules to optimize empiric antibiotic treatment in patients with sepsis.

Prior use of cephalosporins or fluoroquinolones has been identified as a risk factor for infections caused by ESBL-producing bacteria in many studies [9]. Yet, apart from 2 case-control studies focusing on patients in whom blood cultures were obtained [10,11], these associations generally have been established in patient cohorts with microbiologically proven Enterobacterales infections only. These studies, therefore, do not offer guidance for physicians at the moment that empiric antibiotics must be initiated. To the best of our knowledge, there is only 1 other study in which a prediction rule for presence of ESBL-producing Enterobacterales was derived [12], but it included all patients upon hospital admission, which may not necessarily coincide with patients for whom empiric therapy for suspected Gram-negative sepsis is prescribed. In another study, focusing like we did on septic patients, an automated decision support system, called TREAT, was used to comprehensively predict pathogens and resistance patterns, including ESBL-producing pathogens [13]. It provided individual advice on antibiotic treatment based on a causal probabilistic model calibrated on data from literature, large databases, and local epidemiology, and taking clinical and laboratory data as input. Unfortunately, performance data on predicting specific resistant variants of Gram-negative organisms are not available.

Empiric regimens for an infectious syndrome are generally based on the expected susceptibility of pathogens most likely to be involved [14]. For some infections, thresholds have been recommended for adapting empiric regimens, such as a 10% threshold for penicillin-intermediate strains for *Streptococcus pneumoniae* in meningitis [15], and a 20% threshold for trimethoprim-sulfamethoxazole resistance among *Escherichia coli* in uncomplicated cystitis [16]. Yet, these cutoff percentages are limited to single pathogens, whereas, as acknowledged by the recently proposed weighted-incidence syndromic combination antibiogram (WISCA), it is essential to determine the proportion of pathogens that will be covered by a certain empiric regimen [17]. Still, WISCAs are not geared toward the clinical scenario to which prescription guidelines apply. Inclusion of all episodes (including those with negative culture results) is essential to establish the effect of guidelines on

antibiotic prescribing in clinical practice. Although culture-negative infections may also be due to resistant microorganisms, restricting analyses to culture-positive infections only introduces poor generalizability of such episodes to culture-negative infections [18].

Balancing appropriateness of therapy and antibiotic overuse is a challenge [19]. Reports on the consequences of inappropriate empiric therapy differ. In a meta-analysis, inappropriate treatment appeared to be detrimental to the outcome of patients with sepsis [20], which was not confirmed in a study on ESBL-producing Enterobacterales bacteremia in Dutch hospitals [21]. On the other hand, antibiotic use may have adverse effects on an individual level (ie, resistance development and adverse effects), as well as the population level by increasing resistance. In particular, unnecessary use of carbapenems should be avoided as it selects for carbapenemase-producing isolates [22,23]. As demonstrated in this study, strict adherence to current guideline recommendations may stimulate overuse of antibiotics, and proposed treatment algorithms in guidelines should be improved. In this respect, it seems logical to include the severity of illness in the risk stratification, as is in fact the case in many guidelines (**Supplementary Table 1**). Another strategy might be to increase screening for resistant microorganisms to guide empiric therapy, which will increase sensitivity for detecting carriage in those proceeding to infection during hospitalization. Yet, given the low rate of such infections, such a strategy might not be cost-effective [24,25].

Several limitations of the current study must be addressed. First, we analyzed 3GC-R Enterobacterales instead of ESBL-producing Enterobacterales, although Dutch guidelines specifically refer to ESBL. Although risk factors might deviate slightly, 3GC resistance and not ESBL positivity is the only relevant clinical outcome. In a Dutch national survey, 80% of 3GC-R Enterobacterales harbored ESBL genes [26].

Second, using 3GC-R Enterobacterales bacteremia as outcome of interest might be too narrow a definition of severe 3GC-R Enterobacterales infection. Therefore, we also performed a sensitivity analysis involving a very broad definition of 3GC-R Enterobacterales infection, in which guideline performance was equal with regard to sensitivity.

Third, from our random sample of community-onset infections, it appears that 15% of our cohort may consist of community-acquired pneumonia, as blood cultures are usually obtained and treatment often consists of broad-spectrum β-lactams or fluoroquinolones. As Enterobacterales play a minor role in the etiology of community-acquired pneumonia [27], these episodes might be considered less relevant for our study domain.

Fourth, we considered all included episodes to be sepsis of unknown origin, whereas in practice, these episodes might be classified as specific syndromes or occur in specific wards, which warrants different empiric treatment regimens, such as in the case of neutropenic sepsis.

Fifth, antibiotic records were not complete for outpatient antibiotic use or antibiotic use in other hospitals, which could have led to misclassification of prior antibiotic use. The same may have occurred for microbiological culture results. However, better information would only have increased the prevalence of the risk factors, and, based on our sensitivity analyses (**Supplementary Table 3**), this would not have led to substantial improvement of sensitivity for 3GC-R Enterobacterales bacteremias. Moreover, much of this information would not be promptly available to treating physicians in daily practice either.

Last, this study has been performed in the Netherlands, a country with low resistance rates for most nosocomial pathogens [1]. However, the epidemiology of infections caused by 3GC-R Enterobacterales in the Netherlands is not that different from other countries. For instance, in 2012, resistance rates to 3GCs of invasive *E. coli* and *Klebsiella pneumoniae* isolates were comparable to those from Germany and the United Kingdom [1]. In addition, prevalence of carriage with ESBL-producing Enterobacterales in nonhospitalized subjects in the Netherlands was 5.1% [28], which is also similar to reported prevalences from other Western European countries, such as Germany (6.3%) [29], and France (6%) [30]. Yet even in countries with higher proportions of resistance among Gram-negative organisms in patients with documented infections, the actual proportion of infections caused by 3GC-R Enterobacterales would still represent a minor part of all sepsis episodes.

In conclusion, current guideline recommendations do not accurately predict the presence of 3GC-R Enterobacterales as a cause of infection. Therefore, they do not promote the prudent use of antibiotics. Better prediction rules are needed, and these should be developed for the relevant scenario, being a clinical suspicion of infection in which Enterobacterales are considered as a potential cause.

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CHAPTER 6
SUPPLEMENTARY MATERIAL

Predictive value of prior colonization and antibiotic use for third-generation cephalosporin-resistant Enterobacterales bacteremia in patients with sepsis

Modifications of CLSI 2012 breakpoints for MICs

All Enterobacterales obtained in hospital UMCU between 2007 and 2011 with a minimum inhibitory concentration (MIC) ≤2 mg/L for cefotaxime (692 of 43,115 isolates, 1.6%) were considered susceptible to this antibiotic. If susceptibility to cefazoline was relevant for determining appropriateness of therapy, isolates with ≤4 mg/L (*n* = 8) were considered susceptible.

Similarly, all Enterobacterales obtained in hospital TGH between 2007 and 2011 with a ceftriaxone MIC ≤8 or ≤4 mg/L (177 of 27,234 isolates, 0.6%) or a ceftazidime MIC ≤8 mg/L (176 of 38,240 isolates, 0.4%) were considered susceptible to the concerned antibiotic. No modifications to the CLSI 2012 interpretative criteria were made in relation to determining appropriateness of therapy for this hospital.

Data from UPOD used for UMCU

For this study, data from the Utrecht Patient Oriented Database (UPOD) were used. UPOD is an infrastructure of relational databases comprising data on patient characteristics, hospital discharge diagnoses, medical procedures, medication orders and laboratory tests for all patients treated at UMCU since 2004. UPOD data acquisition and management is in accordance with current regulations concerning privacy and ethics. The structure and content of UPOD have been described in more detail elsewhere (ten Berg MJ, Huisman A, van den Bemt PMLA, Schobben AFAM, Egberts ACG, van Solinge WW. Linking laboratory and medication data: new opportunities for pharmacoepidemiological research. Clin Chem Lab Med 2007;45(1):13–9.).

Supplementary Table 1. Overview of a convenience sample of national sepsis or antimicrobial guidelines with regard to antibacterial therapy in case of (severe) non-neutropenic sepsis of unknown origin

Guideline	Condition	Main risk stratification and antibiotic recommendations	Further risk stratification	Access information and date of access
Surviving Sepsis Campaign International guidelines for management of severe sepsis and septic shock: 2012 2012	Severe sepsis and septic shock	*One or more drugs that have activity against all likely pathogens and that penetrate in adequate concentrations into the tissues presumed to be the source of sepsis*	*Choice of therapy depends on the patient's history, including drug intolerances, recent receipt of antibiotics (previous 3 months)*, underlying disease, the clinical syndrome, and susceptibility patterns of pathogens in the community and hospital**, and that previously have been documented to colonize or infect the patient* ** Recently used anti-infective agents should generally be avoided* *** Within regions in which the prevalence of MRSA and Gram-negatives resistant to broad-spectrum β-lactams and carbapenems is significant, empiric therapy adequate to cover these pathogens is warranted*	http://www.survivingsepsis.org/Guidelines/Pages/default.aspx 2 June 2014
Sanford Guide The Sanford guide to Antimicrobial Therapy 2013 (43rd edition) 2013	Sepsis of unknown origin	*No septic shock:* Ertapenem/imipenem/meropenem + vancomycin/linezolid Daptomycin + cefepime/PITZ/TCCL *Septic shock:* Reference made to Surviving Sepsis Campaign	*Septic shock and post-splenectomy or functional asplenia:* Ceftriaxone Levofloxacin/moxifloxacin	Antimicrobial Therapy, Inc. Sperryville, VA, USA http://www.sanfordguide.com/publications/the-sanford-guide-to-antimicrobial-therapy
Australia		Therapeutic Guidelines: Antibiotic (Version 15; 2014; Therapeutic Guidelines Limited) not available online		http://www.tg.org.au/?sectionid=41 23 January 2015
Belgium		No guidelines available from Belgian Antibiotic Policy Coordination Committee		www.bapcoc-hospitalcare.be 25 January 2015

Supplementary Table 1 (continued)

Guideline	Condition	Main risk stratification and antibiotic recommendations	Further risk stratification	Access information and date of access
Canada		Bugs & Drugs 2012 (5th edition; Alberta Health Service) not available online, and Canadian Association Of Emergency Physicians Sepsis Guidelines does not offer specific guidance on antibiotics		http://antibioticawareness.ca/?page_id=58 http://caep.ca/resources/position-statements-and-guidelines/sepsis-guideline 25 January 2015
Denmark Danish Society of Infectious Diseases Rekommen-dationer for initial behandling af svær sepsis og septisk shock 2014	Severe sepsis and septic shock of unknown origin	PITZ (+ gentamicin)	*Penicillin allergy:* Meropenem *Travel abroad during prior 3 months:* Keep in mind resistant bacteria	http://www.infmed.dk/guidelines 23 January 2015
France French Society of Anesthesia and Reanimation Antibiothérapie probabiliste des états septiques graves 2004	Severe sepsis and septic shock of unknown origin	*Community-onset:* Cefotaxim/ceftriaxone + gentamicine/netilmicin + metronidazole *Nosocomial, institutionalized patients, and patients hospitalized in prior 30 days:* Ceftazidim/cefepime + amikacine + vancomycin (+ metronidazole) Imipenem + amikacine + vancomycine		http://www.infectiologie.com/site/consensus_recos.php 23 January 2015

Supplementary Table 1 (continued)

Guideline	Condition	Main risk stratification and antibiotic recommendations	Further risk stratification	Access information and date of access
Germany				
Paul-Ehrlich-Society of Chemo-therapy Empfehlung-en zur kalkulierten parenteralen Initialtherapie bakterieller Erkrankungen bei Erwachsenen – Update 2010 2010	Sepsis of unknown origin	*Community-onset:* Cefuroxime/cefotiam Cefotaxime/ceftriaxone AMCL/AMSU + ciprofloxacin/levofloxacin PITZ *Nosocomial:* PITZ Cefepime Imipenem/meropenem/doripenem	*Severe sepsis and/or widening of spectrum desired:* Add ciprofloxacin/levofloxacin *Severe sepsis/septic shock, in combination with risk factors (mechanical ventilation, prior antibiotics, major surgery, long ICU stay) and high MRSA prevalence:* Add daptomycin, vancomycin or teicoplanin *Initial choice of therapy determined by suspected infection source, underlying diseases, and risk factors, such as origin of infection (community-onset vs. nosocomial), time point of infection occurrence, and prior antibiotic therapy*	http://www.p-e-g.org/econtext/leitlinien 2 June 2014
Japan				
Sepsis Registry Committee of The Japanese Society of Intensive Care Medicine The Japanese guidelines for the management of sepsis 2013	Severe sepsis and septic shock of unknown origin	*Community-onset:* Ceftriaxone/cefotaxime + gentamicin *Nosocomial or nursing/healthcare-associated:* Cefepime + vancomycin (+ amikacin) PITZ + vancomycin (+ amikacin)	*Community-onset and meningitis is likely; patient <50 years and no alcoholism:* Add vancomycin *Community-onset and meningitis is likely; patient ≥50 years or alcoholism:* Add vancomycin and ampicillin *Nosocomial and (i) history of being treated with both cefepime and PITZ/piperacillin in prior 3 months, (ii) high prevalence of P. aeruginosa resistant to both cefepime and PITZ/piperacillin, (iii) high prevalence of ESBL-producers and A. baumannii, or (iv) known colonization of Gram-negatives susceptible only to carbapenems:* Meropenem/doripenem/imipenem + vancomycin (+ amikacin)	English translation published by Oda *et al.* in Journal of Intensive Care 2014;2(1):55

Supplementary Table 1 (continued)

Guideline	Condition	Main risk stratification and antibiotic recommendations	Further risk stratification	Access information and date of access
Netherlands				
Dutch Working Party on Antibiotic Policy Antibacterial therapy of adult patients with sepsis 2010	Sepsis of unknown origin	*Community-onset:* 2GC/3GC AMCL + aminoglycoside *Nosocomial:* PITZ 2GC/3GC (except ceftazidime) + aminoglycoside 2GC/3GC (except ceftazidime) + antipseudomonal fluoroquinolone	*High prevalence of ESBL-producing Enterobacterales and suspicion of infection by ESBL-producing bacteria:* Imipenem/meropenem *Prior use of cephalosporins or quinolones within prior 30 days or colonized with ESBL-producing bacteria:* Add aminoglycoside, or treat with imipenem/meropenem *Ultimate choice of therapy should depend on local epidemiology and resistance data*	http://www.swab.nl/richtlijnen 2 June 2014
Norway Norwegian Directorate of Health Nasjonal faglig retningslinje for bruk av antibiotika i sykehus 2012	Sepsis of unknown origin	Benzylpenicillin + gentamicin	*Suspicion of Pseudomonas involvement:* Tobramycin instead of gentamicin *Severe renal failure:* Benzylpenicillin + ciprofloxacin, PITZ, or cefotaxime *Alternative regime, e.g. in case of high prevalence of gentamicin resistance:* PITZ (add aminoglycoside in case of severe sepsis or septic shock), or cefotaxime *High prevalence of ESBL-producing Enterobacterales:* Carbapenem *Type I penicillin allergy:* Clindamycin + gentamicin *Non-type I penicillin allergy:* Cefotaxime (aminoglycoside or ciprofloxacin may be added in case of septic shock or suspected Gram-negative etiology)	http://helsedirektoratet.no/sites/antibiotikabruk-i-sykehus/terapikapitler/sepsis/Sider/default.aspx 23 January 2015

Supplementary Table 1 (continued)

Guideline	Condition	Main risk stratification and antibiotic recommendations	Further risk stratification	Access information and date of access
			COMMUNITY-ONSET	
			Severe sepsis/septic shock and high local ESBL prevalence in community:	
		COMMUNITY-ONSET	Ertapenem	
		Sepsis:		
		AMCL		
Spain		*Severe sepsis/septic shock:*	*HEALTHCARE-ASSOCIATED:*	
		Ceftriaxone	*Severe sepsis/septic shock and permanent urinary catheter:*	
Spanish Society of Infectious Diseases and Clinical Microbiology		*HEALTHCARE-ASSOCIATED*	Imipenem/meropenem + vancomycin	
		Sepsis:	PITZ + vancomycin	
		AMCL		http://www.seimc.org/documentoscientificos.php?mn_MP=3&mn_MS=111
	Suspicion of bacteremia of unknown origin	Ceftriaxone	*NOSOCOMIAL*	
		Severe sepsis/septic shock:	*Sepsis and in ICU*	
Guía para el diagnóstico y tratamiento del paciente con bacteriemia		Ertapenem	Imipenem/meropenem/PITZ	
			Sepsis and presence of ESBL within hospital	23 January 2015
		NOSOCOMIAL	Imipenem/meropenem/PITZ	
		Sepsis:	*Sepsis and MRSA suspicion:*	
		Ceftriaxone	Add vancomycin	
		Cefepime	*Severe sepsis/septic shock and high prevalence of multiresistant Gram-negative (especially P. aeruginosa):*	
2006		*Severe sepsis/septic shock:*	Aminoglycoside may be added	
		Imipenem/meropenem + vancomycin		
		PITZ + vancomycin		
			SEVERAL OTHER PATIENT CATEGORIES defined, but as these did not specifically involve resistant Enterobacterales, they are not included in this overview	

Supplementary Table 1 (continued)

Guideline	Condition	Main risk stratification and antibiotic recommendations	Further risk stratification	Access information and date of access
Sweden Swedish Society of Infectious Diseases Vårdprogram svår sepsis och septisk chock 2013	Severe sepsis and septic shock of unknown origin	*Community-onset:* Cefepime Imipenem/meropenem PITZ *Nosocomial or with complicating underlying factors:* Cefuroxime Cefotaxime/ceftazidime Cefepime Imipenem/meropenem Quinolone PITZ	*Septic shock or at risk of septic shock:* Add aminoglycoside *MRSA colonization:* Add vancomycin, linezolid or other effective antibiotic according to resistance *Recently treated with antibiotic:* Avoid any antibiotics from same group *Take into account age, underlying disease, immune suppression, time in hospital or similar, stay abroad, other epidemiological information for choice of antibiotics*	http://www.infektion.net/v%C3%A5rdprogram-f%C3%B6r-sv%C3%A5r-sepsisseptisk-chock 23 January 2015
United Kingdom		Sepsis guidelines in preparation by National Institute for Health and Care Excellence		https://www.nice.org.uk/guidance/indevelopment/gid-cgwave0686 23 January 2015

Abbreviations: 2GC, second-generation cephalosporin; 3GC, third-generation cephalosporin; AMCL, amoxicillin/clavulanic acid; AMSU, ampicillin/sulbactam; ESBL, extended-spectrum β-lactamase; ICU, intensive care unit; MRSA, methicillin-resistant *Staphylococcus aureus*; PITZ, piperacillin/tazobactam; TCCL, ticarcillin/clavulanic acid

Supplementary Table 2. Full performance overview of predictors for third-generation cephalosporin-resistant Enterobacterales infection in suspected Gram-negative sepsis

Predictor	Outcome												Prevalence of predictor, %
	3GC-R EB bacteremia						Any 3GC-R EB infection						
	Sens, %	Spec, %	PPV, %	NPV, %	LR+	LR-	Sens, %	Spec, %	PPV, %	NPV, %	LR+	LR-	
Prior colonization with 3GC-R EB: 90 days	42	96	7.4	99.6	11.7	0.6	38	97	34.4	98	14.4	0.6	3.9
Prior colonization with 3GC-R EB: 1 year	48	95	6.1	99.6	9.5	0.5	44	96	28.2	98	10.8	0.6	5.4
Prior 2GC or 3GC use: 30 days	23	90	1.5	99.4	2.2	0.9	26	90	8.5	97	2.6	0.8	10.6
Prior FQ use: 30 days	16	91	1.2	99.4	1.7	0.9	14	91	5.4	97	1.6	0.9	9.2
Prior 2GC, 3GC or FQ use: 30 days	31	83	1.3	99.4	1.9	0.8	34	84	6.9	97	2.1	0.8	17.0
Prior 2GC, 3GC or FQ use: 90 days	52	77	1.5	99.6	2.2	0.6	49	77	7.3	98	2.2	0.7	23.5
Prior colonization with 3GC-R EB (90 days), or prior 2GC, 3GC or FQ use (30 days)	50	81	1.8	99.6	2.7	0.6	52	82	9.7	98	3.0	0.6	18.7
Prior colonization with 3GC-R EB (1 year), or prior 2GC, 3GC or FQ use (90 days)	66	75	1.8	99.7	2.6	0.5	63	76	8.8	98	2.6	0.5	25.5

Abbreviations: 2GC, second-generation cephalosporin; 3GC, third-generation cephalosporin resistance; 3GC-R EB, third-generation cephalosporin resistant Enterobacterales; FQ, fluoroquinolone; LR-, negative likelihood ratio; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity

Supplementary Table 3. Sensitivity analyses with regard to cultures from general practitioners (available in hospital TGH) and outpatient antibiotic use (available in hospital UMCU)

		Outcome			Suspected Gram-negative sepsis	Positive predictive value for:		Positive likelihood ratio for:	
		3GC-R EB bacteremia	Any 3GC-R EB infection	3GC-S EB bacteremia					
Hospital		**Prevalence, *n* (% among all episodes)**							
Both hospitals		64 (0.7)	331 (3.5)	709 (7.5)	9,422 (100)				
Hospital UMCU		50 (1.0)	235 (4.7)	307 (6.2)	4,959 (100)				
Hospital TGH		14 (0.3)	96 (2.2)	402 (9.0)	4,463 (100)				
Predictor		**Sensitivity, *n* (% among outcome)**			**Prevalence, *n* (%)**	**3GC-R EB bacteremia, %**	**Any 3GC-R EB infection, %**	**3GC-R EB bacteremia**	**Any 3GC-R EB infection**
Prior colonization with 3GC-R EB: 90 days									
Both hospitals	w/o GP cultures	27 (42)	125 (38)	30 (4)	363 (4)	7.4	34.4	11.7	14.4
Hospital UMCU	w/o GP cultures	24 (48)	100 (43)	22 (7)	302 (6)	7.9	33.1	8.5	10
Hospital TGH	w/o GP cultures	3 (21)	25 (26)	8 (2)	61 (1)	4.9	41.0	16.4	31.6
Hospital TGH	with GP cultures	3 (21)	26 (27)	8 (2)	66 (1)	4.5	39.4	15.1	29.6
Prior 2GC, 3GC or FQ use: 30 days									
Both hospitals	w/o outpatient AB	20 (31)	111 (34)	88 (12)	1,598 (17)	1.3	6.9	1.9	2.1
Hospital UMCU	w/o outpatient AB	16 (32)	79 (34)	38 (12)	1,023 (21)	1.6	7.7	1.6	1.7
Hospital UMCU	with outpatient AB	18 (36)	83 (35)	45 (15)	1,111 (22)	1.6	7.5	1.6	1.6
Hospital TGH	w/o outpatients AB	4 (29)	32 (33)	50 (12)	575 (13)	0.7	5.6	2.2	2.7

Abbreviations: 2GC, second-generation cephalosporin; 3GC, third-generation cephalosporin; 3GC-R EB, third-generation cephalosporin-resistant Enterobacterales; 3GC-S EB, third-generation cephalosporin-susceptible Enterobacterales; AB, antibiotics; FQ, fluoroquinolone; GP, general practitioner.

Supplementary Table 4. Prior colonization and antibiotic use as predictors for third-generation cephalosporin-resistant Enterobacterales bacteremia in suspected Gram-negative sepsis presenting with bacteremia

	All episodes						
	Bacteremia episodes					Suspected Gram-negative sepsis	
	EB bacteremia episodes			Bacteremia			
	3GC-R EB bacteremia	EB bacteremia					
Prevalence, n (% among all episodes)	64 (0.7)	773 (8.2)		1,657 (17.6)		9,422 (100)	
Predictor	**Sensitivity, n (%)**	**Prevalence, n (%)**	**LR+[a]**	**Prevalence, n (%)**	**LR+[a]**	**Prevalence, n (%)**	**LR+[a]**
Prior colonization with 3GC-R EB: 90 days	27 (42)	57 (7)	10	101 (6)	9.1	363 (4)	11.7
Prior colonization with 3GC-R EB: 1 year	31 (48)	72 (9)	8.4	132 (8)	7.6	510 (5)	9.5
Prior 2GC or 3GC use: 30 days	15 (23)	76 (10)	2.7	170 (10)	2.4	997 (11)	2.2
Prior FQ use: 30 days	10 (16)	51 (7)	2.7	163 (10)	2.7	865 (9)	1.7
Prior 2GC, 3GC or FQ use: 30 days	20 (31)	108 (14)	2.5	289 (17)	1.9	1,598 (17)	1.9
Prior 2GC, 3GC or FQ use: 90 days	33 (52)	191 (25)	2.3	431 (26)	2.1	2,211 (23)	2.2
Prior colonization with 3GC-R EB (90 days), or prior 2GC, 3GC or FQ use (30 days)	32 (50)	139 (18)	3.3	337 (20)	2.6	1,766 (19)	2.7
Prior colonization with 3GC-R EB (1 year), or prior 2GC, 3GC or FQ use (90 days)	42 (66)	218 (28)	2.6	474 (29)	2.4	2,400 (25)	2.6

Abbreviations: 2GC, second-generation cephalosporin; 3GC, third-generation cephalosporin; 3GC-R: third-generation cephalosporin-resistant; EB, Enterobacterales; FQ: fluoroquinolone; LR+: positive likelihood ratio

[a] With regard to prediction of 3GC-R EB bacteremia.

CHAPTER 7

Development of diagnostic prediction tools for bacteremia caused by third-generation cephalosporin-resistant Enterobacterales in suspected bacterial infections: a nested case-control study

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Abstract

Objectives: Current guidelines for the empiric antibiotic treatment predict the presence of third-generation cephalosporin-resistant Enterobacterales bacteremia (3GCR-E-Bac) in case of infection only poorly, thereby increasing unnecessary carbapenem use. We aimed to develop diagnostic scoring systems which can better predict the presence of 3GCR-E-Bac.

Methods: A retrospective nested case-control study was performed that included patients ≥18 years of age from eight Dutch hospitals in whom blood cultures were obtained and intravenous antibiotics were initiated. Each patient with 3GCR-E-Bac was matched to four control infection episodes within the same hospital, based on blood-culture date and onset location (community or hospital). Starting from 32 commonly described clinical risk factors at infection onset, selection strategies were used to derive scoring systems for the probability of community- and hospital-onset 3GCR-E-Bac.

Results: 3GCR-E-Bac occurred in 90 of 22,506 (0.4%) community-onset infections and in 82 of 8,110 (1.0%) hospital-onset infections, and these cases were matched to 360 community-onset and 328 hospital-onset control episodes. The derived community-onset and hospital-onset scoring systems consisted of six and nine predictors, respectively. With selected score cut-offs, the models identified 3GCR-E-Bac with sensitivity equal to existing guidelines (community-onset: 54.3%; hospital-onset: 81.5%). However, they reduced the proportion of patients classified as at risk for 3GCR-E-Bac (i.e. eligible for empiric carbapenem therapy) with 40% (95% confidence interval (CI) 21–56%) and 49% (95% CI 39–58%) in, respectively, community-onset and hospital-onset infections.

Conclusions: These prediction scores for 3GCR-E-Bac, specifically geared towards the initiation of empiric antibiotic treatment, may improve the balance between inappropriate antibiotics and carbapenem overuse.

Introduction

As a consequence of the emergence of infections caused by third-generation cephalosporin-resistant Enterobacterales (3GCR-E; in this paper used synonymously with extended-spectrum β-lactamase (ESBL) producing Enterobacterales), physicians are increasingly faced with the question of which patients need empiric antibiotic treatment to cover these pathogens. Current Dutch empiric treatment guidelines designate patients at risk of infection caused by 3GCR-E on the basis of prior colonization or infection with 3GCR-E, or prior exposure to cephalosporins or fluoroquinolones, as these were identified as risk factors in patients with bacteremia caused by these pathogens [1]. Applying these recommendations to patients needing empiric antibiotic treatment in a setting with a prior probability of 3GCR-E bacteremia (3GCR-E-Bac) of 0.7% revealed that 19% of all patients were classified as being at risk for 3GCR-E infection and thus eligible for empiric carbapenem therapy (referred to as test positivity rate, TePR), while at the same time only 50% of patients with proven 3GCR-E-Bac were classified as at risk (referred to as sensitivity) [2]. Using only prior identification of 3GCR-E carriage as a risk factor reduced the TePR to 4%, at the cost of a reduction in sensitivity to 42%.

As carbapenems are the treatment of choice for 3GCR-E, adherence to these guidelines may result in overuse of these antibiotics. We aimed to develop prediction rules to better identify, among patients needing intravenous empiric antibiotic therapy, those having 3GCR-E-Bac. We were specifically interested in the balance between sensitivity and TePR. In this derivation study, we compared these quantities to those of the two basic strategies introduced above, which rely on prior identification alone (*prior identification model*) or in combination with prior exposure to cephalosporins and fluoroquinolones (*two-predictor model*). We decided to derive separate prediction rules for community-onset and hospital-onset infections, as we assumed that factors driving the spread of 3GCR-E within these two settings are distinct.

Methods

Settings and patients

This was a retrospective nested case-control study involving eight hospitals, of which three were university hospitals, in The Netherlands. Between 1 January 2008 and 31 December 2010, we included all consecutive patients ≥18 years of age in whom a blood culture was obtained and intravenous broad-spectrum β-lactam antibiotics (i.e. not penicillin or flucloxacillin), aminoglycosides, and/or fluoroquinolones were started on the day of, or the day after, blood

culture, irrespective of duration. Patients who had already initiated these antibiotics before the day of blood culture were excluded (see **Supplementary Table 1** for examples; see **Supplementary Material** for additional information on hospital characteristics, study periods, inclusion criteria, sample size, and databases used).

Infection episodes were separated into two cohorts: the community-onset cohort comprising episodes in which the first blood culture was collected during the first 3 days of hospitalization, and the hospital-onset cohort comprising episodes in which blood cultures were obtained later during hospitalization. The causative pathogen of each episode was based on the results of blood cultures obtained on the day that antibiotics were started and the day before. In both cohorts, the case population included all consecutive infection episodes with 3GCR-E-Bac (see **Supplementary Table 2** for definition of 3GC resistance in each of the hospitals). The control population was defined as 'all other infection episodes', including non-bacteremic episodes and episodes with blood cultures yielding non-resistant Enterobacterales, other bacteria, or fungi. From this population, four controls were selected for each case matched on hospital, being in the community- or hospital-onset cohort, and being closest in time to the case episode.

Because of the retrospective nature of this study the Dutch Medical Research Involving Human Subjects Act did not apply to it. Informed consent was waived for the study. In each of the participating hospitals, applicable local guidelines for non-interventional studies were followed. Reporting of this study was in accordance with the TRIPOD Statement [3,4].

Data collection

All selected cases and controls were subjected to chart review to obtain information that was available at the time the initial antibiotics were prescribed (referred to as infection onset). Blinding for the outcome during chart review was not considered feasible. Definitions of collected variables are listed in **Supplementary Table 3**.

Statistical analysis

Two separate prediction models were constructed: one for community-onset infections and one for hospital-onset infections. After observing the data, we first selected ten promising variables, followed by a backward stepwise logistic regression analysis in which only variables with p <0.2 were retained. A simplified score was created by multiplying the regression coefficients with a constant chosen such that, after rounding, the resulting values would be relatively easy to add up.

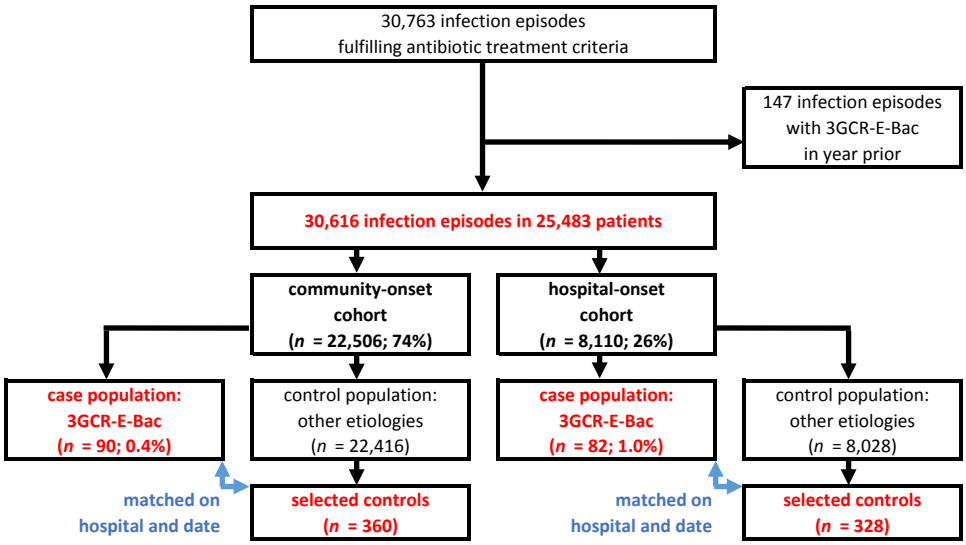

Figure 1. Patient flowchart.

Discrimination of this score was assessed with the area under the receiver operating characteristic curves (referred to as C-statistic). Sensitivity, specificity, positive and negative predictive values, and TePR were calculated at different score cut-offs. These performance characteristics were compared with those of the *prior identification model* (classifying patients with identification of 3GCR-E in the year prior to the infection episode as test-positive) and the *two-predictor model* (classifying patients also as test-positive in the case of cephalosporin or fluoroquinolone use during the prior 2 months).

More details regarding the statistical procedures (including handling of missing variables, performance evaluation, and internal validation) are provided in the **Supplementary Material**.

Results

Probabilities of 3GCR-E-Bac were 0.4% (*n* = 90) for the community-onset infection cohort (22,506 episodes) and 1.0% (*n* = 82) for the hospital-onset infection cohort (8,110 episodes) (**Figure 1**). These case populations were matched to 360 community-onset control episodes and 328 hospital-onset control episodes (**Table 1**). Initial antibiotic therapy and isolated pathogens from blood cultures are presented in **Supplementary Tables 5 and 6**.

Table 1. Clinical characteristics of cases and controls from the community-onset and hospital-onset cohorts

Predictor	Community-onset infection			Hospital-onset infection		
	Cases (*N* = 90)[a], n/N with data (%)	Controls (*N* = 360)[b], n/N with data (%)	Odds ratio (95% CI)[c]	Cases (*N* = 82)[a], n/N with data (%)	Controls (*N* = 328)[b], n/N with data	Odds ratio (95%CI)[c]
Age in years, median (IQR)	69 (61–76)	63 (50–76)	1.02 (1.00–1.03)	64 (55–73)	64 (52–75)	1.00 (0.99–1.02)
Healthcare-associated infection	50/90 (56)	141/353 (40)	1.81 (1.13–2.89)	NA	NA	
Length of hospital stay prior to infection in days, median (IQR)	NA	NA		20 (10–48)	11 (6–19)	1.03 (1.02–1.04)
Diabetes mellitus	28/90 (31)	83/358 (23)	1.48 (0.89–2.46)	16/81 (20)	62/328 (19)	1.10 (0.60–2.03)
Any solid malignancy[d]	16/90 (18)	60/358 (17)	1.07 (0.58–1.97)	25/81 (31)	70/328 (21)	1.67 (0.97–2.87)
Hematological malignancy	11/90 (12)	28/358 (8)	1.62 (0.77–3.40)	9/81 (11)	44/328 (13)	0.85 (0.40–1.82)
Renal disease	13/90 (14)	21/358 (6)	2.54 (1.22–5.27)	14/81 (17)	17/328 (5)	3.98 (1.87–8.45)
Immuno-compromised[e]	27/87 (31)	62/356 (17)	2.03 (1.19–3.46)	16/80 (20)	76/323 (24)	0.85 (0.47–1.56)
Any transplant[f]	14/90 (16)	22/358 (6)	2.67 (1.31–5.45)	15/81 (18)	23/327 (7)	3.10 (1.54–6.23)
Urological patient[g]	25/90 (28)	40/357 (11)	2.96 (1.68–5.22)	5/81 (6)	21/323 (6)	1.05 (0.39–2.83)
Surgical procedure (prior 30 days)	4/90 (4)	34/357 (10)	0.43 (0.15–1.24)	37/82 (45)	116/327 (36)	1.50 (0.92–2.46)
Central vascular catheter (at infection onset)	5/89 (6)	20/344 (6)	0.93 (0.34–2.55)	46/75 (61)	106/299 (36)	2.72 (1.62–4.57)
Signs of hypoperfusion (at infection onset)	12/86 (14)	35/340 (10)	1.46 (0.73–2.93)	25/77 (32)	38/296 (13)	2.82 (1.57–5.06)
Suspected source of infection (at infection onset)						
Urinary tract infection or intra-abdominal infection	55/90 (61)	94/359 (26)	4.44 (2.73–7.22)	26/80 (32)	46/325 (14)	3.00 (1.71–5.26)
Urinary tract infection	41/90 (46)	48/359 (13)	5.44 (3.25–9.11)	12/80 (15)	20/325 (6)	2.85 (1.35–6.04)
Intra-abdominal infection	14/90 (16)	46/359 (13)	1.26 (0.66–2.41)	14/80 (18)	26/325 (8)	2.42 (1.20–4.89)

Table 1 (continued)

Predictor	Community-onset infection			Hospital-onset infection		
	Cases (*N* = 90)[a], n/N with data (%)	Controls (*N* = 360)[b], n/N with data (%)	Odds ratio (95% CI)[c]	Cases (*N* = 82)[a], n/N with data (%)	Controls (*N* = 328)[b], n/N with data	Odds ratio (95%CI)[c]
Lower respiratory tract infection	8/90 (9)	111/359 (31)	0.22 (0.10–0.46)	4/80 (5)	86/325 (26)	0.14 (0.05–0.40)
Other infection	5/90 (6)	42/359 (12)	0.45 (0.17–1.16)	11/80 (14)	35/325 (11)	1.37 (0.66–2.85)
Unknown	22/90 (24)	112/359 (31)	0.71 (0.42–1.21)	39/80 (49)	159/325 (49)	0.98 (0.60–1.60)
Prior identification of 3GCR-E (prior one year)	22/90 (24)	9/359 (2)	11.82 (5.25–26.63)	29/82 (35)	16/328 (5)	10.67 (5.41–21.03)
Any use of antibiotics (prior 2 months)	51/85 (60)	140/346 (40)	2.22 (1.37–3.60)	68/82 (83)	228/324 (70)	2.02 (1.08–3.77)
Cephalosporins or fluoroquinolones	28/85 (33)	66/346 (19)	2.12 (1.26–3.55)	58/82 (71)	165/323 (51)	2.27 (1.34–3.84)
Cephalosporins	14/86 (16)	33/351 (9)	1.91 (0.99–3.68)	49/82 (60)	114/322 (35)	2.67 (1.62–4.39)
Fluoro-quinolones	17/85 (20)	44/346 (13)	1.81 (0.98–3.35)	25/82 (30)	81/322 (25)	1.28 (0.75–2.18)
Carbapenems	4/86 (5)	2/351 (1)	4.95 (1.02–24.02)	12/82 (15)	29/321 (9)	1.66 (0.81–3.42)
At risk of 3GCR-E-Bac according to *two-predictor model*[h]	46/86 (54)	71/347 (20)	4.32 (2.63–7.09)	65/82 (79)	168/323 (52)	3.46 (1.94–6.17)

Abbreviations: IQR, interquartile range.

[a] Patients with 3GCR-E-Bac.

[b] Sample of patients without bacteremia or with blood cultures yielding non-resistant Enterobacterales, other bacteria or fungi.

[c] OR calculated with imputed datasets, and hence its value cannot be derived from presented numbers.

[d] Aggregated variable combining *malignancies with* and *without metastases*.

[e] Aggregated variable combining *immunosuppressant use*, *neutropenia (at infection onset)* and *solid organ transplant*.

[f] Aggregated variable combining *solid organ* and *stem-cell transplants*.

[g] Aggregated variable combining *recurrent urinary tract infection*, *obstructive urinary disease*, and *urological procedure (prior 30 days)*.

[h] Patients scoring positive on *use of cephalosporins or fluoroquinolones (prior 2 months)* and/or *prior identification of 3GCR-E (prior 1 year)*.

Community-onset infection

The prediction model for 3GCR-E-Bac in community-onset infection consisted of six variables (**Table 2**). It showed adequate discrimination (*c*-statistic 0.775, 95% confidence interval (CI) 0.705–0.839). The derived scoring system had a very similar performance (**Supplementary Figure 1a**). **Table 3** and **Figure 2a** depict the trade-off between sensitivity and TePR at different cut-offs for being at risk of 3GCR-E-Bac. These can be contrasted with the fixed values

Table 2. Regression model and scoring system for prediction of 3GCR-E-Bac in community-onset infection

Predictor	β coefficient	Odds ratio (95%CI)	Score
Intercept	–7.248		
Prior identification of 3GCR-E (prior 1 year)	1.963	7.12 (2.88–17.62)	100
Suspected source of infection: urinary tract infection	1.081	2.95 (1.64–5.29)	50
Immunocompromised	0.491	1.63 (0.87–3.08)	25
Any use of antibiotics (prior 2 months)	0.314	1.37 (0.78–2.39)	25
Age (per 1 year of age)	0.018	1.02 (1.00–1.04)	1
Suspected source of infection: lower respiratory tract infection	–0.896	0.41 (0.18–0.94)	–50

The regression analysis was pooled over 20 imputed datasets reflecting 450 infection episodes (of which 90 cases had 3GCR-E-Bac), and was subsequently corrected for the sampling fraction of controls and overoptimism (see **Supplementary Material** for a full explanation).
The predicted probability of 3GCR-E-Bac can be calculated with the following formula: 1/(1 + exp(-(-7.248 + 1.963 x *prior identification of 3GCR-E (prior 1 year)* + 1.081 x *suspected source of infection: urinary tract infection* + 0.491 x *immunocompromised* + 0.314 x *any use of antibiotics (prior 2 months)* + 0.018 x *age in years* - 0.896 x *suspected source of infection: lower respiratory tract infection*))). For categorical predictors, fill in 1 if present, and 0 if absent.
Similarly, the derived score can be calculated with the following formula: 100 x *prior identification of 3GCR-E (prior 1 year)* + 50 x *suspected source of infection: urinary tract infection* + 25 x *immunocompromised* + 25 x *any use of antibiotics (prior 2 months)* + *age in years* - 50 x *suspected source of infection: lower respiratory tract infection*.

for the *prior identification model* (sensitivity 24.4% and TePR 2.8%) and the *two-predictor model* (sensitivity 53.9% and TePR 21.5%). For instance, patients with a score of ≥120 would have a probability of 1.7% (positive predictive value) of having 3GCR-E-Bac, and with this score as a

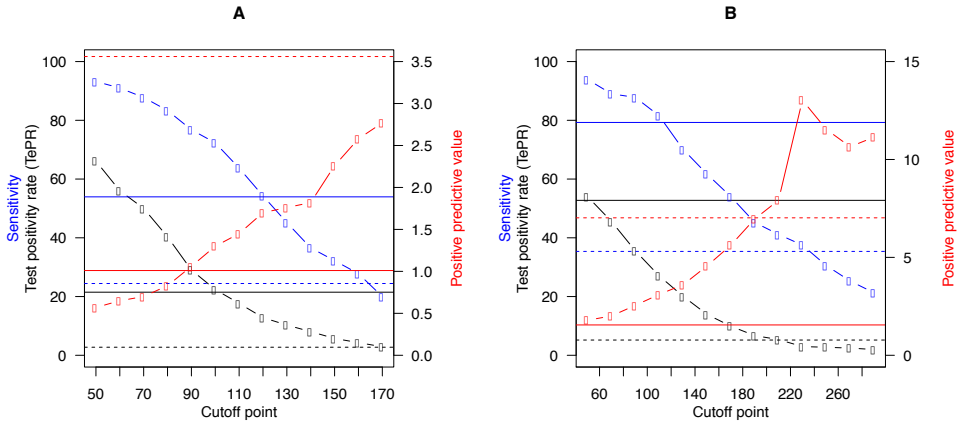

Figure 2. Performance of community-onset (A) and hospital-onset (B) scoring systems at different cutoff values. Figures show sensitivities (blue), test positivity rates (TePR; black), and positive predictive values (red) at different cutoffs for derived scoring systems above which patients are categorized as at risk of 3GCR-E-Bac. These are compared to the (constant) sensitivities, TePR values, and positive predictive values for the basic *two-predictor model* (solid lines) and *prior identification model* (dashed lines). See **Tables 3 and 5** for exact values at the score cutoffs.

Table 3. Performance of scoring system for 3GCR-E-Bac in community-onset infection

	Score													
	–31[a]	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**	**130**	**140**	**150**	**160**	**170**	**267[b]**
	Characteristics of interval [prior value, current value)														
Proportion of cohort, %		33.9	10.1	6.0	9.7	11.3	6.7	4.7	4.8	2.5	2.2	2.3	1.4	1.4	2.9
Probability of 3GCR-E-Bac, %	0.1	0.1	0.2	0.2	0.2	0.3	0.7	0.8	1.4	1.5	0.8	1.3	2.2	2.6	
	Characteristics of cut-off ≥current value for classification as *at risk of 3GCR-E-Bac*														
TePR, %		66.1	56.0	50.0	40.3	29.0	22.4	17.7	12.8	10.3	8.1	5.7	4.3	2.9	0.0
Sensitivity, %		93.2	91.0	87.8	83.3	76.8	72.3	63.7	54.3	45.2	36.6	32.2	27.8	20.0	1.1
Specificity, %		34.0	44.1	50.1	59.9	71.2	77.8	82.5	87.3	89.8	92.1	94.4	95.8	97.2	100.0
Positive predictive value, %		0.6	0.6	0.7	0.8	1.1	1.3	1.4	1.7	1.8	1.8	2.3	2.6	2.8	100.0
Negative predictive value, %		99.9	99.9	99.9	99.9	99.9	99.9	99.8	99.8	99.8	99.7	99.7	99.7	99.7	99.6

These values (means of 20 imputed datasets) have been corrected for the sampling fraction of the controls (meaning that they have been extrapolated to the full community-onset cohort and hence reflect the values as observed in clinical practice), but they have not been corrected for overoptimism (see **Supplementary Material** for a full explanation).

The upper part of the table shows the *calibration* of the score. For example, 33.9% of all patients in the community-onset cohort have scores between -31 and 50. The probability of having 3GCR-E-Bac is low within this interval (0.1%; e.g. compared to 2.9% within the interval between 170 and 267).

The lower part of the table shows how a specific cut-off of the score would perform with regard to detecting 3GCR-E-Bac. For example, 66.1% of the cohort has a score of ≥50 (1 - 33.9%); this is the *TePR*. The *sensitivity* of this cut-off is 93.2%, implying that 6.8% of patients with 3GCR-E-Bac have a score <50. *Specificity* is low because of the ones not having 3GCR-E-Bac; only 34.0% have scores <50. This, combined with the fact that only 0.4% of the cohort has 3GCR-E-Bac, leads to a low *positive predictive value*: only 0.6% of patients with scores ≥50 have 3GCR-E-Bac. Increasing the score cut-off leads to a lower TePR, higher specificity, and higher positive predictive value, but at the cost of a lower sensitivity. A similar overview relating to the underlying regression model instead of the score is available in **Supplementary Table 7**.

[a] Minimum score within the study sample.

[b] Maximum score within the study sample.

cut-off, 45.7% of all patients with 3GCR-E-Bac would be missed (1 - sensitivity). This sensitivity (or proportion missed) is comparable to the simpler *two-predictor model*; however, the scoring system reduces eligibility for carbapenem use (TePR) by 40% (95% CI 21–56%) from 21.5% to 12.8% (**Supplementary Table 12**).

Table 4. Regression model and scoring system for prediction of 3GCR-E-Bac in hospital-onset infection

Predictor	β coefficient	Odds ratio (95%CI)	Score
Intercept	–5.807		
Renal disease	1.372	3.94 (1.55–10.05)	120
Prior identification of 3GCR-E (prior 1 year)	1.353	3.87 (1.67–8.95)	120
Any solid malignancy	0.722	2.06 (1.06–4.01)	80
Signs of hypoperfusion (at infection onset)	0.509	1.66 (0.79–3.49)	40
Surgical procedure (prior 30 days)	0.444	1.56 (0.84–2.91)	40
Central vascular catheter (at infection onset)	0.420	1.52 (0.78–2.95)	40
Use of cephalosporins (prior 2 months)	0.415	1.51 (0.81–2.83)	40
Length of hospital stay prior to infection (per day)	0.011	1.01 (1.00–1.03)	1
Suspected source of infection: lower respiratory tract infection	–1.729	0.18 (0.06–0.56)	–160

The regression analysis was pooled over 20 imputed datasets reflecting 410 infection episodes (of which 82 cases had 3GCR-E-Bac), and was subsequently corrected for the sampling fraction of controls and over-optimism (see Supplementary material for an explanation).

The predicted probability of 3GCR-E-Bac can be calculated with the following formula: 1/(1 + exp(-(-5.807 + 1.372 x *renal disease* + 1.353 x *prior identification of 3GCR-E (prior 1 year)* + 0.722 x *any solid malignancy* + 0.509 x *signs of hypoperfusion (at infection onset)* + 0.444 x *surgical procedure (prior 30 days)* + 0.420 x *central vascular catheter (at infection onset)* + 0.415 x *use of cephalosporins (prior 2 months)* + 0.011 x *length of hospital stay prior to infection in days* - 1.729 x *suspected source of infection: lower respiratory tract infection*))). For categorical predictors, fill in 1 if present, and 0 if absent.

Similarly, the derived score can be calculated with the following formula: 120 x *renal disease* + 120 x *prior identification of 3GCR-E (prior 1 year)* + 80 x *any solid malignancy* + 40 x *signs of hypoperfusion (at infection onset)* + 40 x *surgical procedure (prior 30 days)* + 40 x *central vascular catheter (at infection onset)* + 40 x *use of cephalosporins (prior 2 months)* + *length of hospital stay prior to infection in days* - 160 x *suspected source of infection: lower respiratory tract infection*.

Hospital-onset infection

The hospital-onset prediction model contained nine variables (**Table 4**), and also showed adequate discrimination (*c*-statistic 0.811, 95% CI 0.742–0.873). The derived scoring system again performed very similarly (**Supplementary Figure 1b**). In **Table 5** and **Figure 2b**, sensitivity and TePR at different score cut-offs are compared to the *prior identification model* (sensitivity 35.4% and TePR 5.2%) and the *two-predictor model* (sensitivity 79.3% and TePR 52.8%).

Patients with scores ≥110 have a 3.1% probability of 3GCR-E-Bac, and with this cut-off, 18.5% of all patients with 3GCR-E-Bac would be missed, similarly to the *two-predictor model*.

Table 5. Performance of scoring system for 3GCR-E-Bac in hospital-onset infection

	Score														
	–159[a]	**50**	**70**	**90**	**110**	**130**	**150**	**170**	**190**	**210**	**230**	**250**	**270**	**290**	**432[b]**
	Characteristics of interval [prior value, current value)														
Proportion of cohort, %		46.0	8.4	10.0	8.5	6.9	6.2	4.0	3.2	1.3	2.4	0.2	0.3	0.5	2.0
Probability of 3GCR-E-Bac, %		0.1	0.6	0.1	0.8	1.7	1.4	2.0	2.8	3.1	1.6	30.2	19.3	8.7	10.6
	Characteristics of cut-off ≥current value for classification as *at risk of 3GCR-E-Bac*														
TePR, %		54.0	45.6	35.6	27.0	20.1	13.9	9.9	6.7	5.4	3.0	2.7	2.4	2.0	0.0
Sensitivity, %		93.9	89.0	87.8	81.5	70.1	61.7	54.0	45.2	41.2	37.5	30.6	25.3	21.3	1.2
Specificity, %		46.4	54.9	65.0	73.5	80.4	86.5	90.5	93.7	95.0	97.4	97.6	97.8	98.2	100.0
Positive predictive value, %		1.8	2.0	2.5	3.1	3.6	4.6	5.6	7.0	7.9	13.0	11.5	10.6	11.1	100.0
Negative predictive value, %		99.9	99.8	99.8	99.7	99.6	99.5	99.5	99.4	99.4	99.3	99.3	99.2	99.2	99.0

These values (means of 20 imputed datasets) have been corrected for the sampling fraction of the controls (meaning that they reflect the values as observed in clinical practice), but they have not been corrected for over-optimism (see **Supplementary Material** for an explanation). The use of this table is exemplified below **Table 3**. A similar overview relating to the underlying regression model instead of the score is available in **Supplementary Table 8**.

[a] Minimum score within the study sample.

[b] Maximum score within the study sample.

However, carbapenem eligibility would be reduced by 49% (95% CI 39–58%) from 52.8% to 27.0% (**Supplementary Table 12**).

Additional analyses

An analysis stratified by suspected source of infection (namely lower respiratory tract infection versus other sources) indicated that the community-onset scoring system was valuable in both subgroups (see **Supplementary Material**). The absolute reduction in carbapenem use achieved by using a score of 120 as the cut-off was equally divided between the pneumonia subgroup and the remaining etiologies. Furthermore, internal validation revealed that in future patient populations both the community-onset and the hospital-onset prediction models should be expected to perform slightly worse due to overoptimism (see **Supplementary Material**).

Discussion

We developed scoring systems to more accurately identify patients with bacteremia caused by 3GCR-E among those in whom empiric intravenous antibiotic therapy aimed at Gram-negative bacteria is initiated. The scores consist of a limited number of clinical predictors that can be assessed on the basis of the information available at the initial examination of a patient presenting with infection, before the prescription of initial antibiotics. The calculated score can be converted directly to a probability that the patient suffers from 3GCR-E-Bac, and depending on this probability, a decision can be made on whether initial antibiotics should include coverage for 3GCR-E or not. Implementing the scoring systems could improve appropriateness of empiric antibiotic therapy and reduce unnecessary use of broad-spectrum antibiotic therapy. Compared to a basic model incorporating only prior 3GCR-E identification and exposure to cephalosporins and/or fluoroquinolones, eligibility for empiric carbapenem use could be reduced by 40–49% while maintaining a similar risk of missing patients with 3GCR-E-Bac.

With the global emergence of antibiotic resistance, physicians must assess the risks of missing resistant causative pathogens when starting empiric antibiotic treatment [5]. Risk avoidance, albeit imaginable in many situations, is one of the driving forces for broad-spectrum antibiotic use, fueling the global pandemic of antimicrobial resistance. Better prediction rules for infections caused by antibiotic-resistant pathogens are therefore needed. The strength of our study is that it focused on prediction in all patients receiving their first dose of antibiotic therapy aimed at Enterobacterales. This contrasts with previously published prediction systems which have focused on carriage of or infection with ESBL-producing Enterobacterales at hospital admission [6–8], or on distinguishing bacteremia with ESBL- or carbapenemase-producing pathogens from bacteremia with susceptible Enterobacterales [9–12]. A recently published flow chart for initiating empiric therapy with carbapenem in critically ill patients with suspected Gram-negative infection proposed to apply two of these prediction systems in the decision-making process [13], without acknowledging that these have never been formally evaluated in the setting of prescription of initial antibiotic therapy.

Predicting the probability that a patient is suffering from 3GCR-E-Bac at the moment of presentation involves combining the probabilities that (i) the patient has bacteremia, (ii) the infection is caused by Enterobacterales, and (iii) these Enterobacterales are antibiotic-resistant. Furthermore, because of this dilution effect, the prevalence of 3GCR-E-Bac is an order of magnitude lower (0.4–1.0%) than in patients who, in retrospect, had bacteremia. In a previous

study we calculated that an 8.3% 3GC resistance rate among Enterobacterales bacteremia isolates resulted in a 0.7% probability of 3GCR-E-Bac in cases of suspected Gram-negative infection [2].

Although our data originated in 2008–2010, we believe that the prior and predicted probabilities are relevant to the present-day situation, also in other countries. Importantly, the aforementioned dilution process is always in place when initiating empiric therapy. On top of that, the prevalence of 3GC resistance among Enterobacterales has only marginally increased in The Netherlands since 2010, and most Western European countries currently have similar prevalence rates of 3GC resistance among Enterobacterales, namely between 5% and 15% [14].

Two aspects regarding the patient population in this study should be discussed. First, a large proportion of community-acquired pneumonia (CAP) patients have blood cultures obtained and receive treatment categorized by us as covering Gram-negative bacteria [15]. When setting the patient domain for our community-onset prediction rule, the inclusion of true CAP is debatable since Gram-negatives are rarely encountered as pathogens [16]. However, we found that in the case of a working diagnosis of CAP, the probability of 3GCR-E-Bac is non-zero, and data exists that Gram-negative pathogens (and hence resistant variants) have a higher frequency in specific risk groups [17]. Our community-onset scoring system may not be optimally designed to predict 3GCR-E-Bac in CAP, as the risk factors identified by us are a weighted average of the pneumonia subgroup and all other etiologies. Nevertheless, it has diagnostic accuracy even in CAP patients, and at the same time the effected reduction in carbapenem eligibility is not only the result of giving low scores to CAP patients, as demonstrated in the subgroup analysis.

The second aspect is that we applied a nested case-control design for this study, which implies that instead of analyzing the full cohort, a representative subset of patients without 3GCR-E-Bac (i.e. the control population) was analyzed. The case population (i.e. patients with 3GCR-E-Bac), however, was analyzed in full. This design was chosen for efficiency reasons, as it reduced the amount of data collection by 95% while accepting a small loss of precision. Knowing the size of the original cohort, we were able to extrapolate the case-control data to the full cohort, the result being that probabilities are generalizable to clinical practice.

When applying our prediction rules in practice, some issues should be noted. First, the scores have been derived solely for predicting bacteremia, and not for non-bacteremic infections

caused by 3GCR-E; the latter are considerably more common than the former [2]. Future studies may consider classifying non-bacteremic 3GCR-E infections as outcomes. However, because of the anticipated more benign course, initial treatment with carbapenems may not have a high priority in non-bacteremic infections.

Second, empiric coverage of 3GCR-E is just one aspect of the selection of appropriate empiric therapy. Other potential pathogens (such as *Pseudomonas aeruginosa*) and resistance mechanisms might justify alterations to empiric treatment even in the absence of risk factors for 3GCR-E. In some countries, high incidences of infections with carbapenemase-producing Enterobacterales may limit the usefulness of our prediction rules. On the other hand, escape therapy for 3GCR-E might not necessarily involve carbapenems, because of underlying resistance mechanisms other than ESBL, or favorable patterns of co-resistance. Ideally, frameworks for selecting empiric therapy should evaluate the probability of success of many different antibiotic agents. An example of such an approach is TREAT [18], but predictive performance with regard to 3GCR-E as causative pathogens is currently unknown.

Third, our prediction rules are meant for application only when the initial antibiotic therapy is started. This implies that 3GCR-E-Bac presenting as superinfection while antibiotic therapy is in place will be missed. That this is a relevant subgroup of 3GCR-E-Bac is shown by the fact that in two of the hospitals participating in this study (for which these data were available) such cases amounted to 20–34% of all 3GCR-E-Bac for which anti-Gram-negative therapy was administered on the day of blood culture and/or the day after.

Fourth, the newly developed scoring systems may be used to reduce the proportion of patients eligible for broad-spectrum antibiotics (test-positives), but they can also be used to increase sensitivity, which will simultaneously increase the proportion of test-positivity. A definitive cut-off cannot be defined, as each situation may represent a different balance between the risks associated with overprescribing carbapenems and inappropriate empiric antibiotics. For instance, the acceptance for a delay might be different in a clinically stable patient compared to a hemodynamically unstable patient [19]. Taking the long-term population effects of, for instance, carbapenem overuse into the equation is difficult, as these effects have not been sufficiently quantified [20], and they also depend on extraneous factors such as hospital hygiene and the baseline prevalence of carbapenem-resistant microorganisms [21].

Before implementation of these prediction rules, prospective external validation is required. Our study prone to information bias due to its retrospective nature, relied on data available in

medical charts, and used pragmatic inclusion and exclusion criteria which might not fully reflect intended clinical use. Future studies may try to improve on the definitions of predictors to find a better balance between sensitivity and specificity for 3GCR-E-Bac: for example by modifying the time periods assessed for prior identification of 3GCR-E and prior antibiotic use. Moreover, potentially relevant predictors such as international travel, animal contact, known colonization in household members, dietary preferences, and colonization pressure in the ward were not collected [21,22]. Validation is currently ongoing in regions with a 3GCR-E prevalence comparable to or greater than that in The Netherlands [23]; during this process, it can simultaneously be assessed to what degree model updating is necessary to improve performance in these differing settings [24].

A final limitation of our study is that treating physicians incorporate more factors in their clinical decision-making regarding empiric antibiotics than those provided by current risk-stratification schemes in guidelines. In both this and our previous study [2], empiric carbapenem use was much lower than it would have been with full guideline adherence (**Supplementary Table 5**). As a result, achievable reductions in empiric carbapenem use may in reality be lower than anticipated in our study. Nevertheless, we consider it important that antibiotic guidelines do not stimulate unnecessary broad-spectrum antibiotic use [25].

In conclusion, identification of patients with an infection caused by 3GCR-E amongst all patients that need empiric antibiotic therapy remains a trade-off between acceptably low levels of unnecessary empiric carbapenem use and appropriate treatment in true 3GCR-E-Bac cases. The prediction rules derived in this study quantify this trade-off, and might offer improvement in detecting patients with 3GCR-E-Bac compared to current international guidelines. As such, they provide useful starting points for optimizing empiric antibiotic strategies.

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CHAPTER 7
SUPPLEMENTARY MATERIAL

Development of diagnostic prediction tools for bacteremia caused by third-generation cephalosporin-resistant Enterobacterales in suspected bacterial infections: a nested case-control study

In- and exclusion criteria

Inclusion in this study involved a combination of blood culture obtainment and initiation of what we considered *relevant antibiotics*. *Relevant antibiotics* were defined as antibiotics regularly prescribed in the Netherlands as empiric therapy for cases of sepsis in which Gram-negatives are to be expected as causative pathogens. Among *relevant antibiotics*, we included intravenously administered broad-spectrum β-lactam antibiotics (i.e. not penicillin or flucloxacillin), aminoglycosides, and fluoroquinolones.

Between 1 January 2008 and 31 December 2010, we included all consecutive patients of 18 years of age or older in whom a blood culture was obtained and any of the *relevant antibiotics* were started on the day of the blood culture or the day after, irrespective of duration. Patients receiving any of the *relevant antibiotics* on the day of blood culture obtainment were excluded if these had been initiated prior to this day. In **Supplementary Table 1**, examples are provided of the criteria applied to blood culture obtainment and antibiotic usage to define an infection episode. Also, it is indicated what blood cultures are used for ascertainment of the causative pathogens of an infection episode.

Apart from this, infection episodes in patients with third-generation cephalosporin-resistant (3GCR-E) bacteremia (3GCR-E-Bac) in the year prior were excluded, as it was assumed that treating physicians would always provide therapy aimed at these organisms in case of renewed infection.

Patients could be included more than once, if a subsequent episode complied with in- and exclusion criteria. Within the case and control episodes selected from the community-onset cohort for analysis, multiple selection of individual patients, albeit with different episodes, occurred 8 times, and this number amounted to 9 for the hospital-onset dataset.

Sample size calculation

We estimated that a study period of three years in the participating hospitals would yield 100 patients with 3GCR-E-Bac (case population) in both the community-onset and hospital-onset cohorts, which would allow initial logistic regression with 10 variables, based on the 10 events per variable recommendation [1].

For efficiency reasons, the control populations were not analysed in their entirety. Instead, from these, four controls were matched to each case, a ratio chosen because of minimal gains in statistical power with more controls [2].

Variable handling during data analysis

Data analyses were performed in R (version 3.4.3) [3] including packages *mice* 2.46.0 [4], *rms* 5.1-2 [5], *pROC* 1.10.0 [6], and *xtable* 1.8-2 [7]. Descriptive analyses of predictors were based on non-missing data only. Some variables were aggregated because of high correlation, low prevalence, and/or similar associations with the outcome (indicated in **Supplementary Table 4**). Additionally, the number of categories for suspected sources was reduced to four by combining categories with low frequencies into a single remaining group (original categories in **Supplementary Table 3**), and categories for antibiotic use were created based on prevalence and assumed predictive power for 3GCR-E infection. Twenty imputed datasets were created to deal with missing values during the modelling stage.

Imputation procedure

Reasons for missingness were irretrievability of (parts of) medical files (implying that most predictors were considered missing) or insufficient information in the medical file to accurately assess a specific predictor (mainly affecting the presence of catheters and signs of hypoperfusion). With the latter in mind, we assumed a missing at random (MAR) mechanism, which allowed for imputation methods.

Separately for community-onset and hospital-onset datasets, we created 20 imputation datasets by means of *multivariate imputation by chained equations* (MICE), as implemented in the *mice* package (version 2.46.0) available for R [4]. For imputation, we used all available predictors (see **Supplementary Table 4**) as input, supplemented by study site, the outcome (3GCR-E-Bac), and other indicators of blood culture results (negative blood cultures, Gram-negative bacteremia, polymicrobial bacteremia). Age and length of length of hospital stay prior to infection (hospital-onset dataset only) were included as continuous predictors; study site, suspected source of infection and hospital ward as categorical predictors; and the remainder as binary predictors. Healthcare-associated infection (community-onset dataset only) was represented as two already included predictors (admission from long-term care facility and hemodialysis), supplemented by four binary predictors relating to its remaining components (see **Supplementary Table 3**). Aggregated variables were excluded from the imputation procedure, and were recreated after imputation. No interactions were included.

Numbers of missings per patient, as counted for non-aggregated variables indicated in **Supplementary Table 4**, with prior antibiotic use (including selective digestive/oropharyngeal decontamination) counted as one variable:

Community-onset dataset (31 variables): 381 with 0 missings, 43 with 1 missing, 6 with 2 missings, 9 with 3, 8 with 4, 1 with 7, 1 with 17, 1 with 28.

Hospital-onset dataset (29 variables): 324 with 0 missings, 40 with 1 missing, 14 with 2 missings, 10 with 3, 19 with 4, 1 with 6, 1 with 9, 1 with 18.

Modelling procedures

Starting from 28 and 37 potential predictors in the community-onset and hospital-onset setting respectively, the first step of model creation involved selection of ten relevant predictors based on (i) observing the strength of their associations with 3GCR-E-Bac (without statistical hypothesis testing), and (ii) considerations related to coverage of the entire spectrum of known risk factors for 3GCR-E, and (iii) ease-of-use of any resulting model. These initial selections are indicated in **Supplementary Table 4**.

The second step involved removing redundant variables from the model, which was performed by backward stepwise logistic regression analysis until all remaining predictors had p <0.2 in the Wald test (pooled over 20 imputed datasets by means of Rubin's rules) [8]. Continuous predictors were initially introduced into models with restricted cubic spline functions with three knots to allow for non-linear associations. Finally, we evaluated by means of the Akaike's Information Criterion (AIC; mean of 20 imputed datasets) if simplification to a linear predictor was possible.

Regression coefficients of the final models were pooled over imputed datasets by means of Rubin's rules and shrunk according to model optimism (see description further on). A simplified score was created by multiplying the regression coefficients with a constant, followed by rounding to easy-to-use values.

Analysis of model and score performance

Developing a model in a case-control study artificially increases the prevalence of the outcome, which means that predicted probabilities generated by the model do not reflect true probabilities within the full cohorts. Test positivity rate (TePR) values, and positive and negative predictive values are similarly affected. Therefore, intercepts of the models were adjusted for the sampling fraction of the controls, and controls were weighted by the inverse of the sampling fraction, as previously described [9]. All quantities presented in this paper reflect the values within the original full cohorts.

Calibration of both models (i.e. relating observed probabilities of 3GCR-E-Bac to those predicted by the models within quantiles of the cohorts) was visually inspected for separate imputed datasets (**Supplementary Figure 2**). All other performance parameters were averaged over the imputed datasets. These performance parameters were calculated for both models and both scores.

Discrimination of models and scores (i.e. the ability to differentiate between patients with and without 3GCR-E-Bac) was assessed with the area under the curve for receiver operating characteristic curves (referred to as *c*-statistic; **Supplementary Table 11**). Then, a range of cutoffs was chosen for either calculated scores or predicted probabilities. It was calculated what sensitivity, specificity and positive

and negative predictive values, and TePR (i.e. fraction of the cohort classified as *at risk of 3GCR-E-Bac*) would amount to when these cutoffs would be used to qualify patients as *at risk of 3GCR-E-Bac*. These model performance characteristics were compared to those of the *prior identification model* and *two-predictor model*. Also, for all intervals between cutoffs, it was calculated what proportion of the cohort would be captured by this interval, and what the observed probability of 3GCR-E-Bac within this interval was (another measure of calibration). Results are presented in **Supplementary Tables 7 and 8** (models), **Tables 3 and 5** in the **main text** (scores) and **Figure 2** in the **main text** (scores). A comparison between models and scores is presented in **Supplementary Figure 1**.

Estimation of model overoptimism (internal validation) and confidence intervals

Rationale

Optimism results from the fact that models are developed on a population sample and suffer from overfitting, which jeopardizes generalizability to other populations, including future patients for which a model will be used [10]. By means of a bootstrapping technique, the expected performance loss (e.g. lower sensitivity, specificity, and predictive values, and altered TePR) when applying the model within the total population is quantified. This is a form of internal validation, as opposed to external validation which uses a new dataset to evaluate model performance.

Internal validation could only be performed for the two regression models, and not for the scores because score creation could not be automated. The procedure consisted of creating

2,000 bootstrap samples, creating a new prediction model for each of these samples, and comparing the model's performance in the original and bootstrapped data. In such a way, selecting a sample from a population is mimicked, and with both a sample (the bootstrap sample) and the "population" (in fact the original study sample) at hand, it can be studied how variable selection strategies impacted optimism. This measured optimism can then be subtracted from the values originally calculated within the study sample to obtain optimism-corrected values.

Optimism was estimated for model coefficients, derived odds ratios and *c*-statistics. During the same procedure, the expected overestimation of sensitivity and underestimation of TePR due to optimism was quantified by applying two probability cutoffs above which patients were classified as test-positive. The method was supposed to mimic what would happen if either sensitivity or TePR of the *two-predictor model* would be used to guide choosing a cutoff for the new models' predicted probabilities to qualify patients as *at risk of 3GCR-E-Bac*.

Bootstrap procedure

Bootstrapping was performed separately for the community-onset and hospital-onset models. In each case, 2,000 bootstrap samples of size equal to the original study sample were created by sampling with replacement from the study sample. Cases and controls were sampled separately, and in this way, the 1:4 case:control ratio was retained. Their representations in the previously created 20 imputation instances were all included, and as such, 20 new imputed datasets were constructed during each bootstrap cycle.

On each bootstrap sample, a new model was constructed in two steps, which were supposed to reflect the two-stepped construction of the original models described in the section **Modelling procedures** in this **Supplementary Material**, but in a manner that could be automated:

Step	Realisation during construction of original models	Realisation during bootstrap procedure
1	Selection of ten predictors based on: Observing the strength of their associations with 3GCR-E-Bac (without statistical hypothesis testing) Considerations related to coverage of the entire spectrum of known risk factors for 3GCR-E Ease-of-use of any resulting model	Testing all available predictors (except those indicated in **Supplementary Table 4** as not used for model construction purposes) univariably against the outcome 3GCR-E-Bac. By means of the Wald test, comparing an intercept-only logistic regression model for the outcome to a model including the evaluated predictor. *P*-values pooled over the 20 imputed datasets by means of Rubin's rules. Selection of the ten predictors with the lowest *p*-values.
2	Removal of redundant variables from the model by backward stepwise logistic regression analysis until all remaining predictors had p <0.2 in the Wald test (pooled over 20 imputed datasets by means of Rubin's rules).	

A further difference with the originally created models was that in the latter, continuous variables were initially introduced in models by means of a restricted cubic spline function, and in a final step, it was evaluated whether they could be simplified to a linear form. During the bootstrap procedure, all continuous variables were directly introduced linearly into the models.

The model constructed on the bootstrap sample was used to calculate the predicted probability of 3GCR-E-Bac for each individual within the original study sample. By contrasting performance between the two samples, optimism could be calculated for the following parameters (broadly in accordance with procedures described by Steyerberg [10]):

Parameter	Calculation
Regression coefficients (and odds ratios)	1. Using the model created on the bootstrap sample, calculate linear predictor values for each of the subjects in the original study sample 2. Recalibrate the model by multiplying the linear predictor values with a universal shrinkage factor, so that overall predicted and observed incidences within the study sample match again 3. The median of the 2,000 recalibration slopes represents the shrinkage factor: multiply the regression coefficients of the predictors in the originally created model with this factor to obtain optimism-corrected values (and odds ratios), and recalculate the intercept on the original study sample, so that overall predicted and observed incidences match again

C-statistic	1. Using the model created on the bootstrap sample, calculate the *c*-statistic on the bootstrap sample 2. Using the model created on the bootstrap sample, calculate the *c*-statistic on the original study sample 3. Subtract the median of the 2,000 differences in *c*-statistics from the *c*-statistic of the originally created model (as calculated on the original study sample), to obtain an optimism-correct value
TePR and sensitivity at a probability cutoff with sensitivity or TePR similar to that of the *two-predictor model*	1. Within the bootstrap sample, determine at which cutoff for the probabilities from the model created on the bootstrap sample, the observed sensitivity or the TePR is similar to the sensitivity or the TePR of the *two-predictor model* within the bootstrap sample 2. Using the model created on the bootstrap sample, calculate the sensitivity and TePR when applying the probability cutoff as calculated in the previous step to the original study sample 3. Subtract (or add depending on the sign) the median of the 2,000 differences in the two parameters from the two parameter values as calculated for the originally created model on the original study sample (similar to step 1)

All of the above values were always calculated after correction for the sampling fraction of the controls as described above. Furthermore, because we dealt with 20 imputed datasets, 20 values reflecting the optimism were in fact calculated for each of the above parameters, and the mean of these 20 was used.

Confidence intervals

The bootstrap procedure was also used to obtain 95% confidence intervals for performance parameters of the originally developed prediction models (*c*-statistic, and sensitivity and TePR at different probability/score cutoffs), and the *prior identification model* and *two-predictor model* (sensitivity and TePR). For this, the performance of the originally developed prediction model was also evaluated on each bootstrap sample. For each parameter, the 2.5 and 97.5 percentile values of the 2,000 evaluations were used as boundaries for its confidence interval. Because we again dealt with 20 imputation datasets, a mean of the 20 median values was calculated.

95% confidence intervals for optimism-corrected *c*-statistics, TePR and sensitivity values were calculated by taking 10,000 samples (with replacement) from both the 2,000 optimism estimates (mean of 20 imputation sets), and the 2,000 performance evaluations of the original prediction model (mean of 20 imputation sets). These were subsequently subtracted from each other, and 2.5 and 97.5 percentile values were used as boundaries for the confidence intervals.

A separate bootstrap procedure was performed to obtain 95% confidence intervals for incidences of 3GCR-E-Bac. This procedure involved a fixed cohort size, but variable numbers of cases and controls, and hence control weights. For this, the study cohort was reconstructed, by copying controls according to their sampling fraction. From this reconstructed cohort, 2,000 bootstrap cohorts were sampled with replacement, without any stratification. From these bootstrap cohorts, all available cases were selected and four times as many controls were selected at random. A control weight specific for the bootstrap iteration was then calculated by inverting the sampling fraction of the controls. Using this control weight in the denominator, incidences of 3GCR-E-Bac were calculated for each bootstrap iteration. As always, the procedure worked with 2.5 and 97.5 percentile values, and a final mean of 20 imputation sets.

Results of internal validation

Shrinkage factors for the community-onset model and hospital-onset model were 0.834 and 0.788, respectively. In **Supplementary Tables 9 and 10**, original and optimism-corrected regression coefficients and odds ratios can be compared. Original and optimism-corrected *c*-statistics can be compared in **Supplementary Table 11**.

These optimism-corrected values are also provided in the **main text** and tables. However, for the *c*-statistics of the scores (**Supplementary Table 11**), optimism could not be calculated. Also, for both the models and scores, the values of performance parameters related to cutoffs and intervals (such as sensitivity) could not be corrected for optimism (**Tables 3 and 5** in the **main text** for the scores, and **Supplementary Tables 7 and 8** for the models).

Nevertheless, to give an idea of how models would perform in future patient populations, for both the community-onset and hospital-onset models, two scenarios were evaluated. These scenarios involved selecting a probability cutoff above which patients are classified as *at risk of 3GCR-E-Bac* based on the performance of the *two-predictor model* within the same sample. Either it was attempted to achieve the same sensitivity as the *two-predictor model*, or the same TePR, while hoping for an improvement in the other parameter (lower TePR and higher sensitivity, respectively). Bootstrapping indicated that when applying such cutoffs in future patient population, some performance loss should be expected due to model optimism. Especially sensitivity, and not so much TePR, is affected (**Supplementary Table 13**).

Finally, it should be noted, that even in optimism-corrected parameters, optimism may still be present, as some steps could not be replicated in the bootstrap procedure, such as

aggregation after observing similar associations with the outcome, simplification of continuous variables to linear predictors, and derivation of a scoring system. Also, internal validation cannot help in determining the validity of the models and scores in future populations with a different epidemiology, such as a higher prevalence of 3GCR-E or different distribution of risk factors.

Subgroup analysis: pneumonia vs. other etiologies

For both community-onset and hospital-onset infection, subgroup analyses were performed, in order to evaluate (i) whether the score had any predictive value within the pneumonia subgroup and (ii) whether the potential reduction of TePR associated with the score compared to the two-predictor model was entirely due to not classifying the vast majority of pneumonias as *at risk of 3GCR-E-Bac*.

For each of the two settings, the two subgroups were created based on the variable *suspected source of infection*. Using the same procedure as for the full study sample (including the same control weights used for recreating the cohorts), *c*-statistics of the score were calculated within each subgroup. Due to the small subgroup sizes, calibration was not evaluated. However, sensitivity and TePR of the *two-predictor model*, and at two score cutoffs were evaluated. With these values, the relative reduction in TePR by using 120 (community-onset score) or 110 (hospital-onset score) as a cutoff instead of the *two-predictor model* was calculated for both subgroups, together with how on the level of the entire cohort, the two subgroups contributed to the total TePR reduction. Results are available in **Supplementary Table 12**.

Supplementary Table 1. Examples illustrating inclusion and exclusion criteria

Before day 0	Day 0	Day +1	After day +1	Included as infection episode	Remarks
	BC <ceftriaxone iv---------->			Yes	A blood culture with start of a relevant antibiotic on the same day is included as an episode
	BC <ceftriaxone iv>			Yes	Duration of the initial antibiotic therapy is not relevant for inclusion
	BC	<ceftriaxone iv ------>		Yes	Episodes in which a relevant iv antibiotic regimen is started on the day after the blood culture are also included
	BC	BC <ceftriaxone iv ------>		Yes	In this case, if a blood culture is obtained on the day after the first blood culture, its result is also used for determination of the causative pathogen
BC	BC	BC <ceftriaxone iv ------>		Yes	Blood cultures that have been obtained >1 day before the initiation of a relevant antibiotic are not used for determination of the causative pathogen
	BC <ceftriaxone iv---------->	BC		Yes	Blood cultures that have been obtained on the day after initiation of a relevant iv antibiotic regimen are not used for determination of the causative pathogen
	BC <amoxiclav iv>	BC <ceftriaxone iv---------->		Yes	Switches in relevant antibiotics occurring on the day of initiation of a relevant antibiotic are incorporated into the initial therapy

Supplementary Table 1 (continued)

Before day 0	Day 0	Day +1	After day +1	Included as infection episode	Remarks
	BC **<amoxiclav iv -------->** **<ceftriaxone iv --->**			Yes	In addition, switches in relevant antibiotics occurring the day after the initiation of a relevant antibiotic are incorporated into the initial therapy
BC	**BC** **<amoxiclav iv -------->** <ceftriaxone iv ---->			Yes	This is however not the case if a blood culture has been obtained on the day before the initiation of a relevant antibiotic
	BC <amoxiclav po---------->**<ceftriaxone iv----------**			Yes	Oral antibiotics (even those targeting Gram-negatives) do not affect inclusion of episodes
	BC <flucloxacillin iv---------->**<ceftriaxone iv --->**			Yes	Other iv antibiotics (i.e. not broad-spectrum β-lactam antibiotics, aminoglycosides or fluoroquinolones) do not affect inclusion of episodes
	BC **<amoxiclav iv---------->** <ciprofloxacin po---------->			Yes	Oral antibiotics (even those targeting Gram-negatives) are not included in the description of the initial antibiotic regimen
	BC <penicillin iv---------->**<ciprofloxacin iv---------->**			Yes	Other iv antibiotics (i.e. not broad-spectrum β-lactam antibiotics, aminoglycosides or fluoroquinolones) are not included in the description of the initial antibiotic regimen
	BC <ceftriaxone iv---------->			No	Blood cultures obtained on a day prior to which relevant antibiotics had been prescribed, are not included as episodes

Supplementary Table 1 (continued)

Before day 0	Day 0	Day +1	After day +1	Included as infection episode	Remarks
	BC <ceftriaxone iv--------> <meropenem iv------------------------->			No	Blood cultures obtained on a day on which an antibiotic regimen consisting of relevant antibiotics is switched, are not included as episodes
	BC <ceftriaxone iv---> <ceftriaxone iv------------------------->			No	If a relevant antibiotic is stopped on the day before the blood culture, and the same antibiotic is reinitiated on the day of the blood culture, the antibiotic is assumed to have been continuously prescribed, and the blood culture is not included as an episode
	BC <ciprofloxacin iv-> **<ceftriaxone iv------------------------->**			Yes	This is, however, not the case, if a different relevant antibiotic is started on the day of the blood culture

Explanation of abbreviations and symbols:

relevant antibiotic	any intravenously administered broad-spectrum β-lactam antibiotic (i.e. not penicillin or flucloxacillin), aminoglycoside, or fluoroquinolone
<	start of antibiotic prescription
>	end of antibiotic prescription
BC	blood culture NOT used for determination of the causative pathogen of the infection episode
BC	blood culture used for determination of the causative pathogen of the infection episode
ceftriaxone iv	antibiotic NOT included in initial antibiotic regimen for the infection episode
ceftriaxone iv	antibiotic included in initial antibiotic regimen for the infection episode
iv	intravenous
po	oral
amoxiclav	amoxicillin/clavulanic acid

Supplementary Table 2. Characteristics of study sites

Hospital description			Study period					Community-onset infection				Hospital-onset infection			
Hospital and location	Category	No. of beds in 2008[a]	Start date	End date	Study duration, months	Definition of resistance[b] against cefotaxime/ ceftriaxone/ ceftazidime	Resources used for retrieval of antibiotics	Total No. of episodes	No. of episodes/ month	No. of 3GCR-E-Bac episodes	3GCR-E-Bac incidence, %	Total No. of episodes	No. of episodes/ month	No. of 3GCR-E-Bac episodes	3GCR-E-Bac incidence, %
Academic Medical Center - Amsterdam	University hospital	1,002	1 Mar 2008	31 Dec 2010	34	As reported to clinic; laboratory used higher CLSI breakpoints for 3GCs until June 2009 [12]; thereafter lowered CLSI breakpoints were used [13]; all ESBL producers were manually converted to 3GC-resistant	1. Medication orders from general wards as registered by pharmacy 2. Medication doses registered in ICU PDMS	3,851	113	27	0.70	1,825	54	22	1.21
Amphia Hospital (Breda)	General hospital	1,368	1 Jan 2008	31 Dec 2010	36	Interpretation of MICs from automated systems according to CLSI 2012 [13]	Medication orders from all wards (including ICU) as registered by pharmacy	6,388	177	23	0.36	1,661	46	16	0.96
Antonius Hospital - Nieuwegein & Utrecht	General hospital	1,113	1 Feb 2010	31 Dec 2010	11	Interpretation of MICs from automated systems according to CLSI 2012 [13]	1. Medication orders from general wards as registered by pharmacy 2. Medication doses registered in ICU PDMS	1,750	159	5	0.29	518	47	3	0.58
Diako-nessen huis (Utrecht)	General hospital	627	1 Oct 2009	30 Sep 2010	12	Interpretation of MICs from automated systems according to CLSI 2012 [13]	1. Medication orders from general wards as registered by pharmacy 2. Medication doses registered in ICU PDMS	1,153	96	7	0.61	271	23	1	0.37

Supplementary Table 2 (continued)

Hospital description			Study period					Community-onset infection				Hospital-onset infection			
Hospital and location	Category	No. of beds in 2008[a]	Start date	End date	Study duration, months	Definition of resistance[b] against cefotaxime/ ceftriaxone/ ceftazidime	Resources used for retrieval of antibiotics	Total No. of episodes	No. of episodes/ month	No. of 3GCR-E-Bac episodes	3GCR-E-Bac incidence, %	Total No. of episodes	No. of episodes/ month	No. of 3GCR-E-Bac episodes	3GCR-E-Bac incidence, %
Erasmus Medical Center - Rotterdam	University hospital	1,320	1 Jan 2010	31 Dec 2010	12	Interpretation of MICs from automated systems according to CLSI 2012 [13]	1. Medication orders from general wards as registered by pharmacy 2. Medication doses registered in ICU PDMS	1,482	124	3	0.20	1,002	84	8	0.80
Gelre Hospitals[c] - Apeldoorn & Zutphen	General hospital	961	1 Oct 2009	30 Sep 2010	12	Interpretation of MICs from automated systems according to CLSI 2012 [13]	Medication orders from general wards (excluding ICU) as registered by pharmacy	967	81	4	0.41	213	18	1	0.47
Tergooi - Hilversum & Blaricum	General hospital	935	1 Jan 2008	31 Dec 2010	36	Interpretation of MICs from automated systems according to CLSI 2012 [13]	Medication orders from all wards (including ICU) as registered by pharmacy	3,693	103	6	0.16	813	23	6	0.74
University Medical Center Utrecht - Utrecht	University hospital	1,262	1 Jan 2008	31 Dec 2010	36	Interpretation of MICs from automated systems according to CLSI 2012 [13]	1. Medication orders from general wards as registered in UPOD database[d] 2. Medication doses registered in ICU PDMS	3,222	90	15	0.47	1,807	50	25	1.38
			Total		**189**			**22,506**	**119**	**90**	**0.40**	**8,110**	**43**	**82**	**1.01**

Abbreviations: 3GC, third-generation cephalosporin-resistant; 3GCR-E-Bac, third-generation cephalosporin-resistant Enterobacterales bacteremia; ICU, intensive care unit; MIC, minimum inhibitory concentration; PDMS, patient data management system.

[a] Source: National Institute for Public Health and the Environment (RIVM) [11].

[b] Resistance includes both intermediate and resistant. Enterobacterales were considered 3GC-resistant if resistant to one or more of the three 3GCs.

[c] ICU department was excluded from this hospital.

[d] UPOD is an infrastructure of relational databases comprising data on patient characteristics, hospital discharge diagnoses, medical procedures, medication orders and laboratory tests for all patients treated at the University Medical Center Utrecht since 2004. UPOD data acquisition and management is in accordance with current regulations concerning privacy and ethics. The structure and content of UPOD have been described in more detail elsewhere [14].

Supplementary Table 3. Definitions of study terms and included predictors

Variable	Definition
Relevant antibiotics	Any intravenously administered broad-spectrum β-lactam antibiotic (i.e. not penicillin or flucloxacillin), aminoglycoside, or fluoroquinolone
Initial antibiotics of infection episode	Relevant antibiotics prescribed on the day of a blood culture that fulfils inclusion criteria (see **Supplementary Table 1**), or the day after.
Causative pathogens of infection episode	Combined results of blood cultures fulfilling inclusion criteria (see **Supplementary Table 1**), supplemented with blood cultures obtained the day after if initial antibiotics were only prescribed on the day after the first of these blood cultures. If common skin contaminants were isolated (i.e. *Corynebacterium* spp., *Bacillus* spp., *Propionibacterium* spp., coagulase-negative staphylococci, viridans group streptococci, *Aerococcus* spp., *Micrococcus* spp. [15]), two separately obtained blood cultures with these isolates were required to qualify as causative pathogen.
Infection onset	The moment that decision was made to prescribe (the first of) the antibiotics qualifying as initial antibiotics.
Hospital ward (at infection onset)	Treating specialism at infection onset, except if this was in the emergency room. In the latter case, emergency room was recorded. Originally categorized with 15 categories; during modelling reduced to 4. The following list contains the original categories with the category after reduction for modelling purposes between brackets: internal medicine (internal medicine), oncology (internal medicine), hematology (internal medicine), nephrology (internal medicine), gastroenterology (internal medicine), surgery (surgery), gynecology (surgery), urology (surgery), cardiology (internal medicine), pulmonology (internal medicine), neurology (internal medicine), intensive care unit (intensive care unit), geriatrics (internal medicine), emergency room (emergency room), other (final categorization depended on recorded specification).
Healthcare-associated infection	For community-onset infection cohort only. Patients fulfilling ≥1 of the following criteria: • Intravenous therapy within 30 days prior to infection onset • Wound care within 30 days prior to infection onset • Specialized nursing at home or in a day-care hospital during 30 days prior to infection onset • Any admission to long term care facility during year prior to infection onset • On hemodialysis • Any hospital admission >2 days during 3 months prior to infection onset Adapted from Friedman *et al.* [16]
Admission from long-term care facility	For community-onset infection cohort only. Patients admitted from a nursing home or rehabilitation center.
Hospital admission (prior one year)	Hospital admission of ≥1 night (i.e. day-care excluded).

Supplementary Table 3 (continued)

Variable	Definition
Chronic pulmonary disease	Patients fulfilling ≥1 of the following criteria: • Patients who are dyspnoeic with moderate activity, also including those who are dyspnoeic with light activity, or even at rest. Whether patients are treated or not is not taken into account. • Patients who are dyspnoeic only with attacks (e.g. asthma). • Patients who require constant oxygen, patients with CO_2 retention, patients with a baseline pO_2 below 50 mmHg (6.7 kPa). Adapted from Charlson *et al.* [17]
Diabetes mellitus	Patients treated with insulin or oral hypoglycemic. Adapted from Charlson *et al.* [17]
Liver disease	Patients with cirrhosis and portal hypertension (with or without a history of variceal bleeding). Adapted from Charlson *et al.* [17]
Biliary tract disease	Patients with cholestasis, for example due to recurrent gall stones, malignancies in or around the biliary tract, medication, inherited conditions, pregnancy, primary sclerosing cholangitis, primary biliary sclerosis, chronic pancreatitis.
Solid malignancy – without metastases	Patients with malignancies without documented metastases, but initially treated in the last five years, including breast, colon, lung, and a variety of other tumours. Leukemia and lymphoma are not included. Adapted from Charlson *et al.* [17]
Solid malignancy – metastasized	Patients with metastatic solid tumours, including breast, lung, colon and other tumours. Adapted from Charlson *et al.* [17]
Hematological malignancy	Patients with leukemia, lymphoma or multiple myeloma. Adapted from Charlson *et al.* [17]
Renal disease	Patients on dialysis, those who had a transplant, those with uremia (renal failure), and those with serum creatinines of >265 µmol/L (documented as chronic renal disease in medical file). Adapted from Charlson *et al.* [17]
Hemodialysis	Patients on chronic hemodialysis.
Immuno-suppressant use	Patients chronically treated with corticosteroids, and those treated with chemotherapeutics, high-dose corticosteroids, or other immunosuppressive drugs in the 30 days prior to infection onset.
Neutropenia (at infection onset)	Patients with $<500\times10^9$ neutrophils/L at infection onset.
Solid organ transplant	Patients with a history of any solid organ transplant.
Stem cell transplant	Patients with a history of a stem cell transplant.
Recurrent urinary tract infection	Patients with a history of ≥3 urinary tract infections for which antibiotics were prescribed.

Supplementary Table 3 (continued)

Variable	Definition
Obstructive urinary disease	Patients with conditions such as stones in the urinary tract, malignancies in or around the urinary tract, benign prostate hypertrophy, inherited conditions (e.g. urethral valves), or hydronephrosis of pregnancy.
Urological procedure (prior 30 days)	Patients having had procedures for which a cystoscope was used, such as cystoscopy, transurethral prostatectomy and insertion of a JJ catheter. In addition, insertion of a suprapubic catheter and nephrostomy are included.
Surgical procedure (prior 30 days)	Patient having been in an operating room, but not having had a urological procedure, simple procedures (such as insertion of a central venous catheter, incision and drainage), nor interventional cardiology or radiology.
Endoscopic procedure (prior two days)	Patients having had esophagogastroduodenoscopy, endoscopic retrograde cholangiopancreatography, sigmoidoscopy, colonoscopy, or bronchoscopy. Transesophageal echocardiograms are excluded.
Central vascular catheter (at infection onset)	Patients with any form of central venous catheter or arterial catheter at infection onset, including Hickman catheters, peripherally inserted central catheters, Port-a-Caths, catheters used for renal replacement therapy.
Urinary catheter (at infection onset)	Patients with an indwelling urinary catheter, ureteral stent, suprapubic catheter or nephrostomy at infection onset.
Other catheter/drain (at infection onset)	Patients with a surgical drain (including negative pressure wound therapy), neurosurgical drain, chest tube, or percutaneous endoscopic gastrostomy tube at infection onset.
Signs of hypoperfusion (at infection onset)	Patients with infection associated at onset with organ dysfunction, hypoperfusion, or hypotension (≥1 of the following criteria). *Organ dysfunction variables:* • Arterial hypoxemia (PaO_2/FiO_2 <300) • Acute oliguria (urine output <0.5 mL/(kg x h) or 45 mmol/L for at least 2 hrs) • Creatinine >2.0 mg/dL • Coagulation abnormalities (INR >1.5 or aPTT >60 s) • Thrombocytopenia (platelet count <$100x10^3/\mu L$) • Hyperbilirubinemia (plasma total bilirubin >2.0 mg/dL or >35 mmol/L) *Tissue perfusion variables:* • Hyperlactatemia (>2 mmol/L) *Hemodynamic variables:* • Arterial hypotension (systolic blood pressure <90 mmHg, mean arterial pressure <70 mmHg, or decrease in systolic blood pressure >40 mmHg) Adapted from: Levy *et al.* [18]

Supplementary Table 3 (continued)

Variable	Definition
Suspected source of infection (at infection onset)	The working diagnosis recorded in the medical chart. In the absence of an identifiable working diagnosis, the infection source was classified as 'unknown'. Originally recorded with 11 categories: primary bacteremia, urinary tract infection, intra-abdominal infection, lower respiratory tract infection, meningitis, catheter-related infection, surgical wound infection, other skin and soft tissue infection, arthritis/osteomyelitis, unknown, and other. During modelling urinary tract infection, intra-abdominal infection, and lower respiratory tract infection were retained, primary bacteremias were added to the unknown category, and the remainder was categorized as other.
Prior identification of 3GCR-E (prior one year)	Patients with any prior culture with Enterobacterales reported as 3GC-resistant to the clinic, obtained within the year prior to onset, and with results available at infection onset

Abbreviations: 3GC, third-generation cephalosporin; 3GCR-E, third-generation cephalosporin-resistant Enterobacterales; aPTT, activated partial thromboplastin time; INR, international normalized ratio.

Supplementary Table 4. Full clinical characteristics of cases and controls from the community-onset and hospital-onset cohorts

Predictor[a]	Community-onset infection			Hospital-onset infection		
	Cases (*N* = 90)[b], *n/N* with data (%)	Controls (*N* = 360)[c], *n/N* with data (%)	OR (95% CI)[d]	Cases (*N* = 82)[b], *n/N* with data (%)	Controls (*N* = 328)[c], *n/N* with data (%)	OR (95% CI)[d]
Female gender	39/90 (43)	158/360 (44)	0.98 (0.61–1.56)	32/82 (39)	129/328 (39)	0.99 (0.60–1.62)
Age in years, median (IQR)	69 (61–76)[e]	63 (50–76)[e]	1.02 (1.00–1.03)	64 (55–73)	64 (52–75)	1.00 (0.99–1.02)
Hospital ward (at infection onset)						
Emergency room	58/90 (64)[f]	216/360 (60)[f]	1.21 (0.75–1.96)	0/82 (0)[f]	1/328 (0)[f]	
Internal medicine	18/90 (20)[f]	78/360 (22)[f]	0.90 (0.51–1.61)	31/82 (38)[f]	193/328 (59)[f]	0.42 (0.26–0.69)
Surgery	11/90 (12)[f]	40/360 (11)[f]	1.11 (0.55–2.27)	33/82 (40)[f]	82/328 (25)[f]	2.01 (1.21–3.34)
Intensive care unit	3/90 (3)[f]	26/360 (7)[f]	0.44 (0.13–1.50)	18/82 (22)	52/328 (16)	1.49 (0.82–2.73)
Healthcare-associated infection	50/90 (56)[e]	141/353 (40)[e]	1.81 (1.13–2.89)	[g]	[g]	
Admission from long-term care facility	9/90 (10)	16/353 (4)	2.09 (0.89–4.95)	[g]	[g]	
Hospital admission (prior one year)	60/87 (69)	186/353 (53)	1.97 (1.20–3.23)	45/81 (56)	129/318 (41)	1.85 (1.13–3.02)
Length of hospital stay prior to infection in days, median (IQR)	[g]	[g]		20 (10–48)[e]	11 (6–19)[e]	1.03 (1.02–1.04)
Chronic pulmonary disease	8/90 (9)	68/358 (19)	0.42 (0.19–0.91)	10/81 (12)	39/328 (12)	1.09 (0.52–2.29)
Diabetes mellitus	28/90 (31)[e]	83/358 (23)[e]	1.48 (0.89–2.46)	16/81 (20)	62/328 (19)	1.10 (0.60–2.03)
Liver disease	2/90 (2)	5/358 (1)	1.42 (0.27–7.37)	4/81 (5)	4/328 (1)	4.62 (1.14–18.78)
Biliary tract disease	2/90 (2)	4/358 (1)	1.76 (0.32–9.83)	1/81 (1)	4/328 (1)	1.33 (0.15–11.43)
Any solid malignancy[h]	16/90 (18)	60/358 (17)	1.07 (0.58–1.97)	25/81 (31)[e]	70/328 (21)[e]	1.67 (0.97–2.87)
Without metastases	9/90 (10)[i]	34/358 (10)[i]	1.06 (0.49–2.30)	17/81 (21)[i]	45/328 (14)[i]	1.71 (0.92–3.18)
Metastasized	7/90 (8)[i]	26/358 (7)[i]	1.07 (0.45–2.55)	9/81 (11)[i]	25/328 (8)[i]	1.56 (0.70–3.49)
Hematological malignancy	11/90 (12)	28/358 (8)	1.62 (0.77–3.40)	9/81 (11)	44/328 (13)	0.85 (0.40–1.82)
Renal disease	13/90 (14)[e]	21/358 (6)[e]	2.54 (1.22–5.27)	14/81 (17)[e]	17/328 (5)[e]	3.98 (1.87–8.45)
Hemodialysis	1/90 (1)	5/353 (1)	0.55 (0.06–4.76)	[g]	[g]	
Immunocompromised[j]	27/87 (31)[e]	62/356 (17)[e]	2.03 (1.19–3.46)	16/80 (20)	76/323 (24)	0.85 (0.47–1.56)
Immunosuppressant use	23/90 (26)[i]	59/358 (16)[i]	1.71 (0.98–2.96)	16/81 (20)[i]	74/328 (23)[i]	0.89 (0.49–1.62)
Neutropenia (at infection onset)	7/87 (8)[i]	14/357 (4)[i]	2.09 (0.81–5.40)	5/81 (6)[i]	35/323 (11)[i]	0.53 (0.20–1.42)

Supplementary Table 4 (continued)

Predictor[a]	Community-onset infection			Hospital-onset infection		
	Cases (*N* = 90)[b], *n/N* with data (%)	Controls (*N* = 360)[c], *n/N* with data (%)	OR (95% CI)[d]	Cases (*N* = 82)[b], *n/N* with data (%)	Controls (*N* = 328)[c], *n/N* with data (%)	OR (95% CI)[d]
Any transplant[h]	14/90 (16)[k]	22/358 (6)[k]	2.67 (1.31–5.45)	15/81 (18)[e]	23/327 (7)[e]	3.10 (1.54–6.23)
Solid organ transplant	11/90 (12)[i]	12/358 (3)[i]	3.71 (1.58–8.70)	9/81 (11)[i]	14/327 (4)[i]	2.93 (1.23–6.99)
Stem cell transplant	3/90 (3)	10/358 (3)	1.13 (0.30–4.21)	7/81 (9)[i]	9/327 (3)[i]	3.50 (1.26–9.68)
Urological patient[h]	25/90 (28)[e]	40/357 (11)[e]	2.96 (1.68–5.22)	5/81 (6)[k]	21/323 (6)[k]	1.05 (0.39–2.83)
Recurrent urinary tract infection	16/90 (18)[i]	25/358 (7)[i]	2.81 (1.43–5.53)	2/81 (2)	8/324 (2)	0.96 (0.20–4.63)
Obstructive urinary disease	5/90 (6)[i]	9/358 (2)[i]	2.13 (0.70–6.52)	0/81 (0)	6/328 (2)	Not available
Urological procedure (prior 30 days)	7/90 (8)[i]	7/357 (2)[i]	4.01 (1.36–11.79)	3/82 (4)	7/326 (2)	1.71 (0.43–6.77)
Surgical procedure (prior 30 days)	4/90 (4)	34/357 (10)	0.43 (0.15–1.24)	37/82 (45)[e]	116/327 (36)[e]	1.50 (0.92–2.46)
Endoscopic procedure (prior two days)	1/90 (1)	4/358 (1)	0.84 (0.09–7.60)	6/82 (7)	9/326 (3)	2.65 (0.92–7.66)
Central vascular catheter (at infection onset)	5/89 (6)	20/344 (6)	0.93 (0.34–2.55)	46/75 (61)[e]	106/299 (36)[e]	2.72 (1.62–4.57)
Urinary catheter (at infection onset)	22/88 (25)	61/342 (18)	1.47 (0.84–2.56)	38/71 (54)	142/291 (49)	1.21 (0.73–2.00)
Other catheter/drain (at infection onset)	4/90 (4)	15/347 (4)	0.89 (0.29–2.73)	17/74 (23)	72/300 (24)	0.99 (0.54–1.80)
Signs of hypoperfusion (at infection onset)	12/86 (14)	35/340 (10)	1.46 (0.73–2.93)	25/77 (32)[e]	38/296 (13)[e]	2.82 (1.57–5.06)
Suspected source of infection (at infection onset)						
Urinary tract infection or intra-abdominal infection[h]	55/90 (61)[k]	94/359 (26)[k]	4.44 (2.73–7.22)	26/80 (32)	46/325 (14)	3.00 (1.71–5.26)
Urinary tract infection	41/90 (46)[e]	48/359 (13)[e]	5.44 (3.25–9.11)	12/80 (15)[i]	20/325 (6)[i]	2.85 (1.35–6.04)
Intra-abdominal infection	14/90 (16)	46/359 (13)	1.26 (0.66–2.41)	14/80 (18)[i]	26/325 (8)[i]	2.42 (1.20–4.89)
Lower respiratory tract infection	8/90 (9)[e]	111/359 (31)[e]	0.22 (0.10–0.46)	4/80 (5)[e]	86/325 (26)[e]	0.14 (0.05–0.40)
Other infection	5/90 (6)	42/359 (12)	0.45 (0.17–1.16)	11/80 (14)	35/325 (11)	1.37 (0.66–2.85)
Unknown	22/90 (24)[l]	112/359 (31)[l]	0.71 (0.42–1.21)	39/80 (49)[l]	159/325 (49)[l]	0.98 (0.60–1.60)
Prior identification of 3GCR-E (prior one year)	22/90 (24)[e]	9/359 (2)[e]	11.82 (5.25–26.63)	29/82 (35)[e]	16/328 (5)[e]	10.67 (5.41–21.03)

Supplementary Table 4 (continued)

Predictor[a]	Community-onset infection			Hospital-onset infection		
	Cases (*N* = 90)[b], *n/N* with data (%)	**Controls (*N* = 360)[c], *n/N* with data (%)**	**OR (95% CI)[d]**	**Cases (*N* = 82)[b], *n/N* with data (%)**	**Controls (*N* = 328)[c], *n/N* with data (%)**	**OR (95% CI)[d]**
Any use of antibiotics (prior two months)[h]	51/85 (60)[e]	140/346 (40)[e]	2.22 (1.37–3.60)	68/82 (83)	228/324 (70)	2.02 (1.08–3.77)
Cephalosporins or fluoroquinolones[h]	28/85 (33)[i]	66/346 (19)[i]	2.12 (1.26–3.55)	58/82 (71)	165/323 (51)	2.27 (1.34–3.84)
Cephalosporins	14/86 (16)[i]	33/351 (9)[i]	1.91 (0.99–3.68)	49/82 (60)[e]	114/322 (35)[e]	2.67 (1.62–4.39)
Fluoroquinolones	17/85 (20)[i]	44/346 (13)[i]	1.81 (0.98–3.35)	25/82 (30)	81/322 (25)	1.28 (0.75–2.18)
Carbapenems	4/86 (5)[i]	2/351 (1)[i]	4.95 (1.02–24.02)	12/82 (15)	29/321 (9)	1.66 (0.81–3.42)
Other β-lactams	25/85 (29)[i]	72/345 (21)[i]	1.65 (0.97–2.80)	29/82 (35)	110/320 (34)	1.04 (0.62–1.72)
Aminoglycosides, macrolides or other antibiotics[h]	33/85 (39)[i]	73/345 (21)[i]	2.31 (1.39–3.84)	56/82 (68)[k]	131/323 (41)[k]	3.11 (1.85–5.21)
Aminoglycosides	4/86 (5)[i]	13/351 (4)[i]	1.21 (0.40–3.67)	13/81 (16)	35/319 (11)	1.49 (0.75–2.98)
Macrolides	3/86 (4)[i]	18/347 (5)[i]	0.75 (0.23–2.44)	17/81 (21)	37/320 (12)	2.01 (1.06–3.82)
Other antibiotics	29/85 (34)[i]	57/345 (16)[i]	2.57 (1.51–4.39)	49/82 (60)	98/323 (30)	3.38 (2.04–5.58)
Selective digestive/oropharyngeal decontamination (prior two months)	1/86 (1)[k]	2/351 (1)[k]	1.63 (0.24–11.12)	10/82 (12)	26/325 (8)	1.56 (0.72–3.40)
At risk of 3GCR-E-Bac according to *two-predictor model*[m]	46/86 (54)[n]	71/347 (20)[n]	4.32 (2.63–7.09)	65/82 (79)[n]	168/323 (52)[n]	3.46 (1.94–6.17)

Abbreviations: 3GCR-E, third-generation cephalosporin-resistant Enterobacterales; 3GCR-E-Bac, third-generation cephalosporin-resistant Enterobacterales bacteremia; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

[a] See **Supplementary Table 3** for definitions used.

[b] Patients with 3GCR-E-Bac.

[c] Sample of patients without bacteremia or with blood cultures yielding non-resistant Enterobacterales, other bacteria or fungi.

[d] OR calculated with 20 imputed datasets, combined by means of Rubin's rules.

[e] One of ten predictors selected during the first step of model creation.

[f] Predictor not considered for model construction purposes because of expected problems in generalization to other settings. This implies that is was neither used for univariable preselection during the bootstrapping procedure.

[g] Predictor not recorded for this setting.

[h] Aggregated variable combining indented variables below.

[i] Predictor not considered for model construction purposes (see [f] for implications) because of aggregation.

[j] Aggregated variable combining *immunosuppressant use*, *neutropenia (at infection onset)*, and *solid organ transplant*.

[k] Predictor only shown for comparison with other cohort and not considered for model construction purposes (see [f] for implications).

[l] Predictor not considered for model construction purposes (see [f] for implications) because it was used as reference category.

[m] Aggregated variable combining *use of cephalosporins or fluoroquinolones (prior two months)*, and *prior identification of 3GCR-E (prior one year)*.

[n] Predictor only shown to evaluate performance of *two-predictor model* and not considered for model construction purposes (see [f] for implications).

Supplementary Table 5. Initial antibiotics of infection episodes

	Community-onset infection		Hospital-onset infection	
	Cases[a] (*N* = 90), *n* (%)	**Controls[b] (*N* = 360), *n* (%)**	**Cases[a] (*N* = 82), *n* (%)**	**Controls[b] (*N* = 328), *n* (%)**
Monotherapy				
2nd/3rd gen cephalosporin	25 (28)	121 (34)	16 (20)	121 (37)
BLBLI	5 (6)	85 (24)	6 (7)	58 (18)
Carbapenem	16 (18)	9 (3)	20 (24)	30 (9)
Fluoroquinolone	2 (2)	14 (4)	7 (9)	13 (4)
Broad-spectrum penicillin		11 (3)	2 (2)	1 (0)
Aminoglycoside		6 (2)	4 (5)	17 (5)
1st gen cephalosporin		4 (1)		1 (0)
Aztreonam	1 (1)			
Combination therapy				
β-Lactam with aminoglycoside	15 (17)	51 (14)	6 (7)	38 (12)
β-Lactam with fluoroquinolone	4 (4)	14 (4)	4 (5)	13 (4)
Switches within initial antibiotics	22 (24)	45 (13)	17 (21)	36 (11)

Table includes relevant antibiotics only (defined as broad-spectrum β-lactams, fluoroquinolones and aminoglycosides).
Abbreviations: BLBLI, β-lactam/β-lactamase inhibitor combination; gen, generation

[a] Patients with third-generation cephalosporin-resistant Enterobacterales bacteremia (3GCR-E-Bac).

[b] Sample of patients without bacteremia or with blood cultures yielding non-resistant Enterobacterales, other bacteria or fungi.

Supplementary Table 6. Causative pathogens of infection episodes

	Community-onset infection		Hospital-onset infection	
	Cases[a] (*N* = 90), *n* (%)	**Controls[b] (*N* = 360), *n* (%)**	**Cases[a] (*N* = 82), *n* (%)**	**Controls[b] (*N* = 328), *n* (%)**
No bacteremia[c]		309 (86)		272 (83)
Monomicrobial bacteremia				
Escherichia coli	58 (64)	17 (5)	25 (30)	11 (3)
Klebsiella pneumoniae	6 (7)	5 (1)	11 (13)	1 (0)
Enterobacter cloacae	6 (7)	1 (0)	12 (15)	
Enterococcus spp.		4 (1)		9 (3)
Staphylococcus aureus		4 (1)		2 (1)
Pseudomonas spp.		2 (1)		4 (1)
Other Enterobacterales	8 (9)	2 (1)	17 (21)	3 (1)
Others		13 (4)		16 (5)
Polymicrobial bacteremia[d]	12 (13)	3 (1)	17 (21)	10 (3)

[a] Patients with third-generation cephalosporin-resistant Enterobacterales bacteremia (3GCR-E-Bac).

[b] Sample of patients without bacteremia or with blood cultures yielding non-resistant Enterobacterales, other bacteria or fungi.

[c] Includes instances of single isolation of potential skin contaminants (*Corynebacterium* spp., *Bacillus* spp., *Propionibacterium* spp., coagulase-negative staphylococci, viridans group streptococci, *Aerococcus* spp., *Micrococcus* spp. [15]).

[d] Cases with polymicrobial bacteremia may include isolates not being Enterobacterales.

Supplementary Table 7. Performance of regression model in community-onset infection

	Predicted probability															
	0	**0.001**	**0.002**	**0.003**	**0.004**	**0.005**	**0.006**	**0.007**	**0.008**	**0.009**	**0.010**	**0.011**	**0.012**	**0.013**	**0.014**	**0.015**	**0.107[a]**
	Characteristics of interval [prior value, current value)																
Proportion of cohort, %		10.9	33.1	20.9	11.7	7.4	2.4	2.7	1.0	1.4	0.7	1.9	0.6	1.0	1.0	0.3	3.1
Probability of 3GCR-E-Bac, %		0.0	0.1	0.2	0.3	0.5	0.6	1.1	2.0	1.2	2.2	1.1	1.5	1.0	0.5	1.3	3.3
	Characteristics of cutoff ≥ current value for classification as *at risk of 3GCR-E-Bac*																
TePR, %		89.1	56.1	35.1	23.5	16.1	13.7	11.1	10.0	8.6	7.9	6.0	5.4	4.4	3.5	3.1	0.0
Sensitivity, %		99.9	89.2	80.2	71.2	61.5	57.9	50.8	45.6	41.4	37.7	32.3	30.2	27.8	26.7	25.6	0.1
Specificity, %		10.9	44.1	65.1	76.7	84.1	86.5	89.1	90.1	91.5	92.2	94.1	94.7	95.7	96.6	97.0	100.0
Positive predictive value, %		0.4	0.6	0.9	1.2	1.5	1.7	1.8	1.8	1.9	1.9	2.2	2.2	2.5	3.1	3.3	100.0
Negative predictive value,%		100.0	99.9	99.9	99.8	99.8	99.8	99.8	99.8	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.6

These values (means of 20 imputed datasets) reflect performance of the regression model after correction of coefficients for the sampling fraction of the controls and model shrinkage for optimism, and have been extrapolated to the full community-onset cohort. Values of performance parameters themselves have, however, not been corrected for overoptimism.

Abbreviations: 3GCR-E-Bac, third-generation cephalosporin-resistant Enterobacterales bacteremia; TePR, test positivity rate.

[a] Maximum predicted probability within the study sample.

Supplementary Table 8. Performance of regression model in hospital-onset infection

	Predicted probability															
	0	**0.004**	**0.008**	**0.012**	**0.016**	**0.020**	**0.024**	**0.028**	**0.032**	**0.036**	**0.040**	**0.044**	**0.048**	**0.052**	**0.056**	**0.060**	**0.239[a]**
	Characteristics of interval [prior value, current value)															
Proportion of cohort, %		39.9	26.4	14.5	7.5	3.8	1.1	1.9	1.5	0.6	0.1	0.1	0.1	0.0	0.0	0.3	2.3
Probability of 3GCR-E-Bac, %		0.1	0.4	1.1	1.5	2.5	5.9	1.0	1.7	4.8	6.2	24.2	60.9	100.0	27.3	3.6	10.8
	Characteristics of cutoff ≥ current value for classification as *at risk of 3GCR-E-Bac*															
TePR, %		60.1	33.8	19.2	11.8	8.0	6.9	5.0	3.6	3.0	2.9	2.8	2.7	2.7	2.6	2.3	0.0
Sensitivity, %		95.1	85.0	70.1	59.3	50.2	44.0	42.2	39.8	37.0	36.3	34.9	28.1	25.9	25.3	24.1	0.1
Specificity, %		40.2	66.8	81.3	88.8	92.5	93.5	95.3	96.8	97.4	97.5	97.5	97.6	97.6	97.6	97.9	100.0
Positive predictive value, %		1.6	2.6	3.8	5.2	6.5	6.6	8.6	11.4	12.8	13.1	12.8	10.8	10.0	9.9	10.8	100.0
Negative predictive value, %		99.9	99.8	99.6	99.5	99.4	99.4	99.4	99.4	99.3	99.3	99.3	99.2	99.2	99.2	99.2	99.0

These values (means of 20 imputed datasets) reflect performance of the regression model after correction of coefficients for the sampling fraction of the controls and model shrinkage for optimism, and have been extrapolated to the full hospital-onset cohort. Values of performance parameters themselves have, however, not been corrected for overoptimism.

Abbreviations: 3GCR-E-Bac, third-generation cephalosporin-resistant Enterobacterales bacteremia; TePR, test positivity rate.

[a] Maximum predicted probability within the study sample.

Supplementary Table 9. Original and optimism-corrected community-onset regression model

Predictor	Original model		Optimism-corrected model	
	β coefficient	OR (95% CI)	β coefficient	OR (95% CI)
Intercept	-7.632		-7.248	
Prior identification of 3GCR-E (prior one year)	2.355	10.53 (4.26–26.08)	1.963	7.12 (2.88–17.62)
Suspected source of infection: Urinary tract infection	1.297	3.66 (2.04–6.57)	1.081	2.95 (1.64–5.29)
Immunocompromised	0.590	1.80 (0.96–3.39)	0.491	1.63 (0.87–3.08)
Any use of antibiotics (prior two months)	0.377	1.46 (0.83–2.55)	0.314	1.37 (0.78–2.39)
Age (per one year of age)	0.022	1.02 (1.01–1.04)	0.018	1.02 (1.00–1.04)
Suspected source of infection: Lower respiratory tract infection	-1.075	0.34 (0.15–0.78)	-0.896	0.41 (0.18–0.94)

The original model was pooled over 20 imputed datasets reflecting 450 infection episodes (of which 90 cases with 3GCR-E bacteremia (3GCR-E-Bac)), and was subsequently corrected for the sampling fraction of controls. The optimism-corrected model was derived by multiplication of the β coefficients with a shrinkage factor (0.834), followed by re-estimation of the intercept.

Abbreviations: 3GCR-E, third-generation cephalosporin-resistant Enterobacterales; CI, confidence interval; OR, odds ratio.

Supplementary Table 10. Original and optimism-corrected hospital-onset regression model

Predictor	Original model		Optimism-corrected model	
	β coefficient	OR (95% CI)	β coefficient	OR (95% CI)
Intercept	-6.210		-5.807	
Renal disease	1.743	5.71 (2.24–14.55)	1.372	3.94 (1.55–10.05)
Prior identification of 3GCR-E (prior one year)	1.718	5.57 (2.41–12.89)	1.353	3.87 (1.67–8.95)
Any solid malignancy	0.917	2.50 (1.29–4.87)	0.722	2.06 (1.06–4.01)
Signs of hypoperfusion (at infection onset)	0.646	1.91 (0.91–4.01)	0.509	1.66 (0.79–3.49)
Surgical procedure (prior 30 days)	0.564	1.76 (0.94–3.28)	0.444	1.56 (0.84–2.91)
Central vascular catheter (at infection onset)	0.533	1.70 (0.88–3.31)	0.420	1.52 (0.78–2.95)
Use of cephalosporins (prior two months)	0.527	1.69 (0.90–3.17)	0.415	1.51 (0.81–2.83)
Length of hospital stay prior to infection (per day)	0.014	1.01 (1.00–1.03)	0.011	1.01 (1.00–1.03)
Suspected source of infection: Lower respiratory tract infection	-2.196	0.11 (0.04–0.35)	-1.729	0.18 (0.06–0.56)

The original model was pooled over 20 imputed datasets reflecting 410 infection episodes (of which 82 cases with 3GCR-E bacteremia (3GCR-E-Bac)), and was subsequently corrected for the sampling fraction of controls. The optimism-corrected model was derived by multiplication of the β coefficients with a shrinkage factor (0.788), followed by re-estimation of the intercept.

Abbreviations: 3GCR-E, third-generation cephalosporin-resistant Enterobacterales; CI, confidence interval; OR, odds ratio.

Supplementary Table 11. Original and optimism-corrected *c*-statistics

	***C*-statistic (95% CI)**	
	Original	**Optimism-corrected**
Community-onset infection		
Regression model	0.808 (0.756–0.855)	0.775 (0.705–0.839)
Score	0.807 (0.756–0.855)	[a]
Hospital-onset infection		
Regression model	0.842 (0.793–0.886)	0.811 (0.742–0.873)
Score	0.842 (0.794–0.887)	[a]

Presented values are means of 20 imputed datasets. 95% CIs may differ slightly from those in **Supplementary Table 12**, as they were obtained in separate bootstrap procedures.

Abbreviations: CI, confidence interval.

[a] Value could not be calculated.

Supplementary Table 12. Performance of scoring systems and *two-predictor models* in subgroups

		Community-onset infection			Hospital-onset infection		
		Full cohort	**Subgroup: pneumonia[a]**	**Subgroup: other[a]**	**Full cohort**	**Subgroup: pneumonia[a]**	**Subgroup: other[a]**
Proportion of full cohort, % (95% CI)[b]			30.9 (26.2–35.3)	69.1 (64.7–73.8)		26.4 (21.8–31.1)	73.6 (68.9–78.2)
Prevalence of 3GCR-E-Bac, % (95% CI)[b]		0.40 (0.32–0.49)	0.12 (0.04–0.21)	0.53 (0.41–0.66)	1.03 (0.83–1.30)	0.19 (0.04–0.41)	1.33 (1.04–1.66)
		Two-predictor model					
Sensitivity, % (95% CI)		53.9 (43.5–63.9)	73.8 (38.9–100)	52.0 (41.0–62.5)	79.3 (69.5–87.8)	75.0 (50.0–100)	79.5 (70.0–87.8)
TePR, % (95% CI)		21.5 (17.4–25.7)	17.8 (11.1–25.6)	23.1 (18.2–28.4)	52.8 (47.7–58.2)	47.7 (37.5–58.9)	54.6 (48.4–60.8)
		Scoring system					
C-statistic (95% CI)		0.807 (0.756–0.850)	0.817[c] (0.672–0.933)	0.772 (0.712–0.827)	0.842 (0.794–0.888)	0.753[d] (0.520–1.000)	0.812 (0.753–0.867)
Score cutoff 100/50[e]	Sensitivity, % (95% CI)	72.3 (62.3–81.2)	37.5 (0.0–75.0)	75.7 (66.0–84.4)	93.9 (87.8–98.8)	75.0 (0.0–100)	94.9 (89.3–98.8)
	TePR, % (95% CI)	22.4 (18.2–26.7)	2.2 (0.0–5.3)	31.4 (26.0–37.0)	54.0 (48.6–59.1)	11.6 (5.6–18.7)	69.2 (63.4–74.9)
Score cutoff 120/110[f]	Sensitivity, % (95 %CI)	54.3 (44.3–64.3)	25.0 (0.0–60.0)	57.1 (46.7–67.5)	81.5 (73.0–89.2)	25.0 (0.0–100)	84.4 (76.1–92.0)
	TePR, % (95% CI)	12.8 (9.7–16.4)	2.2 (0.0–5.3)	17.6 (13.0–22.5)	27.0 (22.3–31.8)	2.6 (0.0–6.3)	35.8 (29.7–41.6%)
		Score cutoff 120/110[f] compared to *two-predictor model*					
Relative TePR reduction, % (95% CI)[g]		40.2 (20.9–56.0)	87.8 (71.1–99.9)	23.9 (-2.8–44.6)	48.8 (39.4–57.9)	94.5 (86.6–100)	34.4 (22.8–45.9)
Resulting absolute TePR reduction within full cohort (95% CI)[h]		8.6 (3.9–13.5)	4.8 (2.8–7.3)	3.8 (-0.4–8.2)	25.7 (19.7–32.1)	11.9 (8.6–15.6)	13.8 (8.4–19.6)

This table contrasts performance of two cutoffs of the scoring systems (used for classification as at risk of (3GCR-E-Bac) to the performance of the *two-predictor models*. This is done separately for both community-onset and hospital-onset infection, and two subgroups within each setting, based on the variable *suspected source of infection*. Presented values are means of 20 imputed datasets and have not been corrected for overoptimism. 95% CIs may differ slightly from those in **Supplementary Tables 11 and 13**, as they were obtained in separate bootstrap procedures.

Abbreviations: 3GCR-E-Bac, third-generation cephalosporin-resistant Enterobacterales bacteremia; CI, confidence interval; TePR, test-positivity rate.

[a] Defined by the variable *suspected source of infection*: the pneumonia subgroup contains all episodes designated *lower respiratory tract infection*, and the remaining sources of infection are grouped in the other subgroup.

[b] Unlike other 95% CIs, 95% CIs for these parameters were obtained in a bootstrap procedure without a fixed case:control ratio. See the section **Confidence intervals** in this **Supplementary Material** for a description.

[c] Cases within this subgroup (patients with 3GCR-E-Bac) had scores of 29–35–41–47–59–117–119–123, whereas 75% of controls had scores up to 42, and 90% of controls had scores up to 60.

[d] Cases within this subgroup (patients with 3GCR-E-Bac) had scores of -151–59–89–237, whereas 75% of controls had scores up to -4, and 90% of controls had scores up to 55.

[e] The score above which patients are classified as *at risk of 3GCR-E-Bac*. It is chosen such that the resulting sensitivity is as close as possible to the sensitivity of the two-predictor model within the full cohort. The first value is the cutoff used in community-onset infection, the second value is used in hospital-onset infection.
[f] The score above which patients are classified as *at risk of 3GCR-E-Bac*. It is chosen such that the resulting TePR is as close as possible to the TePR of the two-predictor model within the full cohort. The first value is the cutoff used in community-onset infection, the second value is used in hospital-onset infection.
[g] Calculated as the relative difference between TePR with the score cutoff mentioned and TePR with the *two-predictor model*.
[h] Calculated as the absolute difference between TePR with the score cutoff mentioned and TePR with the *two-predictor model*, which in case of a subgroup, is subsequently multiplied by the proportion of the full cohort within that subgroup.

Supplementary Table 13. Expected optimism when selecting cutoffs for predicted probability based on *two-predictor models*

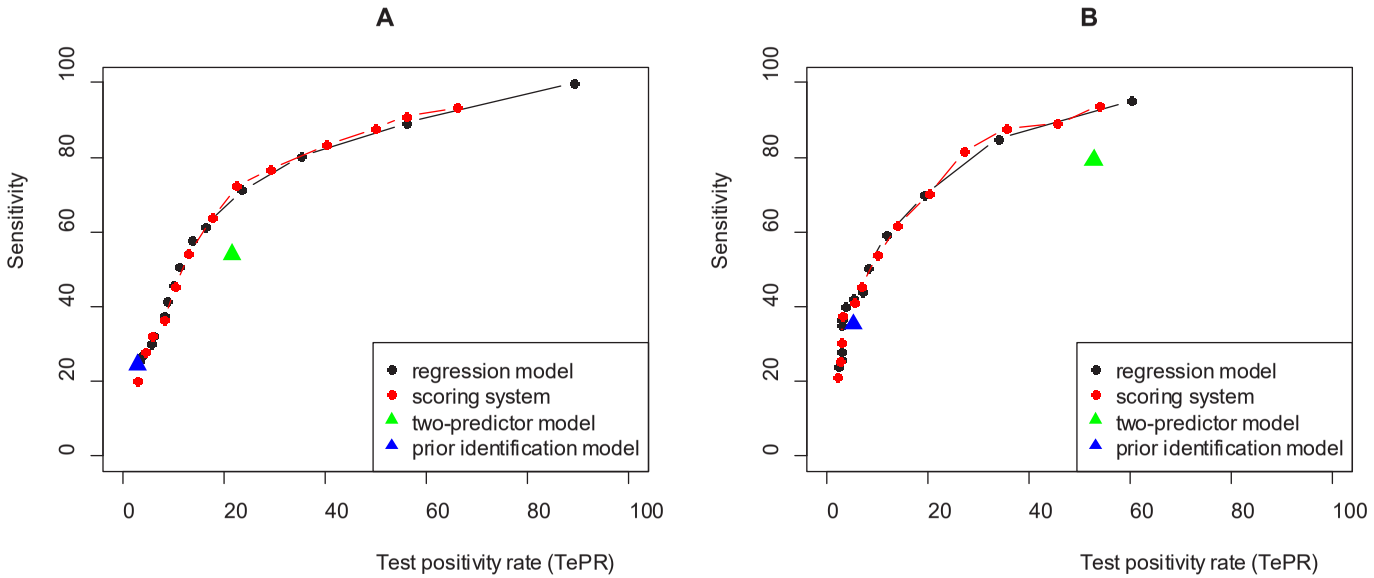
	Community-onset infection			Hospital-onset infection		
	Two-predictor model	**Probability cutoff 0.0067[a]**	**Probability cutoff 0.0042[b]**	***Two-predictor model***	**Probability cutoff 0.0086[a]**	**Probability cutoff 0.0040[b]**
	Apparent performance in study sample					
Sensitivity, % (95% CI)	53.9 (44.2–63.9)	55.2 (43.7–63.7)	68.3 (58.2–78.2)	79.3 (70.7–87.8)	80.6 (71.8–88.8)	91.6 (86.6–97.6)
TePR, % (95% CI)	21.5 (17.3–25.8)	12.8 (9.8–16.7)	21.0 (16.9–25.4)	52.8 (47.3–57.9)	27.6 (22.6–32.1)	52.3 (47.3–57.6)
	Optimism-corrected performance					
Sensitivity, % (95% CI)	53.9[c] (44.2–63.9)	49.0 (32.3–62.2)	64.3 (50.3–77.8)	79.3[c] (70.7–87.8)	75.3 (61.8–86.6)	89.8 (82.0–98.9)
TePR, % (95% CI)	21.5[c] (17.3–25.8)	13.2 (6.9–18.6)	22.5 (16.5–29.2)	52.8[c] (47.3–57.9)	28.2 (18.7–35.0)	53.8 (46.6–62.8)

This table contrasts performance of cutoffs of the predicted probability (used for classification as *at risk of third-generation cephalosporin-resistant Enterobacterales bacteremia* (3GCR-E-Bac)) in the current study sample, to expected performance of these cutoffs in future similar patient populations. The differences in sensitivity in TePR are the result of overoptimism during model development. Predicted probabilities were calculated with the original regression models, i.e. not optimism-corrected. Presented performance values are means of 20 imputed datasets. 95% CIs may differ slightly from those in **Supplementary Table 12**, as they were obtained in separate bootstrap procedures. Abbreviations: CI, confidence interval; TePR, test-positivity rate.

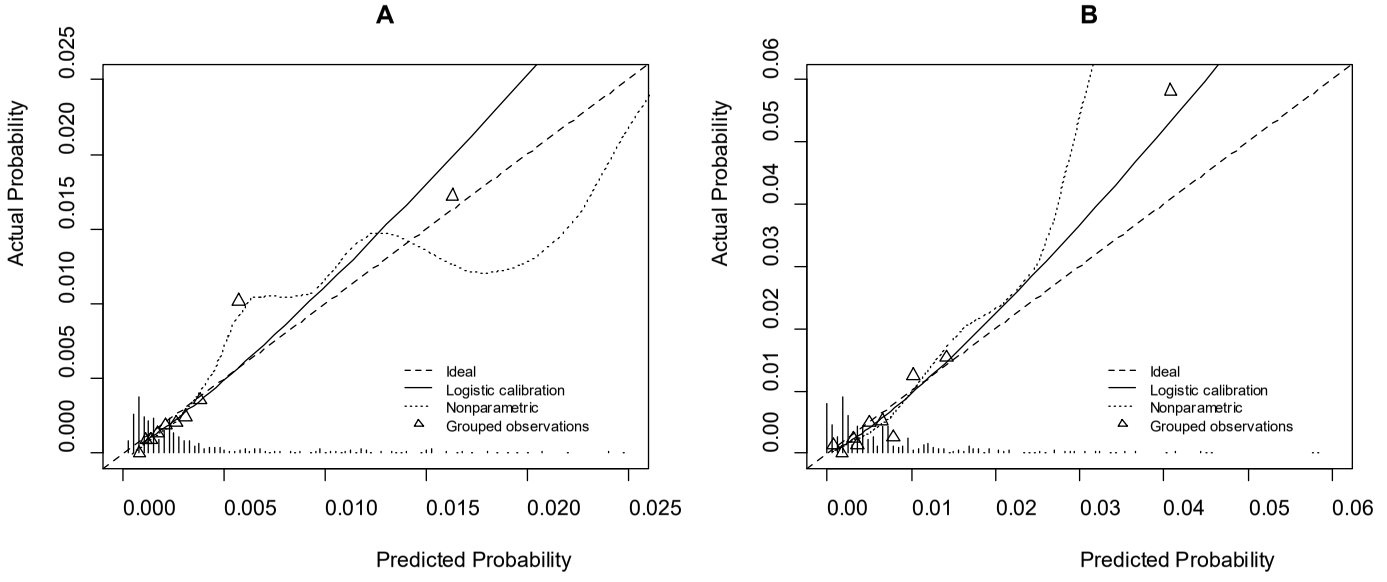
[a] The predicted probability above which patients are classified as *at risk of 3GCR-E-Bac*. It is chosen such that the resulting sensitivity is as close as possible to the sensitivity of the *two-predictor model* in the study sample.

[b] The predicted probability above which patients are classified as *at risk of 3GCR-E-Bac*. It is chosen such that the resulting TePR is as close as possible to the TePR of the *two-predictor model* in the study sample.

[c] Not affected by overoptimism due to pre-specification of models.



Supplementary Figure 1. Comparison of performance at different cutoffs of community-onset (A) and hospital-onset (B) regression models and derived scoring systems. Figures show the association between sensitivity and test positivity rate (TePR) when moving cutoffs above which patients are categorized as *at risk of third-generation cephalosporin-resistant Enterobacterales bacteremia (3GCR-E-Bac)*, both for probabilities resulting from the (optimism-corrected) regression models, and for the derived scoring systems. Performance is compared to the two basic models (*two-predictor model* and *prior identification model*). As TePR approximates 1 - specificity in these cohorts, figures are identical to receiver operating characteristic (ROC) curves. All values for sensitivity and TePR have been extrapolated to the full community-onset and hospital-onset cohorts. See **Tables 3 and 5** in the **main text** for exact values at score cutoffs, and **Supplementary Tables 7 and 8** for exact values at cutoffs for probabilities calculated with the regression models.



Supplementary Figure 2. Calibration of community-onset (A) and hospital-onset (B) regression models. Figures show calibration (predicted probability vs. actual probability of third-generation cephalosporin-resistant Enterobacterales bacteremia (3GCR-E-Bac)) of the regression models after correction for the sampling fraction of the controls and model shrinkage for optimism, for one of the 20 imputed datasets. Triangles denote deciles of the cohorts ordered according to predicted probability. Along the *x*-axis, vertical bars represent a histogram for predicted probabilities. Created with *valprob* function from *rms* package version 5.1-2 for R [5]. Calibration is presented numerically in **Supplementary Tables 7 and 8**.

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CHAPTER 8

General discussion

Antibiotic resistance is considered a worldwide public health concern [1]. The studies in this thesis provided information on some aspects of the way in which antibiotic resistance manifests itself in Dutch hospitals. Several multicenter studies in this thesis provided information on mortality attributable to two relevant antibiotic resistance problems in the Netherlands. In **Chapter 4**, it was shown that pathogens exhibiting different forms of multidrug resistance (MDR), mainly involving extended-spectrum β-lactamase (ESBL) production, were not associated with increased mortality in all forms of Gram-negative infection. These data are corroborated by the study presented in **Chapter 3**. It showed no impact of inappropriate empiric antibiotic therapy in case of bacteremia with ESBL-producing pathogens, although the effect estimate in **Chapter 3** should be interpreted with caution due to the small study size.

The general discussion will first analyze existing estimates of the number of deaths resulting from antibiotic resistance, and the relative increases in mortality on which these estimates are based. As such, a context for the surprising negative results of many studies in this thesis is provided. Then, a separate section is devoted to the issue of vancomycin-resistant *Enterococcus faecium* (VRE), the attributable burden of which was studied in **Chapter 5**. Here, in contrast to the issue of Gram-negative resistance, the microbiology community in the Netherlands has had a continuous debate over the last years whether the current control efforts are justified. Then, recommendations will be made for future studies on the attributable mortality of antibiotic resistance. The final issue that will be covered is the anticipation of antibiotic resistance in patients presenting with infection, studied in **Chapters 6 and 7** in this thesis.

Estimates of deaths attributable to antibiotic resistance

Many studies on the attributable mortality of antibiotic resistance, with a design similar to ours, have been published. Some have taken such estimates to provide estimates of the number of deaths due to specific antibiotic resistance problems (**Table 1**). The most conspicuous example is probably one of the reports published by the AMR Review in December 2014. This committee was installed by the British government to propose strategies to confront the glooming worldwide antimicrobial resistance (AMR) crisis and was headed by the renowned economist Terence James O'Neill. Their initial report, titled *Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations*, set the scene for the actual task of the committee, and included an estimate of the burden of AMR by 2050 [2]. This widely

Table 1. Estimates of numbers of deaths due to antimicrobial resistance

First author and year of publication	Region	Year	Resistance problem	Estimated No. of deaths
ECDC 2009 [49]	European Union, Iceland and Norway	2007	Infection due to: • MRSA • VRE • 3GC-R *E. coli* • 3GC-R *K. pneumoniae* • Carbapenem-resistant *P. aeruginosa*	25,100
De Kraker 2011 [50]	31 European countries participating in EARS-Net	2007	Bacteremia due to: • 3GC-R *E. coli* • MRSA	8,215
Laxminarayan 2013 [14]	India	?	Neonatal sepsis due to: • ESBL+ bacteria • MRSA	58,319
CDC 2013 [48]	United States	2011	Infection due to resistant bacteria	23,488
AMR Review 2014 [2]	Worldwide	2014	Infection due to: • MRSA • 3GC-R *E. coli* • 3GC-R *K. pneumonia* • Resistant *M. tuberculosis* • Resistant HIV • Resistant malaria	700,000
	Worldwide	2050		10,000,000
Lim 2016 [16]	Thailand	2010	Hospital-acquired infection due to MDR bacteria	19,122
Cassini 2018 [6]	30 countries in the European Union and European Economic Area	2015	Infection due to: • Resistant *Acinetobacter* spp. • Resistant *E. coli* • Resistant *K. pneumoniae* • Resistant *P. aeruginosa* • VRE • MRSA • Resistant *S. pneumoniae*	33,110

Abbreviations: 3GC-R, third-generation cephalosporin-resistant; ESBL+, extended-spectrum β-lactamase-producing; HIV, human immunodeficiency virus; MDR, multidrug-resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococcus.

cited number of potentially 10 million deaths yearly was based on the reports of two consultancy firms, namely RAND Europe and KPMG [3,4]. The KPMG report, the best known of the two, uses the following calculation to obtain its estimate of deaths due to AMR:

$$\#\,Infections \times Resistance\ rate \times Attributable\ mortality\ rate = Mortality$$

Six resistant pathogens were included in these calculations, namely methicillin-resistant *Staphylococcus aureus* (MRSA), third-generation cephalosporin-resistant (largely equivalent to ESBL-producing) *Escherichia coli* and *Klebsiella pneumoniae*, and drug-resistant variants of *Mycobacterium tuberculosis*, malaria and human immunodeficiency virus (HIV). Several future scenarios were projected (including 100% resistance rates and doubling of current infection rates) and other data sources were reviewed for a.o. estimates of attributable mortality rates, in order to obtain mortality estimates with help of the formula. AMR has a much wider scope than the antibiotic-resistant bacteria studied in this thesis, and although the KPMG report does not provide exact numbers, it can be observed from available graphs that more than half of the projected mortality burden by 2050 is the result of drug-resistant tuberculosis, malaria, and HIV infection. On the other hand, the future burden of carbapenemase-producing Enterobacterales was excluded. The remainder of this discussion will focus on the more common opportunistic pathogens that reside within the normal human flora, which are a major cause of infection in the community and pose particular problems within the healthcare setting.

The estimates cited by the AMR Review have been criticized by the scientific community. De Kraker *et al.* focused their critique on six aspects, thereby particularly focusing on the hospital-associated resistant bacteria: (i) that worldwide estimates of bacteremia numbers are based on unrepresentative European data from EARS-Net; (ii) that estimates of worldwide resistant rates may be biased due to differential culture rates; (iii) that extrapolation of the number of bacteremias to infections at other sites is based on very sparse data; (iv) that much of the literature on the attributable mortality of resistance is biased; (v) that projected future scenarios are unlikely; and (vi) that essential elements of the scientific method, such as uncertainty parameters and peer review are lacking [5].

Alongside the release of the AMR Review report, the scientific community has been inspired to come up with more accurate estimates of the global burden of resistance. Whereas previous efforts often limited themselves to obtaining the increase in the risk of mortality for individual patients, the field has obtained a more public health oriented scope. The figures of attributable mortality then serve as one of the starting points to derive worldwide and region-specific burden estimates. In essence, such estimates rely on the elements that can also be found in the aforementioned KPMG formula.

Recently, the European Center for Disease Prevention and Control (ECDC) published its most up-to-date estimation of the burden of antibiotic resistance in European countries, which showed a large disparity between countries with regard to mortality rates [6]. Another recent study attempted to improve on the aspect of the number of infections, and derived more accurate global rates of resistant Enterobacterales infections [7]. Unfortunately, these estimates remain dependent on European incidence rates of infection and questionable extrapolation of bacteremias to other types of infection, and are hampered by the absence of reliable AMR surveillance in many parts of the world. An important step forward may the incorporation of the issue of AMR into the Global Burden of Diseases, Injuries, and Risk Factors Study [8]. Bringing together more data sources than ever before, the aim is to obtain more reliable figures for the global distribution of infections with resistant micro-organisms, which can also be updated periodically.

Attributable mortality of antibiotic resistance

From the aforementioned formula to calculate the of burden resistance, it appears that, apart from the incidence of AMR, an essential aspect remains the attributable mortality rate. Meta-analyses evaluating the impact of MRSA and ESBL-producing Gram-negatives in bacteremia, including ours (**Chapter 2**), have consistently found an increase in mortality associated with these pathogens [9,10]. However, most of these meta-analyses only incorporated effect estimates without correction for confounding, or relied heavily on single center studies that applied inappropriate statistical techniques to derive causal estimates, such as stepwise selection of significant variables.

More recently, several large-scale, rigorous, multinational European studies have been published on the attributable mortality of several antibiotic-resistant pathogens (**Table 2**). Also, large-scale data on several types of resistant Gram-negatives in the United States have been published [11]. Nevertheless, effect estimates for the contribution of resistance to outcome are conflicting. Interestingly, these large-scale efforts include numerous studies, next to ours, that show limited additional impact of antibiotic resistance on mortality. The most notable exceptions are the studies by De Kraker *et al.* [12,13].

Table 2. Recent large-scale studies on the attributable mortality of antibiotic-resistant pathogens

First author and year of publication	Setting	Antibiotic-resistant pathogen and infection	No. of study sites		No. of infections with:		Adjusted association measure (95% CI) for mortality for:	
			Total	Tertiary centers	S phenotype	R phenotype	S phenotype vs no infection	R phenotype vs S phenotype
Ammerlaan 2009 [51]	Europe	MRSA bacteremia	60	?	257	77	-	OR 0.98[a] (0.50–1.94)
De Kraker 2011 [12]	Europe	MRSA bacteremia	13	13	618	248	OR 2.4 (1.7–3.3)	OR 1.8 (1.04–3.2)
Lambert 2011 [52]	Europe ICU only	MRSA bacteremia	537	?	284	171	HR 2.1[b] (1.6–2.6)	HR 1.6[b] (1.1–2.3)
Stewardson 2016 [53]	Europe	MRSA bacteremia	10	9	885	163	HR 1.81[b] (1.49–2.20)	HR 1.26[b] (0.82–1.94)
Lambert 2011 [52]	Europe ICU only	Ceftazidime-R *Pseudomonas aeruginosa* bacteremia	537	?	282	82	HR 3.2[b] (2.6–4.0)	HR 1.2[b] (0.8–1.9)
Lambert 2011 [52]	Europe ICU only	3GC-R *Escherichia coli* bacteremia	537	?	218	42	HR 2.7[b] (2.1–3.4)	HR 1.3[b] (0.8–2.2)
De Kraker 2012 [13]	Europe	3GC-R *Escherichia coli* bacteremia	13	13	1,110	111	OR 1.9 (1.4–2.5)	OR 2.5 (0.9–6.8)
Stewardson 2016 [53]	Europe	3GC-R Enterobacterales bacteremia	10	9	2,100	360	HR 1.16[b] (0.98–1.36)	HR 1.63[b] (1.13–2.35)
Kadri 2018 [11]	USA	3/4GC-R Gram-negative bacteremia	173	?	21,410[c]	2,756	-	RR 1.13 (1.05–1.22)
Rottier **Chapter 4**	Netherlands	Multidrug-resistant Gram-negative infection	8	1	1,711	243	RR 1.33 (1.07–1.65)	RR 1.05 (0.46–2.35)

Abbreviations: 3GC-R, third-generation cephalosporin-resistant; 3/4GC-R, third- and/or fourth-generation cephalosporin-resistant; CI, confidence interval; HR, hazard ratio; ICU, intensive care unit; MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odds ratio; R, resistant; RR, risk ratio; S, susceptible.

[a] Included inappropriate empiric therapy in the model (OR 0.69, 95% CI 0.36–1.32).

[b] Cause-specific HR for in-hospital mortality. In most cases, infection also impacted the cause-specific HR for hospital discharge, implying that the overall effect on the cumulative incidence of in-hospital mortality may be larger.

[c] Excluding carbapenem- and fluoroquinolone-resistant Gram-negative isolates.

These studies all pertain to high income countries, and there remains a large knowledge gap with regard to the burden of antimicrobial resistance in low and middle income countries (LMICs), where the consequences are assumed to be most pronounced [14]. They may be further exacerbated as alternative antibiotics in many instances are not available [15]. Fortunately, two recent publications have allowed us to obtain more insight in the situation. First, Lim *et al.* studied the consequences of MDR in community- and hospital-onset bacteremia in nine Thai public hospitals, and showed that in MDR bacteremia, 30-day mortality was increased from 34% to 44% [16]. The authors estimated that, nationally, approximately 19,000 deaths in 2010 would be attributable to MDR in hospital-acquired bacteremia, in a country with a population of 66 million at that time. Second, Gandra *et al.* provided much needed data on the situation in India [17]. In infections studied in ten hospitals, MDR Gram-negatives were associated with 2–3 times increased mortality rates. Data applying more rigorous methods for causal inference from a wide variety settings is much needed. For now, we get an incomplete picture of the seriousness of the situation with help of the two named studies, combined with the sparse information on the prevalence of antibiotic resistance in clinical infection [18], and reports on the enormous challenges in infection prevention and control [19–21].

The results presented in this thesis are in large contrast to the estimates from LMICs, and, as noted, estimates from high income countries are also conflicting (**Table 2**). An explanation for this may be that antibiotic resistance is only relevant in relation to the antibiotics that are provided. If antibiotic resistance is perfectly anticipated in all affected patients by the choice of antibiotic regimens, there is unlikely to be any burden at all, except if resistant pathogens were to be notably more virulent, for which there is little evidence [22,23]. Currently, perfect anticipation at the moment that infection presents is not possible, as shown in **Chapters 6 and 7**, and empiric antibiotic treatment guidelines differ between settings. Logistics of diagnostic facilities vary, resulting in different turnaround times in different settings, and guidance of treating physician's decisions by expert consultation may also differ between settings. This all implies that the impact of resistance in one setting may be poorly generalizable to another setting. The results presented in this thesis with regard to the burden of Gram-negatives should therefore mainly be interpreted as evidence that currently, in the Netherlands, the issue is confronted in an appropriate manner, and not necessarily as evidence that studies that did find increased mortality in case of antibiotic resistance, are flawed.

Vancomycin-resistant *Enterococcus faecium*

Worldwide, the estimation of the burden of antimicrobial resistance has particularly focused on MRSA and resistant Gram-negatives. Yet, many European hospitals are facing outbreaks of VRE or have reached VRE endemicity [24,25]. Controlling the spread of VRE within hospitals involves a large financial burden [26], which is at odds with the general perception that enterococci are poorly pathogenic [27]. There is a fierce debate ongoing whether contact precautions for VRE are necessary [28]. Also, in the Netherlands, it is debated whether VRE should still be regarded as a highly resistant micro-organism (HRMO), implying that it is a target for infection prevention measures [29–31]. Relaxation of these measures will most likely lead to a situation in which the amoxicillin-resistant *E. faecium* (ARE) population currently endemic in Dutch hospitals is supplanted by VRE.

Our study (**Chapter 5**) provided no indications that any such shift from an ARE to VRE phenotype is associated with an increase in mortality mediated through the later onset of appropriate antibiotic therapy. The observed association between VRE bacteremia and mortality (relative risk 1.54, 95% confidence interval 1.06–2.25) may well be explained by unmeasured confounding, as there are very few indications that pathogenicity differs between *E. faecium* strains. Studies from the late 1990s and early 2000s concluded that mortality was increased in VRE bacteremia [32], but more recent accounts on the attributable of VRE reached similar conclusions as our study [33,34]. Data from one of these newer studies also specifically supported our approach of restriction of the study domain to *E. faecium* only [34]. Mortality was much lower in *E. faecalis* than in *E. faecium* bacteremia, and since vancomycin resistance is particularly rare in this species, inclusion in the study domain would imply an unwarranted advantage for the vancomycin-susceptible control group.

Relaxation of infection prevention measures targeting VRE however poses a more complex question than the attributable mortality of VRE in bacteremia. In a meeting of Dutch infection prevention experts, it was decided that to make a more appropriate risk-benefit assessment, additional information would be required [35]. For one, VRE outbreaks are speculated to be an indicator that hospital hygiene standards are in need of improvement. Also, the burden of VRE may also not have been captured in its entirety, as non-bacteremic infection involving prosthetic joints and valves may be particularly troublesome to treat. Preliminary data on the first aspect indicate that there may indeed be a collateral benefit of measures taken during VRE outbreaks on the incidence of *Clostridium difficile* infections [36]. Preliminary data on the

comparison between complicated ARE and VRE infections showed that a significant additional burden of VRE is unlikely, as the ARE infections are rare and have a very poor prognosis in any case [37].

In conclusion, VRE infections are unlikely to impose an additional burden when compared to ARE infections, questioning the need to maintain VRE as an HRMO. Nevertheless, there may be other arguments to uphold its status. First, therapy for VRE infections may be more expensive than for ARE infections [35]. Second, it is questionable whether a cascade of ever increasing resistance in *E. faecium* should be set in motion, seeing that linezolid-resistant VRE has already been reported, and will gain advantage when linezolid use increases [38]. Third, as discussed, VRE outbreaks may signal an opportunity to improve hospital hygiene standards. Yet, as the current Dutch definition of HRMOs is based on disease burden and effects on empiric therapy [29], these three arguments may carry little validity for the decision to maintain the HRMO status of VRE. Moreover, it should be noted that signals on the state of hospital hygiene may also be derived from other indicators, including the incidence of the more relevant hospital-associated pathogens themselves.

Recommendations for studying the attributable mortality of antibiotic resistance

Several recommendations can be made with regard to future studies on the burden of AMR. As it is hypothesized that effects may differ per setting, understanding of the burden may likely be increased by performing multinational studies which can directly contrast settings in which a high versus a low burden is anticipated, for example Northern and Southern Europe. By means of daily assessment of acute severity of illness and antibiotic therapy, the interplay between these factors can be established, and effect estimates can be obtained for inappropriate therapy corrected for time-varying confounding. Such estimates may be amenable to effect modification, but are less likely to differ fundamentally between settings than effects of antibiotic resistance.

Another important aspect of contrasting settings recommendable to conduct is an analysis of which patients develop infections caused by resistant pathogens. Including non-infected patients may then be a relevant addition in order to describe the source population of infected patients. Here, we would argue against the use of the parallel matched cohort design as a tool to improve correction for confounding, for the reasons described in **Chapter 4**. Especially if community-onset infections are included in the study, it is important to draw non-infected

controls from the community and not from the hospital. If a study is designed in that manner, a population-based approach may provide even more insights when contrasting settings.

Another recommendation is to include the bacterial strain causing infection as a covariate in analyses. Although, as mentioned before, virulence is not likely to play an important role as explanation of the burden antimicrobial resistance, such claims can be further substantiated with the help of molecular epidemiology. Understanding the local epidemiology of circulating strains may also help in explaining differences between settings. Furthermore, analyses conditioning on bacterial strains may serve as control for confounding, as indicated by the directed acyclic graph presented in **Chapter 1**. However, much work probably needs to be invested in creating meaningful categories for the genetic background of bacterial strains. Meanwhile, control for confounding may be improved by appropriate modelling techniques (e.g. not relying on *p*-values for retaining confounders in models). Also, several studies may have omitted important confounders that we identified through studying directed acyclic graphs and performing statistical modelling. These include treatment restrictions, antibiotic exposure, and colonization status as known to treating physicians, all prior to infection. For nosocomial infections, the Acute Physiology Score as defined by the APACHE scoring system may be a relevant confounder, as shown in **Chapter 5**, but establishing the acute illness severity prior to infection (as opposed to data on comorbidities) in case of community-onset infection remains problematic.

Anticipating antibiotic resistance in patients presenting with infection

Chapters 6 and 7 of this thesis show that anticipation of antibiotic resistance at the moment a patient presents with infection is difficult, but that improvements can be achieved by incorporating additional parameters into prediction schemes, and appropriately weighting them. Studies on antibiotic resistance have traditionally focused on contrasting infections with resistant and susceptible pathogens and there are a variety of reasons why this may not always be the right approach [12,13,39]. This problem is especially pronounced in case of prediction of antibiotic resistance in case of infection. Based on the ubiquity of information on risk factors derived with standard resistant vs. susceptible study design [40], existing prediction schemes have mainly focused on discerning bacteremias with resistant pathogens from those with susceptible pathogens [41]. These studies thereby overlook the fact that such prediction schemes are technically only valid at the moment that the pathogen is identified, pending susceptibility results. Moving the point of reference to the moment at which patients present

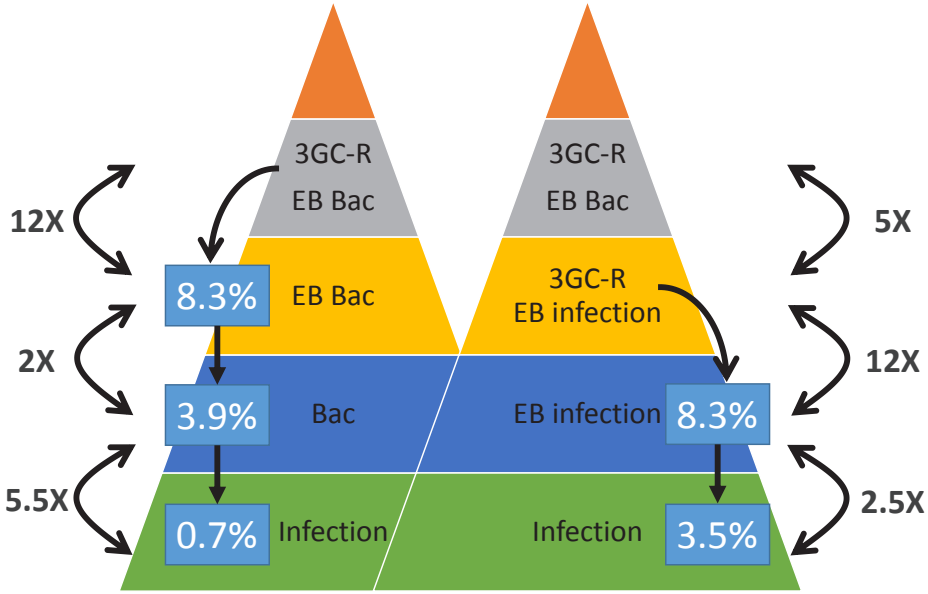

Figure 1. This figure (with data from **Chapter 6**) shows, on the left, that 1:12 (8.3%) of Enterobacterales (EB) bacteremias (Bacs) are caused by third-generation cephalosporin-resistant (3GC-R) EB, but this percentage is subsequently diluted to a 0.7% prior probability of 3GC-R EB Bac in suspected infection, due to the addition of non-EB Bacs, non-bacteremic infections, and suspected infections turning out to be of non-infectious etiology.
A similar reasoning is made on the right, as it was estimated that 3GC-R EB infection (bacteremic or non-bacteremic) was 5 times as common as 3GC-R EB Bac.
Most research on the risk factors for antibiotic resistance has been performed by contrasting the grey to the yellow level in the left pyramid. Yet, for patients, it may be most relevant to identify infections with resistant pathogens among the green level of the pyramid.

with infection may have more impact, as that is the moment that the patient may benefit most from prescribing appropriate antibiotic therapy [42]. Yet, prior probabilities of infections with resistant pathogens may differ by an order of magnitude between these two moments (**Figure 1**), and the prevalence of risk factors among patients not having an infection with resistant pathogens may also diverge. These parameters can have an important impact on the performance of prediction schemes.

A balance between inappropriate empiric antibiotic therapy and unnecessary prescription of broad-spectrum antibiotics is deemed essential in curbing the antibiotic resistance problem [14,43]. Finding such a balance is hampered because it is unknown what downstream effects of prescribing specific classes of antibiotics can be expected with regard to driving antibiotic resistance. As a result, adequately weighting the consequences of specific treatment choices

is impossible. Efforts have recently been undertaken to establish the economic costs of prescribing a specific antibiotic [44], but again, these costs may be highly context-specific. In a setting with appropriate hospital hygiene standards and without endemicity of carbapenemase-producing pathogens, prescribing carbapenems may not carry a particular risk for driving the spread of these organisms. Balancing risks and benefits of antibiotic use has been implemented in the electronic decision support system TREAT [45], but this has not been widely adopted.

Conclusion

The studies in this thesis show that the most commonly encountered forms of antibiotic resistance in the Netherlands are not associated with a mortality burden. Importantly, these studies do not reflect the global burden of antibiotic resistance. Directly contrasting mortality in infections with antibiotic-resistant and antibiotic-susceptible pathogens ignores several aspects. The spread of antibiotic-resistant bacterial lineages may in fact inflate the number of infections [46,47]. Moreover, morbidity and societal costs related to antibiotic resistance are relevant other outcomes, which will be addressed in future publications within the GRAND-ABC project (of which **Chapter 4** is the first publication). The burden of more recent resistance problems which have hardly manifested themselves in the Netherlands, such as carbapenemases, is not included. Nevertheless, the studies in this thesis provide evidence that the burden of antimicrobial resistance is currently manageable within the Netherlands. These conclusions may generalize to some other high-income, mainly Northern European countries. At the same time, they may serve as an incentive to study the burden of the global antibiotic resistance crisis in those settings where it is more likely to manifest itself, especially in low and middle income countries. Whereas it is essential to remain vigilant in the Netherlands with regard to the potential threat, resources spent globally on antibiotic resistance should take into account the extremely skewed distribution of its burden, and the scarcity of information on it in many settings.

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Samenvatting in het Nederlands / Summary in Dutch

In de afgelopen decennia is antibioticaresistentie wereldwijd een groot probleem geworden. Nederland is zeer succesvol geweest met het zoek-en-vernietig-beleid gericht op methicilline-resistente *Staphylococcus aureus* (de MRSA-bacterie). Daarentegen is bij een andere groep resistente bacteriën, de multiresistente Gram-negatieven, de situatie in Nederland niet beduidend anders dan in omliggende West-Europese landen. Deze groep betreft grotendeels darmbewoners, zoals *Escherichia coli*. Een deel van hen is resistent geworden voor veel in ziekenhuizen gebruikte antibiotica, doordat ze extended-spectrum β-lactamases (ESBL's) en andere enzymen aanmaken (produceren). Deze multiresistente bacteriën verspreiden zich niet alleen onder patiënten in ziekenhuizen. Ook in de algemene bevolking is een groot deel van de mensen drager. Een ander belangrijk resistentieprobleem in Nederland betreft de vancomycine-resistente enterokokken (VRE). Dit zijn varianten van *Enterococcus faecium*, een darmbacterie die zich extreem goed heeft aangepast aan de ziekenhuisomgeving. Nederlandse ziekenhuizen maken veelvuldig melding van kleinere en grotere uitbraken met VRE, waarbij na opname van een VRE-positieve patiënt in het ziekenhuis, de bacterie de kans ziet zich te verspreiden naar andere patiënten.

Op dit moment zijn in Nederland voor patiënten met infecties met resistente bacteriën vrijwel altijd effectieve antibiotica beschikbaar. Hierbij is het probleem echter dat een effectieve therapie vaak pas met een vertraging kan worden toegediend. In het algemeen geldt dat als een patiënt zich in het ziekenhuis presenteert met een ernstige infectie, er al direct met antibiotica wordt gestart. Tevens worden er kweken van lichaamsmateriaal ingezet om de ziekteverwekker met zijn resistentiepatroon te identificeren en gericht antibiotica te kunnen geven. De resultaten hiervan laten alleen vaak enkele dagen op zich wachten. In de tussentijd wordt daarom op basis van allerlei factoren een keuze voor een zogenaamd *empirisch antibioticaregime* gemaakt. Dit regime moet in principe de bij de infectie verwachte ziekteverwekkers behandelen, maar een dekkingsgraad van 100% is niet haalbaar. Het is namelijk onverstandig om bij onzekerheid maar veel soorten antibiotica toe te dienen, want het te 'breed' voorschrijven van antibiotica leidt tot bijwerkingen voor de patiënt en zou antibioticaresistentie onnodig aanwakkeren. Omdat resistente bacteriën in Nederland en veel andere landen slechts een klein deel van alle infecties veroorzaken, worden antibiotica die effectief zijn tegen resistente bacteriën niet standaard als empirische antibiotica voorgeschreven. In plaats daarvan wordt op basis van risicofactoren nagegaan of de patiënt een verhoogde kans heeft op een infectie met resistente bacteriën. Als dat het geval is, dan

behoeft het standaard *empirische antibioticaregime* aanpassing. Dit soort risico-indelingen zijn echter feilbaar, waardoor het netto-effect is dat patiënten die geïnfecteerd zijn met resistente bacteriën, later effectieve (adequate) antibiotica krijgen dan patiënten die geïnfecteerd zijn met de gangbare varianten van deze bacteriën.

De studies in dit proefschrift richten zich op twee thema's. In de eerste hoofdstukken wordt bestudeerd wat het effect is van de vertraging van effectieve antibiotica, specifiek voor multiresistente Gram-negatieven en VRE. Op deze wijze kan een inschatting worden gemaakt van de omvang van het antibioticaresistentie-probleem, oftewel de ziektelast, in Nederland. In de laatste twee hoofdstukken wordt onderzocht of de indeling in het risico op resistente ziekteverwekkers bij de keuze van een *empirisch antibioticaregime* kan worden verbeterd. Zodoende kunnen patiënten met resistente infecties eerder adequate antibiotica krijgen, terwijl tegelijkertijd de onnodige behandeling van patiënten met te brede antibiotica wordt teruggedrongen.

Wat betreft het eerste thema, de gevolgen van antibioticaresistentie, geldt dat onderzoek noodgedwongen plaatsvindt met behulp van observationele, niet-experimentele studies. Hierbij worden de natuurlijke variaties die zich voordoen in de medische praktijk, geregistreerd. Het zou namelijk niet ethisch of praktisch haalbaar zijn om experimentele, gerandomiseerde studies te verrichten waarbij patiënten willekeurig een resistente of gevoelige bacterie krijgen toegediend, of willekeurig worden ingedeeld in groepen met vroege of late effectieve antibioticaregimes. In de niet-experimentele studies worden vaak twee groepen patiënten – de een geïnfecteerd met gevoelige, de ander met resistente bacteriën – op een aantal uitkomsten vergeleken. Omdat het ernstige infecties betreft, is sterfte, bijvoorbeeld binnen 30 dagen na het begin van de infectie, een veelgebruikte uitkomstmaat.

Een probleem bij deze opzet is dat voor een eerlijke vergelijking de twee groepen op andere factoren, dus buiten resistentie, niet mogen verschillen. Slechts dan kunnen we het causale effect van antibioticaresistentie op sterfte vaststellen. Los van de infectie zijn patiënten met infecties met resistente bacteriën echter gemiddeld genomen in een slechtere gezondheidstoestand dan patiënten met infecties met gevoelige bacteriën. De reden hiervoor is dat een grotere blootstelling aan antibiotica en de ziekenhuisomgeving ertoe leidt dat patiënten drager worden van resistente bacteriën (anders gezegd: ze zijn gekoloniseerd) en daardoor de kans op infecties met resistente bacteriën hoger is. Voor deze verstoring,

confounding genoemd, moet worden gecorrigeerd in de studie-opzet of door middel van statistische methoden.

In **hoofdstuk 2** hebben wij de bestaande literatuur geanalyseerd en daarbij gekeken naar het effect van het produceren van ESBL's bij ernstige infecties met Gram-negatieven. Het gaat dan om bacteriëmieën, hetgeen betekent dat de infecterende bacterie kan worden aangetoond in het bloed. Er werden 32 studies over dit thema geïdentificeerd in het jaar 2010. Vervolgens hebben we deze studies samengenomen in een meta-analyse. Hieruit blijkt dat de *odds*[1] om te sterven kort na de infectie 2,35 keer verhoogd is bij infecties met ESBL-producerende bacteriën, t.o.v. de gevoelige varianten van de betreffende bacteriën (*odds* ratio (OR); 95% betrouwbaarheidsinterval (BI) 1,90–2,91). Dit gaat dan om een niet voor *confounding* gecorrigeerde schatting; na correctie is de OR 1,52 (95% BI 1,15–2,01). Studies hebben echter verschillende benaderingswijzen gehanteerd bij het corrigeren voor *confounding*. Ze hebben vaak gecorrigeerd voor de ernst van de onderliggende ziekte, maar veel studies hebben ook gecorrigeerd voor de ernst van de infectie en de adequaatheid van de empirische antibiotica. Het blijkt dat hoe meer van deze drie factoren zijn gebruikt in de correctie, des te lager de gevonden OR's zijn. Correctie voor ernst van de infectie en de adequaatheid van de therapie zijn echter niet gewenst, omdat deze factoren intermediairen in de causale keten van resistente infectie naar sterfte zijn. Anders gezegd: infecties met resistente bacteriën leiden tot meer inadequate empirische therapie, en mogelijk ernstigere infecties, met als gevolg dat de sterfte hoger is. Dit is een geheel ander mechanisme dan voor het voornoemde *confounding*. In statistische modellen moeten dergelijke factoren juist niet worden verdisconteerd. De conclusie van deze meta-analyse is daarmee dat schattingen van het effect van resistentie op sterfte in het geval van Gram-negatieve infecties sterk afhangt van hoe de correctie door middel van statistische modellen is opgezet.

Hoofdstuk 3 beschrijft vervolgens een eerste studie naar de epidemiologie van bacteriëmieën die worden veroorzaakt door ESBL-producerende Gram-negatieven in Nederland. In acht ziekenhuizen werd een cohort samengesteld met 232 episodes van dit type infectie, veroorzaakt door *E. coli* (70%), *Klebsiella pneumoniae* (19%) en *Enterobacter cloacae* (11%).

[1] Epidemiologische studies gebruiken vaak de odds in plaats van de kans om risico's uit te drukken, omdat dit voordelen heeft bij de modellering. Als de kans 25% is, d.w.z. 1 op 4, dan is de odds 1 tegen 3, oftewel 33%. De odds van verschillende groepen kan worden gecontrasteerd met de odds ratio, d.w.z. de ratio tussen twee odds. Een odds ratio zal in veel gevallen redelijk overeenkomen met de waarde van het relatief risico (de ratio van twee kansen) berekend op dezelfde gegevens maar mag daar zeker niet aan gelijk gesteld worden. Een odds ratio, of relatief risico, van 1 betekent dat de kans (en dus odds) tussen twee groepen niet verschilt.

Deze ESBL-positieve infecties vormen net iets meer dan 7% van het totaal van alle bacteriëmieën met deze bacteriesoorten. Tien procent van de 232 infecties betreft kinderen onder de 18 jaar. Bij 84% van de infecties blijkt vooraf contact te zijn geweest met de gezondheidszorg, vaak in de vorm van een eerdere opname. Slechts 37% van alle patiënten met infecties krijgt binnen 24 uur na het begin van de infectie adequate antibiotica toegediend. Van 31% van de patiënten is al bekend vanuit een eerder kweek dat zij een ESBL-producerende bacterie bij zich draagt op het moment dat zij zich presenteert met de infectie. Opmerkelijk genoeg krijgt slechts 54% van de groep bekende ESBL-dragers adequate therapie binnen 24 uur. Overigens adviseert de Nederlandse sepsis-richtlijn niet alleen ESBL-dragerschap mee te nemen in de afweging rondom dekking van ESBL-producerende bacteriën bij de empirische therapie, maar daarbij ook te kijken naar recent gebruik van antibiotica uit de cefalosporine- en fluorochinolon-klasse. Daarmee neemt het percentage mensen dat vooraf kan worden geclassificeerd als hebbende een risico op een ESBL-infectie, toe van 31% naar 64%. Slechts 43% van hen krijgt adequate therapie binnen 24 uur. Door de richtlijn strikter te volgen kan het deel van de patiënten met een ESBL-bacteriëmie dat snel adequate therapie krijgt, fors worden uitgebreid. In deze studie wordt echter inadequate antibiotica gedurende de eerste 24 uur van de bacteriëmie niet geassocieerd met een hogere sterfte in de eerste 30 dagen. Na correctie voor verstorende factoren vinden wij een OR van 1,65 met 95% BI 0,76–3,59, d.w.z. een erg onzekere schatting die niet significant verschilt van 1.

Hoofdstuk 4 beschrijft een grote vervolgstudie. In dit geval gaat het erom om specifiek voor Nederland de sterfte binnen 30 dagen na het begin van een infectie te vergelijken tussen infecties met multiresistente Gram-negatieven en de gebruikelijke varianten van Gram-negatieven. Het betreft nu een iets bredere groep van resistente Gram-negatieven dan alleen de eerder genoemde ESBL-producerende Gram-negatieven, namelijk Gram-negatieve bijzonder resistente micro-organismes (BRMO's). Hiertoe behoren ook varianten die geen ESBL's produceren, maar wel resistent zijn voor meerdere andere klassen antibiotica, zoals multiresistente *Pseudomonas aeruginosa*. Dit onderzoek is opgezet in de vorm van een *parallel gematcht cohort*. Dat wil zeggen dat zowel de BRMO-infecties als de niet-BRMO-infecties niet direct met elkaar worden vergeleken, maar elk hun eigen controlegroep van patiënten zonder infectie hebben en daarmee worden vergeleken. In acht Nederlandse ziekenhuizen worden 1.954 Gram-negatieve infecties geïdentificeerd in de periode 2013–2016. In 39% van de gevallen betreft de Gram-negatieve infectie een bacteriëmie; in andere gevallen gaat het vaak om een urineweginfectie waarbij de verwekker niet in het bloed wordt aangetroffen. Twaalf

procent wordt veroorzaakt door een BRMO, in de meeste gevallen toch een ESBL-producerende bacterie. Opnieuw wordt gevonden dat op de dag dat de infectie begint, er vaak geen adequate antibiotica worden gegeven bij resistente bacteriën. Van de BRMO's krijgt 32% direct adequate therapie, tegenover 61% van de niet-BRMO's. De sterfte bij BRMO-infecties is echter niet hoger: 10% tegen 11%. Om te corrigeren voor *confounding* wordt dan gebruik gemaakt van het *parallel gematcht cohort*. De patiënten met BRMO-infectie hebben een hogere sterfte dan hun op ziekenhuis, opnameduur en leeftijd *gematchte* niet-geïnfecteerde controlepatiënten (gecorrigeerd relatief risico (RR) 1,40, 95% BI 0,64–3,05). Hetzelfde geldt echter voor de vergelijking tussen patiënten met een niet-BRMO-infectie en hun controlepatiënten (RR 1,33, 95% BI 1,07–1,65). De eindconclusie is dat BRMO-infecties in Nederland niet tot een verhoogde sterfte leiden (RR 1.05, 95% BI 0,46–2,35), ondanks dat adequate antibiotica later worden gestart. Deze conclusie is tegengesteld aan die van veel andere studies, onder andere onze meta-analyse (**hoofdstuk 2**). Een mogelijke verklaring is dat het toedienen van inadequate antibiotica gedurende de eerste uren van een infectie in veel gevallen minder ernstig is dan voorheen voorgesteld, en dat in Nederlandse ziekenhuizen snel alsnog de juiste antibiotica kunnen worden voorgeschreven.

Hoofdstuk 5 past vervolgens een vergelijkbare vraagstelling toe op het probleem van de VRE's. De studie richt zich op het contrasteren van de 30-dagen-sterfte tussen VRE-bacteriëmieën en bacteriëmieën met de gebruikelijke variant, amoxicilline-resistente *E. faecium* (ARE). VRE-bacteriëmieën zijn echter veel zeldzamer dan Gram-negatieve infecties en er worden 16 Nederlandse en 4 Deense ziekenhuizen bij de studie betrokken. Dat levert 63 VRE-bacteriëmieën op in de periode 2009–2014. Er wordt nu geen gebruik gemaakt van een *parallel gematcht cohort*, maar de VRE-bacteriëmieën worden direct *gematcht* aan 234 ARE-bacteriëmieën, op basis van ziekenhuis, ligafdeling, opnameduur voorafgaand aan de infectie en leeftijd. De sterfte bij VRE-bacteriëmieën is 40%, bij ARE 32%. Na verdere correctie voor *confounding* vinden we een RR van 1,54 (95% BI 1,06–2,25). Opnieuw vinden we een vertraging van de adequate therapie bij de resistente bacteriën. Als we in statistische modellen nagaan of dit de verklaring is van de verhoogde sterfte, blijkt dit niet het geval te zijn. Ook blijken in Denemarken de juiste antibiotica voor VRE-bacteriëmieën sneller te worden gegeven dan in Nederland, maar de sterfte is juist hoger in Denemarken. Er blijven daarom twee verklaringen over voor het RR van 1,54: VRE is virulenter dan ARE (d.w.z. heeft een grote capaciteit voor het veroorzaken van ziekte), of het effect is het gevolg van onvoldoende correctie voor *confounding*. Als argument voor die laatste verklaring geldt dat we specifiek voor de

Nederlandse ziekenhuizen nog nauwkeurigere gegevens hadden om de ernst van ziekte voorafgaand aan de infectie in kaart te brengen, en dat correctie met deze gegevens het RR richting 1 werd gebracht. Daarnaast blijft het effect van VRE op sterfte over de loop van een jaar bestaan. Dit vinden wij passend bij een ongemeten verschil in de ernst van onderliggende ziekte (verklaring twee). Tot slot zijn zowel VRE als ARE goed genetisch in kaart gebracht en zijn zij nauwelijks te onderscheiden. Daarmee lijkt een systematisch hogere virulentie van VRE onwaarschijnlijk, hoewel het niet volledig is uitgesloten.

Hoofdstuk 6 gaat verder in op het voorspellen van ESBL-producerende bacteriën als ziekteverwekker wanneer een patiënt zich presenteert met een infectie in het ziekenhuis en er met intraveneuze (d.w.z. per infuus toegediende) antibiotica wordt gestart. In een perifeer en academisch ziekenhuis werden in de periode 2008–2010 9.422 van dit soort sepsis-episodes geselecteerd. De voorafkans op een bacteriëmie met ESBL-producerende bacteriën is echter laag in deze populatie, slechts 0,7%. Het is daarom moeilijk om het onnodig breed voorschrijven van antibiotica in evenwicht te brengen met het identificeren van deze 0,7%. Zoals eerder gemeld beveelt de Nederlandse sepsis-richtlijn aan om bekende kolonisatie met ESBL-producerende bacteriën en recent gebruik van cefalosporines en fluorochinolonen mee te nemen in deze afweging. Net zoals in **hoofdstuk 2** blijkt dat daarmee 50% van de ESBL-bacteriëmieën vooraf kan worden geïdentificeerd; dit is de sensitiviteit. Maar van de hele sepsis-populatie blijkt 19% aan deze criteria te voldoen (anders gezegd: is test-positief). Dit grote aandeel test-positieven komt met name door voorafgaand antibioticagebruik. Als alleen kolonisatie als criterium wordt gehanteerd, daalt het aantal test-positieven naar 4%. De sensitiviteit daalt slechts in lichte mate, naar 42%. Voorafgaand antibioticagebruik heeft dus weinig toegevoegde waarde voor het identificeren van ESBL-bacteriëmieën en leidt tot veelvuldig onnodig breed antibioticumgebruik. Opnieuw blijkt dat behandelend artsen de sepsis-richtlijn slecht opvolgen: van alle patiënten met de genoemde risicofactoren krijgt slechts 27% therapie die volgens de richtlijn in dat geval gepast zou zijn om het risico op een ESBL af te dekken. Desalniettemin is het resultaat toch dat 56% van de patiënten met ESBL-bacteriëmieën vanaf het begin adequate antibiotica kreeg. De conclusie is daarmee dat striktere opvolging van de richtlijn niet leidt tot een betere initiële behandeling van ESBL-bacteriëmieën, terwijl het aantal onnodige voorschriften van brede antibiotica onterecht zou toenemen.

In **hoofdstuk 7** wordt vervolgens nagegaan of er een betere balans mogelijk is tussen sensitiviteit en test-positieven in de sepsis-populatie. Nu worden in acht Nederlandse

ziekenhuizen gegevens verzameld over patiënten met sepsis in de periode 2008–2010. Patiënten die achteraf een ESBL-bacteriëmie blijken te hebben, worden vergeleken met alle overige sepsis-patiënten, op zoek naar nieuwe risicofactoren die beide groepen kunnen onderscheiden. Dit wordt apart uitgevoerd voor buiten het ziekenhuis opgelopen (*community-onset*) infecties (90 ESBL-bacteriëmieën en 360 controlepatiënten) en in het ziekenhuis opgelopen (*nosocomiale*) infecties (82 ESBL-bacteriëmieën en 328 controlepatiënten). Voor beide groepen wordt een risicoscore ontwikkeld. Het blijkt dan dat de voorspelling sterk kan verbeteren als voorafgaand antibioticagebruik veel minder gewicht krijgt in de score en andere factoren worden meegewogen, zoals de vermoede infectiebron (urineweginfectie, longontsteking etc.), leeftijd, opnameduur in het ziekenhuis en bepaalde vormen van onderliggend lijden en voorafgaande interventies. Bekende kolonisatie met ESBL-producerende bacteriën blijft een zeer belangrijke voorspeller. Door een iets ingewikkelder model te hanteren kan, in vergelijking met de risicofactoren van de Nederlandse sepsis-richtlijn, de sensitiviteit worden gehandhaafd, terwijl het percentage test-positieven wordt gereduceerd met 40–49%. Daarmee kan het onnodig voorschrijven van brede antibiotica veilig worden teruggedrongen. Aan de andere kant wordt tegemoet gekomen aan de noodzaak om bij bepaalde patiënten juist deze bredere antibiotica voor te schrijven. Helaas is daarbij geen dekkingsgraad van 100% haalbaar, omdat sommige patiënten toch een ESBL-infectie ontwikkelen zonder duidelijke risicofactoren. De resultaten moeten nog wel in een tweede studie gevalideerd worden voordat zij daadwerkelijk in de praktijk kunnen worden toegepast. Dit gebeurt inmiddels in Europees verband.

In **hoofdstuk 8** wordt een aantal zaken uit dit proefschrift verder bediscussieerd. Er wordt nog eens gesteld dat wij voor zowel resistente Gram-negatieven als VRE geen effect op sterfte van patiënten konden vinden. In vergelijking met andere grootschalige studies zijn wij niet de enigen met dergelijke conclusies, hoewel sommige studies juist weer een groter effect van resistentie op sterfte rapporteerden. Een mogelijke verklaring voor dit verschil is dat het effect van antibioticaresistentie altijd in de context van het beleid rondom antibiotica en diagnostiek moet worden bezien. Als er goed en snel kan worden geanticipeerd op antibioticaresistentie, zoals vermoedelijk het geval is in Nederlandse ziekenhuizen, is het logisch dat er nauwelijks een ziektelast mee gepaard gaat. Om hier meer zicht op te krijgen wordt de aanbeveling gedaan om bijvoorbeeld te analyseren hoe in verschillende landen op verschillende wijze wordt omgegaan met het antibioticabeleid bij infecties, en wat de wisselwerking daarvan met de toestand van de patiënt is. Om dit goed in kaart te brengen moeten studies zich richten op

de dagelijkse ontwikkeling van de ziekte-ernst bij een infectie, in plaats van alleen te kijken naar een uitkomst als sterfte na 30 dagen. Overigens is vooral in lage- en midden-inkomenslanden weinig bekend over de gevolgen van antibioticaresistentie, terwijl de ziektelast daar naar verwachting het grootst is. De studies in dit proefschrift representeren geenszins de wereldwijde ziektelast van antibioticaresistentie. Het is dus belangrijk dat de aandacht van de onderzoeksgemeenschap zich ook op niet-westerse landen richt.

Specifiek rondom VRE woedt al jaren een debat over de vraag of deze nog als BRMO moeten worden beschouwd. BRMO's worden in Nederlandse ziekenhuizen actief bestreden. Veel ziekenhuizen hebben de afgelopen jaren te maken gehad met uitbraken van VRE die erg moeilijk weer onder controle te brengen waren. VRE wordt echter gezien als weinig virulent, waardoor het nut van de bestrijding in twijfel werd getrokken. Nu blijkt uit ons onderzoek ook nog dat het onwaarschijnlijk is dat VRE leidt tot meer sterfte dan de nu alomtegenwoordige variant van *E. faecium*, ARE. Aan de andere kant geldt wel dat de behandeling van VRE-infecties mogelijk duurder is, dat VRE in de toekomst waarschijnlijk weer extra resistentiemechanismes zal verwerven, en dat problemen met VRE een indicator kunnen zijn van problemen met de hygiëne in het ziekenhuis. Deze argumenten gaan echter voorbij aan de definitie van BRMO's die voor bestrijding in aanmerking komen: BRMO's moeten gepaard gaan met een hoge ziektelast en gevolgen hebben voor de empirische antibioticaregimes in Nederlandse ziekenhuizen. Het nut van bestrijding moet namelijk opwegen tegen de kosten die ermee gemoeid zijn.

Tot slot blijkt uit dit proefschrift dat het mogelijk is om op basis van patiëntkarakteristieken beter te anticiperen op antibioticaresistentie bij de presentatie van een infectie. Onderzoek met alleen die patiënten van wie later blijkt dat ze een bacteriëmie hebben, zoals nog steeds gebruikelijk is, kan echter niet direct worden toegepast op het moment dat de arts de eerste antibioticakeuze maakt. Daarom is het van belang om toekomstig onderzoek te richten op de meest relevante patiëntpopulatie: alle patiënten die zich presenteren met een infectie.

Samenvattend kan geconcludeerd worden dat op dit moment in Nederland het antibioticaresistentieprobleem beheersbaar is en niet tot extra sterfte bij patiënten leidt. Het is echt belangrijk om waakzaam te blijven gezien de op wereldwijde schaal continu veranderende epidemiologie van resistente bacteriën.

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About the author

Wouter Rottier was born on 3 October 1987 in 's-Hertogenbosch, the Netherlands. He followed secondary education at Gymnasium Bernrode, Heeswijk, from which he graduated in 2004. Subsequently, he moved to Utrecht for his bachelor's degree at University College Utrecht. There, he followed a liberal arts and sciences education, majoring in science with a focus on biomedical studies. After graduation in 2007, he enrolled in SUMMA, a master's degree in medicine at Utrecht University. As part of this program, he joined the research group on the epidemiology of antibiotic resistance at the department of Medical Microbiology (University Medical Center Utrecht). There, during a research internship supervised by prof. dr. Marc Bonten, the foundation for this thesis was laid.

After obtaining his medical doctor's degree in 2012, Wouter decided to continue his studies in this field. He started his PhD degree at the Julius Center for Health Sciences and Primary Care (University Medical Center Utrecht), with continuous supervision by prof. dr. Marc Bonten, and co-supervision by dr. Heidi Ammerlaan. During the course of his PhD, he followed the postgraduate master's program in epidemiology at Utrecht University, specializing in the epidemiology of infectious diseases. He also joined prof. dr. Evelina Tacconelli's research group at Universitätsklinikum Tübingen in Germany for a three-month internship in 2015.

At the end of 2016, Wouter moved further north in the Netherlands. At the University Medical Center Groningen, under supervision of prof. dr. Alexander Friedrich and dr. Greetje Kampinga, he started his training in medical microbiology, a medical specialty. Simultaneously, he finished the remainder of his PhD project, which culminated in this thesis and his PhD defense on 19 February 2019. As part of his training in medical microbiology, Wouter is currently engaged in an internship at Izore, Center for Infectious Diseases Friesland, under supervision of dr. Jan van Zeijl. He lives together with his fiancée Indira in Groningen.

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