Gram-Negative Infections
-
Burden, Antibiotic Resistance and Empiric Treatment

J.W.T. Deelen

Gram-Negative Infections - Burden, Antibiotic Resistance and Empiric Treatment
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Gram-Negative Infections - Burden, Antibiotic Resistance and Empiric Treatment

Gram-Negatieve Infecties -
Ziektelast, Antibioticaresistentie en Empirische Behandeling
(met een samenvatting het Nederlands)

Proefschrift

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General introduction

J.W.T. Deelen

Antibiotic resistance is a growing global health threat with potentially major consequences, where bacteria acquire resistance to commonly used antibiotics.[1] By means of spreading and selective pressure, resistant bacteria are becoming a large part of the bacterial ecosystem.[2] Since the development of one of the first antibiotics, penicillin, antibiotic resistance has been a point of concern, with the first report on antibiotic resistance to penicillin stemming from 1940.[3] Antibiotic resistance is a problem for two major reasons: 1. The increase of resistance to commonly used antibiotics leads to more difficult to treat – or even untreatable – infections, 2. Prevention of infections may become impossible, and would increase the risk of post-surgical infections, infections during and after chemotherapy, organ transplantations and ICU admissions. This is worsened by a small pipeline of new antibiotics due to complicated drug development processes and low financial incentive for pharmaceutical companies to invest in new antibiotics.

Gram negative infections

Gram-negative bacteria are a subset of bacteria that cause between 25 and 50% of infections in hospitalized patients.[4] Gram-negative bacteria include the Enterobacterales, which consists of pathogens such as *Escherichia coli, Klebsiella* species and *Enterobacter* spp., and the non-fermenters, entailing opportunistic pathogens such as *Pseudomonas* spp. and *Acinetobacter* spp. Gram-negative infections (GNI) caused by these pathogens are a major source of mortality and morbidity. GNI can originate from the community, most often in the form of urinary tract infections, and occur as complications of hospitalization, including post-operative wound infections and pneumonia. *E. coli* is the most frequent cultured causative pathogen of Gram-negative infections. This species, which normally colonizes human intestinal tract, can cause severe infections either by moving to a previously sterile site (e.g. urinary tract) or translocation from the intestines to the bloodstream.

The most severe, and best studied infections are bloodstream infections (BSI), where bacteria enter the bloodstream and cause a systemic inflammatory reaction. The combination of infection and systemic inflammatory reaction, called sepsis, has a mortality ranging from 15 to 37%, depending on severity of disease.[5,6] Bloodstream infections are studied for several reasons. 1. They are clinically relevant, with significant mortality. 2. They can stem from all body sites, and can be complication of e.g. severe urinary tract infections, pneumonia, cholangitis, intra-abdominal infections and skin infections and 3. Diagnosis is unambiguous. Where in other infections the line between colonization and infection can be unclear, a positive blood culture with Gram-negative bacteria is considered proof of infection. However, the focus on BSI is paired with a relative lack of research on non-BSI, while the incidence of the latter is presumably larger, and which may also accompany a significant burden.

The incidence of Gram-negative infections, both in the community and in already hospitalized patients has been increasing in recent years.[7] An aging population, that is also more frequently admitted to hospitals (both for planned and unplanned reasons) and more often receives immunosuppressive treatment may explain this increase. Considering this trend, it can be

assumed that infections will take up a larger share of causes of death in the population.

Antibiotic resistance in Gram-negative bacteria

Antibiotic resistance in Gram-negative bacteria is considered a major threat to safe healthcare by the WHO.[8] Antibiotic resistance can occur for any antimicrobial agent and/or class. Clinically relevant resistance mechanisms are grouped as *multi-drug resistant organism* (MDRO).[9] A few of them are important to address here.

Extended spectrum beta lactamase (ESBL) is a resistance mechanism mediated by enzymes that hydrolyze beta-lactam antibiotics, including amoxicillin, cephalosporins and monosporins.[10] These enzymes are produced by a variety of genes, which have spread across the world and can be located on plasmids or be located on the chromosome. Beta-lactam antibiotics (penicillins, cephalosporins, carbapenems) have been a preferred empiric treatment for suspected infections for their spectrum (which includes many Gram-positive and Gram-negative bacteria), their effectiveness and safety profile. Resistance to these drugs is thus a clinical problem that has an impact on how doctors prescribe treatment for (severe) infections. The prevalence of ESBL-producing Enterobacterales (with all clinically cultured Enterobacterales as a denominator) varies widely across settings and geography. "Low-resistance countries", including the Scandinavian countries and the Netherlands, report that 5-10% of *E.coli* produces ESBL, and 7-12% of *Klebsiella pneumoniae*.[11] This number is 28.7% in Italy for *E.coli* and 53.7% for *K. pneumoniae*. As such, the potential consequences of antibiotic resistance are not equal across countries.

The increase of ESBL is an important driver of increased use of carbapenem-antibiotics. These drugs, also a beta-lactam class of antibiotics, are considered a last-resort antibiotic for many infections, including those caused by ESBL-producing Enterobacterales and *Pseudomonas/Acinetobacter spp*. However, like ESBL, carbapenem resistance is increasing, with high prevalences reported in South-European countries and increasingly low and middle income countries (LMIC's), limiting treatment options in these countries.[11] Another problem with these resistance mechanisms is co-resistance to other drugs. This includes resistance to quinolones, aminoglycosides and co-trimoxazole, and further limits treatment options.

Quantifying the burden of antibiotic resistance in Gram-negative bacteria

As with any public health issue, estimating the size of that problem is one of the first steps in determining strategies to combat it. Additionally, since resources for health care allocation are limited, assessing the current and future burden is essential for providing an evidence base for healthcare spending. The burden can be estimated in several ways, and most commonly involves mortality, costs and morbidity. In this thesis, we focus primarily on mortality caused by GNI and antibiotic resistance, although morbidity and costs may constitute a major part of the burden.

There are some important observations when investigating the literature on mortality caused

by antibiotic resistance. The first is that there is a strong focus on bloodstream infections. The second is that many studies are from high-resistance countries. While this is not a problem in itself, we don't know if these findings are generalizable to lower-resistance countries. Additionally, the focus on antibiotic resistance may obfuscate that a significant part of the disease burden by Gram-negative infections may be due to infections with susceptible micro-organisms.

Estimating the mortality caused by antibiotic resistance is less easy than it seems. To start, it is a causal question. However, the gold standard in epidemiological research for causal inference is a randomized controlled trial which is unfeasible (and perhaps unethical) for investigating antibiotic resistance.[12] One important reason to conduct a randomized trial is the removal of confounding bias. However, with antibiotic resistance, only observational studies are possible, which means confounders must be understood and measured, so you can adjust for those variables.

Confounding plays a crucial role in antibiotic resistance. Antibiotic resistance is mainly a consequence of healthcare exposure, in the form of hospital admission, exposure to antibiotics, and susceptibility to infections. This means that comorbidities, pathogens, admission related variables (e.g. community onset vs hospital onset), prior surgery/procedures (might introduce pathogens to a sterile site), immunosuppression, prior antibiotic use (which may predispose someone to death since one has been weakened by a previous infection) and infection related variables (infection source) are all important factors to consider. Additionally, with an acute event like infection, timing of measurements of confounders is important, since, for example, disease severity occurring after infection onset, is an intermediate variable in the pathway to mortality, and not a confounder.[13] For a long time, it has also been a discussion whether we should compare resistant infections to sensitive infections or to non-infected patients to assess the mortality burden.[14] The difference in effect sizes between these two control groups may be large.

Aside from these methodological issues, it is important to realize that there are only a few ways through which antibiotic resistance can increase mortality. Inappropriate treatment, side effects from medication (e.g. kidney damage from aminoglycosides), increased virulence/pathogenicity and consequences of isolation strategies are the four ways by which antibiotic resistance can increase mortality. Of these, virulence cannot be measured in a reliable way, and consequences of isolation strategies are rarely measured. If these could be measured, adjusting for these measurements should theoretically remove any effect of antibiotic resistance on mortality, and leaves us with the effect of confounders.

Recent developments have introduced several concepts to improve causal inference from observational studies. For one, it is considered good practice to use prior knowledge of the causal mechanisms and possible confounders, and to state that knowledge explicitly.[15,16] This is contrasted by the 'old-fashioned' way of causal inference, where a stepwise regression analysis,

leading to several 'significant predictors', would be the way to analyze a study. Another point is an explicit definition of the causal contrast. These kind of assumptions, on confounders, can be visualized in directed acyclic graphs, which is a tool to visualize causal relationships, and can assist with selecting variables for the analysis.[17]

There are several studies that have quantified the mortality due to bloodstream infections (BSI) caused by ESBL-producing pathogens. A systematic review from 2012 shows increased mortality in ESBL-BSI, with an OR of 1.52 (95% CI 1.15–2.01).[18] When taking the methodological aspects from the previous paragraphs into account, there are some interesting issues. Many studies adjust for inappropriate therapy, which is one of the ways that antibiotic resistance (in this case ESBL) causes mortality. By adjusting for this intermediate variable, a part of the attributable mortality is adjusted for, and thus results in an underestimation of the mortality burden.[19]

This problem is compounded by the use of individual studies on the burden of resistance in meta-analyses on the burden of disease. In a landmark paper from 2018 by Cassini et al, the European burden for several kinds of antibiotic resistance is calculated in the form of disability adjusted life years (DALY), and specific numbers are given per country.[20] However, since individual studies may use less than optimal methodology (regarding study design and statistical analysis) to estimate the burden, the pooled estimations are biased by unknown amounts.[21] Currently, many studies on the burden of carbapenem resistance are published, and the methods of these studies with regard to causal inference have not been studied separately. [22–25]

Empiric treatment strategies for antibiotic resistant Gram-negative infections

The presence of antibiotic resistance in Gram-negative bacteria causes uncertainty for clinicians by increasing the risk of inappropriate therapy. At the onset of an infection, the causative pathogen is unknown and culturing and determining the resistance pattern may take up to three days. During this time, antibiotic treatment has to be prescribed, while the pathogen is still unknown. This initial antibiotic treatment is called empiric antibiotic therapy. In the worst case, inappropriate empiric treatment can result in the patient's state worsening or death.[26–28]

Every time a doctor sees a patient with a suspected infection, a decision must be made regarding empiric therapy. This choice is more acute when the patient is more severely ill (e.g. suspected bloodstream infection). This choice is codified in guidelines, where the general recommendation is to cover the most common causative pathogens (with a second or third-generation cephalosporin in the Netherlands).[29] However, if a doctor is excessively worried (by a severely ill patient), has assumptions about the causative pathogen (due to prior cultures) or is overall defensive, he may choose for a broader-spectrum antibiotic (e.g. carbapenems) that surely would result in appropriate empirical treatment. However, if all doctors would make this decision, there would be a large increase in carbapenem use, resulting in a higher

risk of developing carbapenem resistance. This makes it a classical tragedy of the commons situation, where individual benefit (adequate treatment of patient) must be weighed against the greater whole (occurrence of resistance).[30]

Current data suggests carbapenems are liberally prescribed, and that this has been increasing in the last decade in European countries (Antimicrobial Consumption database, ESAC-NET). Often, carbapenem use is unnecessary, either prescribed for too long of a time, or prescribed for patients who did not have a proper indication.[31,32] At the same time, there is evidence for increasing use of carbapenems leading to more resistance.[33] Thus, reduction of unnecessary empiric carbapenem use may reduce the growth of antibiotic resistance to carbapenems.

Prediction rules have been considered as potential tools to guide empiric treatment.[34] If prior to initiation of empiric treatment, a risk stratification could be made based on easily available clinical parameters, it could help with selecting the right antibiotic. Currently, a form of risk stratification is being employed in Dutch hospitals. In patients with a suspected bloodstream infection, prior colonization with an ESBL-producing pathogen and/or use of fluoroquinolones in the 30 days prior to infection onset, it is advised to either prescribe a carbapenem or add an aminoglycoside to empiric treatment to mitigate the risk of a potential ESBL-producing pathogen. A study from Rottier et al in 2015 showed that this strategy is suboptimal, and that clinicians both do not follow the guidelines and that actually following the guidelines would lead to a large increase in unnecessary carbapenem use.[35]

There are several other prediction rules for antibiotic resistance in Gram-negative bacteria, and for ESBL BSI in particular.[36–38] However, currently, none of them are actually applied in practice. There are several reasons that may explain this lack of use. Most of the prediction rules have reasonable discrimination, but often poor calibration. Second, they are often developed in a patient population with Gram-negative BSI, thus only providing prediction at the point where you know it is a Gram-negative bloodstream already, which can be 12 hours to two days after initiation of empiric treatment. Third, most of them have not been validated, or validation was unsuccessful (due to overfitting or other problems with the original model. Thus, we are still in need of a prediction rule that can actually help with reducing unnecessary carbapenem use.

Next to carbapenems, the Dutch national sepsis guidelines suggest adjunctive aminoglycosides next to beta-lactam antibiotics as an alternative for the treatment of sepsis/suspected bloodstream infection for patients with a risk of ESBL.[29] This is generally a short course (1-2 days). In locally adapted guidelines however, adjunctive short-course aminoglycosides have become the standard therapy for sepsis (combined with amoxicillin-clavulanic acid, cefuroxime or ceftriaxone). The rationale for this treatment is that it broadens the antimicrobial spectrum and there is a supposed synergistic bactericidal effect from combination treatment.[39] Other hospitals have a policy to reduce 3rd generation cephalosporin and carbapenem use and

prescribe aminoglycosides to maintain adequate levels of appropriate treatment.

In a Cochrane review from 2014, there did not seem to be a beneficial effect from adjunctive aminoglycosides in the treatment of several infectious diseases.[40] However, the use of short course was not explicitly analyzed as part of this review. Thus, it is unclear if this strategy results in better outcomes for patients. The risks of aminoglycosides are, however, well known, and include a risk of kidney damage and hearing loss even with a course of short duration. In a recent study in ICU patients from two hospitals in the Netherlands, adjunctive aminoglycosides were associated with an increased risk of kidney failure in patients with septic shock.[41,42] It is thus interesting that this practice is so widespread and heterogeneous, not based on a lot of evidence, and inconsistent between Dutch hospitals.

Aim and Thesis outline

In this thesis we address the burden of Gram-negative infections and antibiotic resistance in the Netherlands, methods to study the attributable burden of antibiotic resistance, and strategies to optimize empiric treatment in patients with suspected gram-negative infections.

The thesis starts off with a description of the epidemiology of Gram-negative infections in the Netherlands, which is then used to estimate the number of Gram-negative infections in hospitalized patients in the Netherlands (**Chapter 2**). In this study, we provide a broad overview of the clinical aspects of Gram-negative infections, including the causative pathogens, the number of infections caused by highly resistant micro-organisms and finally, the number of infections and deaths per year. This provides the landscape in which clinicians treat their patients.

Chapter 3 then dives deep into the mortality burden of antibiotic resistance in Gram-negative infections in the Netherlands. In this study, using the same data as in chapter 2, we compare MDRO Gram-negative infections with non-MDRO Gram-negative infections and non-infected control patients in the form of a parallel matched cohort study, to answer the question whether MDR-organisms cause additional mortality. In **Chapter 4**, we zoom out and address methodological issues with assessing the burden of antimicrobial resistance. In recent years, increasing interest for causal inference in observational studies led to the development of several frameworks that could improve the quality of causal estimates. In this study, we analysed the methodology of recent studies assessing the burden of carbapenem resistant bloodstream infections, the causal models they present are applied to two datasets, and the findings are put into perspective by relating them to the counterfactual framework.

The second part of the thesis involves the empiric treatment of infections.

In **Chapter 5,** in what can be considered a magnum opus, we describe how two prediction rules for third-generation cephalosporin resistant Enterobacterales that were developed in the Netherlands perform in an international setting. Thirty-three hospitals in thirteen countries were involved in this study to analyze the predictive value of a community onset and hospital

onset prediction rule, and potential reductions in carbapenem us.

We further delve into antibiotic treatment of Gram-negative bloodstream infections in the Netherlands in **Chapter 6**, where we analyze the treatment strategy of adjunctive short-course aminoglycosides as empiric therapy versus beta-lactams alone. Since there is large variation between hospitals in the Netherlands regarding this treatment choice, an analysis of this may show the direction we want to take.

In **Chapter 7**, the general discussion, the findings are put into perspective.

References

1. Roca, I. *et al.* The global threat of antimicrobial resistance: Science for intervention. *New Microbes New Infect.* 6, 22–29 (2015).
2. Davies, J. & Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74, 417–33 (2010).
3. Abraham, E. P. & Chain, E. An enzyme from bacteria able to destroy penicillin. 1940. *Rev. Infect. Dis.* 10, 677–8 (1940).
4. Biedenbach, D. J., Moet, G. J. & Jones, R. N. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). *Diagn. Microbiol. Infect. Dis.* 50, 59–69 (2004).
5. Fitzpatrick, J. M. *et al.* Gram-negative bacteraemia; a multi-centre prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. *Clin. Microbiol. Infect.* 22, 244–251 (2016).
6. Kang, C. *et al.* Bloodstream Infections Caused by Antibiotic-Resistant Gram-Negative Bacilli : Risk Factors for Mortality and Impact of Inappropriate Initial Antimicrobial Therapy on Outcome Bloodstream Infections Caused by Antibiotic-Resistant Gram-Negative Bacilli : Ris. *Antimicrob. Agents Chemother.* 49, 760–766 (2005).
7. Kaye, K. S. & Pogue, J. M. Infections Caused by Resistant Gram-Negative Bacteria: Epidemiology and Management. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* 35, 949–962 (2015).
8. WHO. Antimicrobial resistance: Global report on Surveillance. (2014).
9. Magiorakos, A.-P. *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 18, 268–81 (2012).
10. Paterson, D. L. & Bonomo, R. A. Clinical Update Extended-Spectrum Beta-Lactamases : a Clinical Update. *Clin. Microbiol. Rev.* 18, 657–686 (2005).
11. ECDC. Antimicrobial resistance surveillance in Europe 2014. (2015).
12. Hernán, M. A. & Robins, J. M. *Causal Inference*. (Chapman & Hall/CRC, forthcoming, 2019).
13. Thom, K. A. *et al.* Controlling for Severity of Illness in Outcome Studies Involving Infectious Diseases: Impact of Measurement at Different Time Points. *Infect. Control Hosp. Epidemiol.* 29, 1048–1053 (2008).
14. Kaye, K. S., Engemann, J. J., Mozaffari, E. & Carmeli, Y. Reference group choice and antibiotic resistance outcomes. *Emerg. Infect. Dis.* 10, 1125–8 (2004).
15. Robins, J. M. Data, design, and background knowledge in etiologic inference. *Epidemiology* 12, 313–20 (2001).
16. Hernán, M. a, Hernández-Díaz, S., Werler, M. M. & Mitchell, A. A. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am. J. Epidemiol.* 155, 176–84 (2002).
17. Shrier, I. *et al.* Reducing bias through directed acyclic graphs. *BMC Med. Res. Methodol.* 8, 70 (2008).
18. Rottier, W. C., Ammerlaan, H. S. M. & Bonten, M. J. M. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum ß-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. *J. Antimicrob. Chemother.* 67, 1311–20 (2012).
19. Schwaber, M. J. & Carmeli, Y. Antimicrobial resistance and patient outcomes: the hazards of adjustment. *Crit. Care* 10, 164 (2006).

20. Cassini, A. *et al.* Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect. Dis.* 19, 56–66 (2019).
21. Samuel Shapiro. Meta-analysis Shmeta-analysis.pdf. *Am. J. Epidemiol.* 140, 771–778 (1994).
22. Hussein, K. *et al.* Impact of carbapenem resistance on the outcome of patients' hospital-acquired bacteraemia caused by Klebsiella pneumoniae. *J. Hosp. Infect.* 83, 307–313 (2013).
23. Zheng, S.-H. *et al.* Risk factors, outcomes and genotypes of carbapenem-nonsusceptible *Klebsiella pneumoniae* bloodstream infection: a three-year retrospective study in a large tertiary hospital in Northern China. *Infect. Dis. (Auckl).* 0, 1–9 (2018).
24. Satlin, M. J. *et al.* Bacteremia due to carbapenem-resistant Enterobacteriaceae in neutropenic patients with hematologic malignancies. *J. Infect.* 73, 336–45 (2016).
25. Mouloudi, E. *et al.* Bloodstream infections caused by carbapenemase-producing klebsiella pneumoniae among intensive care unit patients after orthotopic liver transplantation: Risk factors for infection and impact of resistance on outcomes. *Transplant. Proc.* 46, 3216–3218 (2014).
26. Davey, P. G. & Marwick, C. Appropriate vs . inappropriate antimicrobial therapy. *Clin. Microbiol. Infect.* 14, 15–21 (2008).
27. Tellor, B. *et al.* Inadequate Source Control and Inappropriate Antibiotics are Key Determinants of Mortality in Patients with Intra-Abdominal Sepsis and Associated Bacteremia. *Surg. Infect. (Larchmt).* 16, 785–793 (2015).
28. Girometti, N. *et al.* Klebsiella pneumoniae bloodstream infection: epidemiology and impact of inappropriate empirical therapy. *Medicine (Baltimore).* 93, 298–309 (2014).
29. Stichting Werkgroep Antibioticabeleid. SWAB guidelines for Antibacterial therapy of adult patients with Sepsis. (2010).
30. Hardin, G. The tragedy of the commons. The population problem has no technical solution; it requires a fundamental extension in morality. *Science* 162, 1243–8 (1968).
31. Janssen, J., Kinkade, A. & Man, D. Carbapenem utilization evaluation in a large community hospital (CARBON): A quality improvement study. *Can. J. Hosp. Pharm.* 68, 327–331 (2015).
32. Seah, V. X. F. *et al.* Impact of a carbapenem antimicrobial stewardship program on patient outcomes. *Antimicrob. Agents Chemother.* 61, 1–9 (2017).
33. Yang, P. *et al.* Association between antibiotic consumption and the rate of carbapenem-resistant Gram-negative bacteria from China based on 153 tertiary hospitals data in 2014. *Antimicrob. Resist. Infect. Control* 7, 1–7 (2018).
34. Pogue, J. M., Kaye, K. S., Cohen, D. A. & Marchaim, D. Appropriate antimicrobial therapy in the era of multidrug-resistant human pathogens. *Clin. Microbiol. Infect.* 21, 302–312 (2015).
35. Rottier, W. C. *et al.* Predictive value of prior colonization and antibiotic use for third-generation cephalosporin-resistant enterobacteriaceae bacteremia in patients with sepsis. *Clin. Infect. Dis.* 60, 1622–1630 (2015).
36. Kim, M. *et al.* Clinical prediction score for community-onset bloodstream infections caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species. *J. Korean Med. Sci.* 34, 1–13 (2019).
37. Augustine, M. R. *et al.* Clinical Risk Score for Prediction of Extended-Spectrum ß-Lactamase-Producing Enterobacteriaceae in Bloodstream Isolates. *Infect. Control Hosp. Epidemiol.* 38, 266–272 (2017).
38. Johnson, S. W., Anderson, D. J., May, D. B. & Drew, R. H. Utility of a clinical risk factor scoring model in predicting infection with extended-spectrum ß-lactamase-producing enterobacteriaceae on hospital admission. *Infect. Control Hosp. Epidemiol.* 34, 385–92 (2013).
39. Davis, B. D. Bactericidal synergism between ß-Lactams and aminoglycosides: Mechanism and possible therapeutic implications. *Reviews of Infectious Diseases* 4, 237–245 (1982).
40. Paul M, L. L. Cochrane Database of Systematic Reviews Beta lactam antibiotic monotherapy versus beta lactam- aminoglycoside antibiotic combination therapy for sepsis (Review). *Cochrane Database Syst. Rev.* (2014). doi:10.1002/14651858.CD003344.pub3
41. Prayle, A., Watson, A., Fortnum, H. & Smyth, A. Side effects of aminoglycosides on the kidney, ear and balance in cystic fibrosis. *Thorax* 65, 654–658 (2010).
42. Picard, W. *et al.* Propensity-Based Study of Aminoglycoside Nephrotoxicity in Patients with Severe Sepsis or Septic Shock. *Antimicrob. Agents Chemother.* 58, 7468–7474 (2014).

CEPHALOSPORINS CARBAPENEMASE DISEASE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL MORTALITY DISEASE BURDEN GRAM-NEGATIVE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET PROPENSITY SCORE INFECTION COHORT METHODS GRAND-ABC BURDEN CEPHALOSPORINS BACTERIA BLOODSTREAM INFECTION MORTALITY PREDICTION GRAM-NEGATIVE INFECTION PREDICT ANTIBIOTIC CEPHALOSPORINS CARBAPENEMASE DISEASE TRIAL MORTALITY ANTIBIOTIC PREDICT BLOODSTREAM GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL ANTIBIOTIC DISEASE CHAPTER 2 CAUSAL INFERENCE MORTALITY GRAM-NEGATIVE BACTERIA PREDICTION GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM

The burden of bacteremic and non-bacteremic Gram-negative infections: a prospective multicenter cohort study in the Netherlands

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Manuscript under review

Abstract

Introduction

There is a global increase in community and hospital-onset infections caused by Gram-negative bacteria, but the burden of both bacteremic and non-bacteremic Gram-negative infections (GNI) in hospitalized patients has not been determined. We provide a comprehensive description of the epidemiology and burden of bacteremic and non-bacteremic Gram-negative infections in hospitalized patients in the Netherlands.

Methods

We conducted a prospective cohort of patients in eight hospitals with microbiologically confirmed GNI between June 2013 and November 2015. In each hospital the first five adults meeting the eligibility criteria per week were enrolled during a one-year period. We used descriptive statistics for population characteristics, management and patient outcome. We estimated the national incidence and mortality of GNI by combining the cohort data with the national surveillance database for antimicrobial resistance, using data from 2016.

Results

1,954 patients with GNI were included of which 725 (37%) were community-acquired, 681 (35%) healthcare associated, and 548 (28%) hospital-acquired infections. In all, 758 (39%) patients had bloodstream infection (BSI) and the most frequent sources of infection were the urinary tract (52%) and abdomen (19%). Most infections (n=1,661, 85%) were monomicrobial, and caused by *Escherichia coli* (n=997, 60%), and 243 GNI (12%) involved multi-drug resistant pathogens, mainly because of resistance to 3rd-generation cephalosporins (n=189, 78%). Source control was performed in 664 (34%) patients. 30-day mortality rates were 11.1% (n=217) for all patients and 15.6% (n=118) for those with BSI. Estimated national incidences of non-bacteremic GNI and bacteremic GNI in hospitalized adults were 74 (95% CI 58 – 89) and 86 (95% CI 72-100) per 100,000 person years, respectively, yielding estimated annual numbers of 30-day all-cause mortality deaths of 1,528 (95% CI 1,102-1,954) for bacteremic and of 982 (95% CI 688 – 1,276) for non-bacteremic GNI.

Conclusions

The clinical presentation of microbiologically confirmed GNI in hospitalized patients is diverse. The estimated incidences of non-bacteremic GNI and bacteremic GNI were 74 and 86 per 100,000 person years. The estimated annual numbers of deaths occurring in the 30 days after infection onset were 1,528 for bacteremic GNI and 982 for non-bacteremic GNI.

Introduction

Infections caused by Gram-negative bacteria, such as Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* species, also called Gram-negative infections (GNI), are associated with significant mortality, morbidity and health care costs[1] Such infections occur in community-dwelling subjects and may also complicate treatment in healthcare facilities as post-operative wound infections, urinary tract infections (UTI) and catheter-related bloodstream infections (CRBSI).[2] The incidence of these infections is rising partly because of aging, and the global increase in antibiotic resistance increasingly hampers successful treatment.[3]

Our current understanding of the epidemiology of GNI in hospitalized patients is almost exclusively based on bloodstream infections (BSI). Additionally, studies on the burden of Gram-negative infections strongly focused on estimating the burden caused by antibiotic resistance. However, these comprise only a subset of all GNI in hospital settings and consequently, the burden arising from non-bacteremic GNI and non-resistant GNI is unknown.

We therefore aimed to comprehensively evaluate the epidemiology of GNI, including bacteremic and non-bacteremic GNI, associated pathogens, clinical management and patient outcomes in a representative cohort of hospitalized patients in the Netherlands, and used that data to estimate the national incidence and mortality associated with GNI.

Methods

Setting and study population

We conducted a prospective cohort study in eight hospitals in the Netherlands (seven secondary care hospitals, one tertiary care hospital) between June 2013 and November 2015, the GRAND-ABC study. In each hospital, trained research nurses weekly screened consecutive clinical cultures (not taken for screening purposes) of the previous week and included the first five adult patients (>18 years) that met all of the following criteria; (1) culture results involved Enterobacterales and/or non-fermenters (*Pseudomonas, Acinetobacter* and *Stenotrophomonas* spp.; (2) episode constituted a new infection according to the respective CDC-criteria for infection[4]; (3) for skin and pulmonary infections, prescribed antibiotics were required to cover the cultured pathogen.

Definitions

Infections were considered hospital-acquired if the sample was taken =>48h after hospital admission and healthcare-associated if the sample was taken <48h after hospital admission and the patient had been hospitalized ≥2 nights in the last three months, was on dialysis, received intravenous therapy (e.g. chemotherapy) within the last 30 days or resided in a nursing home or other long term care facility.[5] All other infections were considered community-acquired.

Antibiotic susceptibility was tested according to local laboratory practices using EUCAST

criteria and bacteria were considered Highly Resistant Microorganisms (HRMO) according to modified Dutch guidelines (supplement A). GNI were categorized as mono- or polymicrobial. In the latter case all relevant Gram-negative bacteria from index cultures were included in the analyses of antimicrobial susceptibilities.

The source of infection was based on the CDC-criteria for infections.[4] Some infections were initially included as "secondary bloodstream infection". We retrospectively added the specific source as well if we considered the source to be unambiguously clear based on the patient records.

Comorbidities were scored according to the Charlson Comorbidity Index.[6] Patients were considered immunocompromised if they had at least one of the following: (1) chemotherapy in the last 30 days; (2) high dose corticosteroids (≥20 mg prednisone or equivalent per day) for more than two weeks at time of GNI; (3) neutropenia ($<0.5*10^9$ cells/L); or (4) immunosuppressive drugs in the last three months.

Sepsis severity was classified according to the SEPSIS-II-guidelines as sepsis, severe sepsis or septic shock.[7] Every bacteremic GNI was per definition classified as at least sepsis. The occurrence of each of the following source control procedures was registered by trained research nurses: abscess drainage, necrotectomy, amputation, removal of prosthesis, heart valve surgery, joint flushing, other surgical procedures, insertion/exchange of bile duct stent, removal of urinary catheter and removal of central venous catheter. Other source control procedures were included if reported as such in patient charts after adjudication by one of the investigators.

Clinical outcomes included clinical cure at discharge or 14 days after GNI onset, whichever occurred first; 30-day all-cause mortality; length of stay post infection; and ICU admission post-infection. Reasons for not reaching clinical cure at day 14 were recorded.

For estimating the national burden of GNI we used data from the "Infectious Disease Surveillance Information System for Antimicrobial Resistance" (ISIS-AR).[8] This database is maintained at the national institute for public health (RIVM) and contains all positive culture results and susceptibility data from routine diagnostics in participating medical microbiology laboratories. In 2016 it was estimated that participating laboratories covered 60-75% of the hospitalized population in the Netherlands. From the database we extracted data of isolates of Enterobacterales, *Pseudomonas* spp. and *Acinetobacter* spp. from in-hospital (including emergency department) adult patients in 2016. For our analyses we included all culture results obtained in a hospital labeled as 'diagnostic' or 'unknown', thereby excluding screening cultures (e.g. rectal swabs), from hospitalized adults.

Ethics

The Ethics Committee of the University Medical Center Utrecht exempted the study from formal review and waived the requirement of informed consent due to the observational

nature of the study.

Analysis

Continuous data are described in means and standard deviation or median and inter-quartile range, as appropriate. Event rates and proportions are reported as percentage with corresponding 95% confidence intervals. As this is a descriptive study, no formal statistical tests were performed.

To determine the national incidence of GNI, we related our prospective cohort findings to the ISIS-AR database. This database contains isolate data without clinical information, and findings may not reflect a new episode of GNI (but a repeated positive culture instead). In our prospective cohort study, we evaluated, each week, consecutive culture results to identify the first five new episodes of GNI. We thus excluded screening cultures and isolates considered to be part of an ongoing episode of GNI. We also excluded upper-respiratory tract cultures from both databases, as these are not used to clinically assess infection in the participating hospitals. In addition, non-blood isolates that were used to diagnose a source of BSI were excluded from the GRAND-ABC database, and this percentage (6%) was also removed from the non-blood isolates in the ISIS-AR database, since these isolates would have been used to diagnose a new infection and thus be counted twice. As a result, we could determine the average number of isolates (with available resistance data) required to ascertain one new infection. As an example, out of 5 urinary cultures, two may not satisfy the CDC-criteria for infection (no symptoms), two may have been taken a day after a previous urine culture for which therapy was already started (repeated culture), and one did actually involve a new UTI with symptoms and initiation of antibiotic therapy. This means the ratio of "new episode of infection" to "isolates reported" would be 1:5, or 20%.

We applied this ratio to the ISIS-AR database to estimate the total number of GNI in the Netherlands. From this number, we calculated estimates of HRMO infections and 30-day mortality by calculating those respective proportions. To take sampling variation into account, we calculated the distributions (100.000 samples) for isolate:infection ratio (proportion), the different proportions (for prevalence of HRMO and 30-day mortality risk estimated in the GRAND-ABC database; binomial distribution) and number of cultures in the ISIS-AR database (Poisson distribution). By multiplying these distributions and taking the 2.5% and 97.5% percentiles, we calculated the 95% confidence interval. We assumed a national coverage of the ISIS-AR data of 75% for the lower bound and 60% for the upper bound of the 95% CI to generate a conservative confidence interval (see supplement B for a flowchart of this process). The point estimate was calculated by taking the mean of the 95% CI boundaries. Finally, we calculated the national incidence of GNI in 100.000 person-years by extrapolating the total number of GNI to the total Dutch population (13,542,471 people older than 18 years in 2016[9]). We performed all analyses separately for total number of infections, bacteremic and non-bacteremic GNI.

Results

Patients

A total of 1,954 GNI episodes were included, of which 725 (37.1%) were community-acquired, 681 (34.9%) were healthcare-associated, and 548 (28.0%) were hospital-onset infections (Table 1).

Table 1: Patient and infection characteristics in all, non-bacteremic GNI and bacteremic patients with Gram-negative infections in the GRAND-ABC cohort

	Overall	**Non-bacteremic GNI**	**Bacteremic GNI**
n	1954	1196	758
Demographics			
Age (mean (sd))	68.87 (15.7)	67.2 (16.2)	71.6 (14.1)
Female	915 (46.8)	591 (49.2)	324 (42.7)
Comorbidity			
Charlson Comorbidity index (median, IQR)	2 [1 - 3]	2 [0 – 3]	2 [1 - 4]
Immunocompromised	235 (12.0)	133 (11.1)	104 (13.7)
Prior colonization HRMO	129 (6.6)	86 (7.2)	56 (10.4)
Surgery in 30d prior	386 (19.8)	285 (23.8)	101 (13.3)
Origin			
- Community acquired	725 (37.1)	406 (33.9)	319 (42.1)
- Healthcare associated	681 (34.9)	385 (32.2)	296 (39.1)
- Hospital onset	548 (28.0)	405 (33.9)	143 (18.9)
Ward of index culture			
- Surgical ward	544 (27.8)	439 (36.7)	105 (13.9)
- Intensive care unit	74 (3.8)	38 (3.2)	36 (4.7)
- Internal medicine	520 (26.6)	341 (28.5)	179 (23.6)
- Emergency ward	816 (41.8)	378 (31.6)	438 (57.8)
LOS prior to infection (hospital-onset-infections, IQR)	8.0 [5.0 - 15.0]	8 [5.0 – 14.0]	10 [5.0 – 19.5]
Source of infection			
Primary BSI	91 (4.7)	-	91 (12.0)
Urinary tract	1008 (51.6)	602 (50.3)	406 (53.6)
Abdominal	378 (19.3)	206 (17.2)	172 (22.7)
Respiratory	165 (8.4)	142 (11.9)	23 (3.0)
Skin and soft tissue	223 (11.4)	202 (16.9)	21 (2.8)
Bone and joint	25 (1.3)	22 (1.8)	3 (0.4)
Other	64 (3.3)	22 (1.8)	42 (5.5)
Sepsis severity			
No sepsis	460 (18.4)	460 (38.5)	-
Sepsis	1193 (61.1)	614 (51.3)	579 (76.4)
Severe sepsis	164 (8.4)	72 (6.0)	92 (12.1)
Septic shock	157 (8.0)	50 (4.2)	87 (11.5)
30 day mortality	217 (11.1)	99 (8.3)	118 (15.6)

Data are given as N (%) unless otherwise indicated.

In all, 758 (38.8%) episodes were BSI (44.0%, 43.4% and 26.1% of community-acquired, healthcare-associated and hospital-onset, respectively). The most common infection sources were the urinary tract (n=1008, 51.6%), the abdomen (n=378, 19.3%) and skin and soft tissue (n=223, 11.4 %). Samples for microbiological cultures were most often obtained at the emergency department (n=816; 41.7%), in 27.8% from surgical wards (n=544), in 26.6% from medical wards (n=520) and in 74 episodes (3.8%) from intensive care units.

Bacteremic GNI were, compared to non-bacteremic GNI, associated with older age (71.6 vs 67.2), community onset infections (42.1% vs 33.9%) and septic shock (11.5% vs 4%), whereas non-bacteremic GNI were associated with female sex (49.4% vs 42.7%), prior surgery (23.8% vs 13.3%) and hospital-onset infections (33.9% vs 18.9%). For further stratification per infection type, see supplement C.

Second generation cephalosporins were most often prescribed as initial antimicrobial treatment for bacteremic as well as non-bacteremic GNI (overall 31.1%), followed by third-generation cephalosporins (23.3%) (Table 2). In 173 episodes (8.8%), no antibiotics were prescribed in the first 24 hours after sample obtainment. Source control was performed in 664 episodes (34.0%). For a breakdown of types of source control, see supplement D.

Table 2: Antibiotic treatment and source control of Gram-negative infections in the GRAND-ABC cohort

	Total (n (%))	**Non-bacteremic GNI (n=1028) (%)**	**Bacteremic GNI (n=690) (%)**
Initial antibiotic treatment			
Penicillins*	365 (21.7)	202 (20.1)	163 (24.1)
2G-cephalosporins	522 (31.1)	270 (26.9)	252 (37.2)
3G-cephalosporins	392 (23.3)	197 (19.6)	195 (28.8)
Carbapenems	64 (3.8)	33 (3.3)	31 (4.6)
Fluoroquinolones	208 (12.4)	147 (14.6)	61 (9.0)
Co-trimoxazole	60 (3.6)	40 (4.0)	20 (3.0)
No AB first day after culture	173 (8.8)	121 (11.8)	52 (7.5)
Source control**	664 (34.0)	436 (36.5)	228 (30.1)

*Includes amoxicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam. **Medication missing from one hospital. Data on source control is from all hospitals.

Pathogens

The vast majority of infections was caused by a single pathogen (n=1,661, 85.0%), which most frequently was *Escherichia coli* (n=997; 60%), followed by *Klebsiella pneumoniae* (n=144; 8.7%) and *Pseudomonas aeruginosa* (n=144; 8.7%) (Table 3). The same bacterial species were predominant in polymicrobial infections. Overall, 243 infections (12.3%) involved HRMO, according to Dutch definitions; 189 (77.8% of HRMO) Enterobacterales were resistant to 3rdgeneration cephalosporins (116 being *E. coli*); 69 Enterobacterales (28.4%) were resistant to

both aminoglycosides (gentamicin and/or tobramycin) and fluoroquinolones (ciprofloxacin) (48 being *E. coli*) and 2 (0.8%) Enterobacterales were resistant to carbapenems. For the pathogen and HRMO distribution by infection source, see supplement E.

Table 3: Microbiological cause of Gram-negative infections in the Grand-ABC cohort

	Overall (n=1954)	Non-bacteremic GNI	Bacteremic GNI
E. coli	997	551 (46.1)	446 (58.8)
HRMO (%)	116 (11.6)	69 (12.5)	47 (10.5)
E. cloacae	72	48 (4.0)	24 (3.2)
HRMO (%)	25 (34.7)	21 (43.8)	4 (16.7)
K. pneumoniae	144	79 (6.6)	65 (8.6)
HRMO (%)	12 (8.3)	6 (7.6)	6 (9.2)
P. mirabilis	99	80 (6.7)	19 (2.5)
HRMO (%)	3 (3.0)	3 (3.8)	0 (0.0)
P. aeruginosa	144	101 (8.4)	43 (5.7)
HRMO (%)	9 (6.2)	6 (5.9)	3 (7.0)
Other species	205	143 (12.0)	62 (8.2)
HRMO (%)	22 (10.7)	17 (11.9%)	5 (8.1%)
Multiple species	293	194 (16.2)	99 (13.1)
HRMO (%)	56 (19.1)	43 (22.2)	13 (13.1)

Infection outcomes

All-cause 30-day mortality was 11.1% (217 of 1,954); 15.6% (118 of 758) among patients with bacteremic GNI and 8.3% (99 of 1196) among those with non-bacteremic GNI (table 1). Of all patients, 1,523 (78%) were considered clinically cured within 14 days and 113 (6.7%) had died within 14 days. ICU-admission after infection onset occurred in 184 cases (9.4%). Absence of clinical cure at day 14 (ongoing infection) was associated with hospital-onset infections (40.8% hospital-onset in no cure vs 24.4% in patients that were cured?), prior surgery (30.4% vs 17.4%) and skin infections (27.8% vs 6.8%). See supplement F for reasons of non-cure and expansions of infections.

Burden of Gram-negative infections in the Netherlands

In the prospective cohort study, 14,749 Gram-negative cultures were screened to include 1,954 GNI episodes, yielding a screening-infection ratio of 7.5:1. The national database contained (for 2016) 97,751 cultures yielding Gram-negative bacteria. From the screening to infection episode ratio and extrapolated to the total Dutch population assuming coverage of 67.5%, we estimated that this figure represented an overall number of 19,544 GNI. Using the day-30 mortality observed in the prospective cohort study, this would reflect 2,198 associated deaths (table 4). The estimated number of deaths within 30 days of infection onset was 1,528 for bacteremic and 982 for non-bacteremic GNI. Extrapolated to the total Dutch population, the estimated national incidence of GNI was 144 (95% CI: 122-166) per 100,000 person years in 2016, with comparable contribution from bacteremic and non-bacteremic GNI (71

(95% CI: 60-83) and 86 (95% CI: 72-100) per 100,000 person years, respectively).

Table 4: Estimated number (95% confidence interval) of Gram-negative infections and mortality in the Netherlands in 2016

	GNI	GNI incidence*	Non-bacteremic GNI	Non-bacteremic GNI incidence*	Bacteremic GNI	Bacteremic GNI incidence*
Number of GNI	19544 (16574 –22514)	144 (122-166)	11613 (9707–13519)	86 (72 - 100)	9645 (8070 - 11221)	71 (60 – 83)
Number of HRMO infections	2459 (1887–3031)	18 (14 – 22)	1626 (1206–2047)	12 (10 – 15)	1015 (692 – 1340)	7.5 (5.1 – 9.9)
30-day mortality	2198 (1672–2723)	16 (12 – 20)	982 (688 –1276)	7.1 (5.2 –9.4)	1528 (1102-1954)	11 (8-14)
30-day mortality HRMO infections	276 (198 –354)	2.0 (1.5 – 2.6)	137 (90 –184)	1.0 (0.7 – 1.4)	161 (102-220)	1.2 (0.75 – 1.63)

GNI: gram-negative infection; HRMO: highly resistant microorganism; * per 100,000 person years. GNI, non-bacteremic and bacteremic GNI calculated separately.

Discussion

In this study we described the epidemiology, treatment and outcomes of adult hospitalized patients with microbiologically documented GNI and estimated that the incidence of GNI in the Netherlands is 144 per 100,000 person years. Thus, our extrapolations for 2016 imply that annually around 20,000 adults develop microbiologically confirmed GNI that is diagnosed in a hospital setting and that 2,200 of them succumb within 30 days of infection onset. A third of these deaths occur in patients with non-bacteremic GNI.

Previously reported national incidences of bacteremic GNI ranged from 70 to 150 per 100,000 person years in Finland, Sweden and Canada.[10–12] Our study demonstrates that in the Netherlands, there is a more or less similar incidence of GNI not associated with BSI. Although non-bacteremic GNI episodes are associated with a generally less severe presentation of disease, 30-day mortality still is 8.3% in our study. For the Netherlands we estimated that annually 2,198 patients die within 30 days after GNI, which is similar to reported numbers of annual deaths due to diabetes mellitus (n=2,891), prostate cancer (n=2,770) and breast cancer (3,175). There are a few important caveats with this number: first, it is a description of 30-day mortality, and since people may be admitted in a hospital for other reasons, naturally, mortality does not capture the full burden of disease, which also includes surgical procedures, prolonged duration of treatment and permanent disabilities. To

fully capture the burden of GNI, disability adjusted life years (DALY's) and other outcomes next to mortality should be determined.[13,14]

We consider two important aspects related to GNI epidemiology. First, antimicrobial resistance currently receives a lot of attention from researchers and policy makers. Yet, as we demonstrate for the Netherlands, 90% of GNI are caused by antimicrobial susceptible bacteria, and these bacteria are responsible for 90% of the GNI-associated 30-day mortality. We previously estimated that the attributable mortality due to antibiotic resistance in GNI in the Netherlands, which predominantly reflects resistance to third-generation cephalosporins among Enterobacterales, is close to zero.[15] Thus, interventions to solely reduce antibiotic resistant infections and transmission (like isolation strategies) are unlikely to have a major impact on the total burden of disease caused by Gram-negative bacteria. Second, our study underlines the heterogeneity of clinical presentations of GNI, which are usually described as more specific infections, such as BSI or UTI. Our findings demonstrate that such an approach will severely underestimate the true burden of disease caused by GNI.

With such a high disease incidence, prevention of GNI should be considered a public health concern. As a considerable part of the disease burden originates from community-acquired infections, interventions targeting non-hospitalized patients are warranted. Vaccination against *E. coli* infections could be an option, and a 4-valent conjugate *E. coli* vaccine, targeting four E. coli serotypes, was well tolerated and yielded good immunological response in a phase II study.[16]

For hospital-acquired infections, selective decontamination of the digestive tract (SDD) has been associated with a 50% reduction in the incidence of ICU-acquired GNI in the Netherlands.[17] SDD (or selective oral decontamination, SOD), was standard care in the ICUs of the participating hospitals in our study, which may explain the observed lower proportion of GNI diagnosed in ICU than in other studies.[12,18] Similarly, topical antibiotic prophylaxis may prevent surgical site infections in patients who undergo elective colon surgery, which is currently being adopted in Dutch hospitals.[19,20] Other opportunities to reduce hospital-onset GNI include restrictive and proper use of urinary and intravascular catheters, and oral care for prevention of hospital acquired pneumonia.[21]

Some study limitations need to be discussed. Due to difficulties in distinguishing screening and clinical sputum samples obtained in ICU, we did not include infections from ICU patients if the microbiological evidence of infection was limited to sputum cultures. As a result, we may have underestimated the amount of Gram-negative respiratory tract infections in ICUs. Second, we calculated the overall infection, bacteremic and non-bacteremic infection estimates separately, and non-bacteremic and bacteremic GNI do not add up to the overall estimate. This is because of a higher (43%) percentage of bacteremic GNI in the national database than in the cohort study (38%), due to a higher proportion of blood culture isolates in the national database compared to the isolates in our cohort study (13.1% vs 10.3%).

In conclusion, we quantified the burden of Gram-negative infections in the form of incidence and all-cause mortality in a country with low levels of antibiotic resistance. One-third of the all-cause mortality follows a non-bacteremic infection. The most important target for reducing the burden are the community and chronic healthcare settings, where most infections develop.

References

1. Vrijens, F., Hulstaert, F., Devriese, S. & Van De Sande, S. Hospital-acquired infections in Belgian acute-care hospitals: An estimation of their global impact on mortality, length of stay and healthcare costs. *Epidemiol. Infect.* 140, 126–136 (2012).
2. Neidell, M. J. *et al.* Costs of healthcare- and community-associated infections with antimicrobial-resistant versus antimicrobial-susceptible organisms. *Clin. Infect. Dis.* 55, 807–15 (2012).
3. Zaman, S. Bin *et al.* A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus* 9, (2017).
4. CDC. CDC / NHSN Surveillance Definitions for Specific Types of Infections. *Surveill. Defin.* 2015, 1–24 (2016).
5. Cardoso, T. *et al.* Classification of healthcare-associated infection: A systematic review 10 years after the first proposal. *BMC Med.* 12, 1–13 (2014).
6. Quan, H. *et al.* Updating and validating the charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. *Am. J. Epidemiol.* 173, 676–682 (2011).
7. Levy, M. M. *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Med.* 29, 530–538 (2003).
8. Altorf-van der Kuil, W. *et al.* National laboratory-based surveillance system for antimicrobial resistance: a successful tool to support the control of antimicrobial resistance in the Netherlands. *Eurosurveillance* 22, 1–10 (2017).
9. Netherlands, S. Bevolking op eerste van de maand, 2016. *Bevolking op eerste van de maand;* (2016).
10. Laupland, K. B. Incidence of bloodstream infection : a review of population-based studies. *Clin. Microbiol. Infect.* 19, 492–500 (2013).
11. Skogberg, K., Lyytikäinen, O., Ollgren, J., Nuorti, J. P. & Ruutu, P. Population-based burden of bloodstream infections in Finland. *Clin. Microbiol. Infect.* 18, E170–E176 (2012).
12. Holmbom, M. *et al.* 14-Year survey in a Swedish county reveals a pronounced increase in bloodstream infections (BSI). Comorbidity - An independent risk factor for both BSI and mortality. *PLoS One* 11, 1–16 (2016).
13. Leibovici, L. Long-term consequences of severe infections. *Clin. Microbiol. Infect.* 19, 510–512 (2013).
14. Winters, B. D. *et al.* Long-term mortality and quality of life in sepsis: a systematic review. *Crit. Care Med.* 38, 1276–83 (2010).
15. Rottier, W. C. Attributable mortality of antibiotic resistance in Gram-negative infections. (Utrecht University, 2019).
16. Frenck, R. W. *et al.* Safety and immunogenicity of a vaccine for extra-intestinal pathogenic Escherichia coli (ESTELLA): a phase 2 randomised controlled trial. *Lancet Infect. Dis.* 19, 631–640 (2019).
17. de Smet, A. M. G. A. *et al.* Decontamination of the Digestive Tract and Oropharynx in ICU Patients. *N. Engl. J. Med.* 360, 20–31 (2009).
18. Scheuerman, O. *et al.* Comparison of Predictors and Mortality Between Bloodstream Infections Caused by ESBL-Producing Escherichia coli and ESBL-Producing Klebsiella pneumoniae. *Infect. Control Hosp. Epidemiol.* 39, 660–667 (2018).
19. Mulder, T. *et al.* Preoperative oral antibiotic prophylaxis reduces surgical site infections after elective colorectal surgery: results from a before-after study. *Clin. Infect. Dis.* 1–7 (2018). doi:10.1093/cid/ciy839
20. Abis, G. S. A. *et al.* Randomized clinical trial of selective decontamination of the digestive tract in elective colorectal cancer surgery (SELECT trial). *Br. J. Surg.* 106, 355–363 (2019).
21. Fernando, S. A., Gray, T. J. & Gottlieb, T. Healthcare-acquired infections: prevention strategies. *Intern. Med. J.* 47, 1341–1351 (2017).

Chapter 2

Supplementary Materials

A. Definition of Gram-negative highly-resistant micro-organisms (HRMO)

Organism group	HRMO definition based on [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 [27] to minimum inhibitory concentrations obtained through automated systems (VITEK, Biomerieux, or Phoenix, BD), and includes isolates categorized as intermediate to the antibiotic.

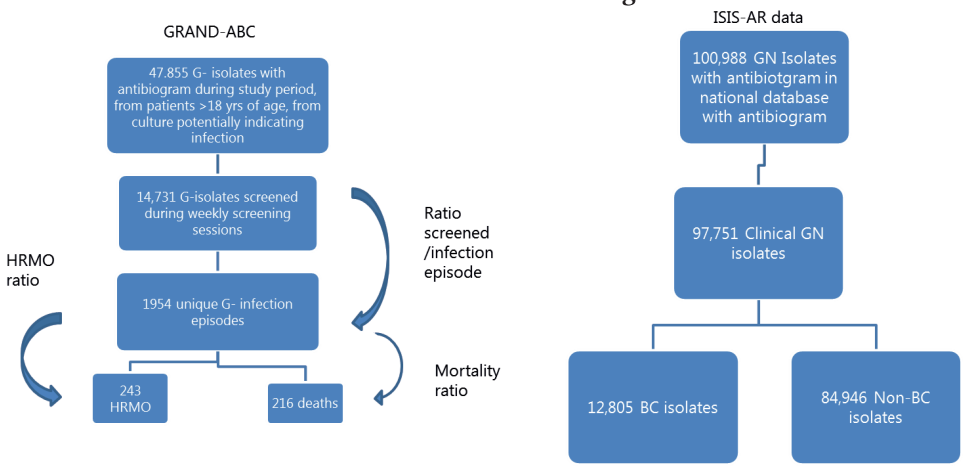
[a] In this study, Enterobacterales (formerly Enterobacteriaceae) included *Citrobacter* spp., *Enterobacter* spp. (including *Enterobacter/Klebsiella aerogenes*, *Enterobacter/Kluyvera intermedia* and *Enterobacter/Cronobacter sakazakii*), *Escherichia* spp., *Hafnia* spp., *Klebsiella* spp. (including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

[b] Dutch HRMO guideline uses extended-spectrum beta-lactamase-positive for this criterion.

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

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

B. Flowchart of estimation of national burden of Gram-Negative infections



On the left the GRAND-ABC (prospective cohort flow chart), on the right the ISIS-AR national surveillance database flow chart. We used the ratio of infection episode/GN-isolates to calculate the number of infection episodes based on the national surveillance data. We similarly used the HRMO ratio's (HRMO infection episodes/all infection episodes) and mortality. It is thus an extrapolation of data from a prospective cohort to national surveillance data.

C. Baseline data per infection type

	Overall	Primary BSI	Urinary tract
n	1954	91	1008
Age (mean (sd))	68.87 (15.67)	67.48 (15.77)	70.78 (15.52)
Sex = f (%)	915 (46.8)	35 (38.5)	525 (52.1)
Charlson Comorbidity index	2.00 [1.00, 3.00]	2.00 [1.00, 4.00]	2.00 [1.00, 3.00]
Immunocompromised	0.12 (0.33)	32 (35.2)	101 (10.0)
Prior colonization MDRO	129 (6.6)	10 (11.0)	66 (6.5)
Surgery prior to infection	386 (19.8)	7 (7.7)	124 (12.3)
Origin			
- Community onset	725 (37.1)	26 (28.6)	410 (44.5)
- Healthcare associated	681 (34.9)	29 (31.9)	337 (36.6)
- Hospital onset	548 (28.0)	36 (39.6)	175 (19.0)
Culture ward			
- Surgical ward	544 (27.8)	5 (5.5)	178 (17.7)
- Intensive care unit	74 (3.8)	7 (7.7)	7 (0.7)
- Internal medicine	520 (26.6)	38 (41.8)	248 (24.6)
- Emergency dep	816 (41.8)	41 (45.1)	575 (57.0)
LOS prior to infection	8.00 [5.00, 15.00]	11.00 [5.00, 19.25]	7.00 [5.00, 13.00]
Bloodstream infection (%)	758 (38.8)	91 (100.0)	406 (40.3)
Sepsis severity			
- No sepsis	586 (30.0)	11 (12.1)	276 (27.4)
- Sepsis	1097 (56.1)136 (7.0)	54 (59.3)	605 (60.0)
- Severe sepsis	135 (6.9)	16 (17.6)	79 (7.8)
- Septic shock		10 (11.0)	48 (4.8)

	Abdominal	Respiratory	Skin and soft tissue	Bone and joint	Other
	378	165	223	25	64
	66.66 (15.82)	69.01 (15.52)	66.40 (14.66)	66.80 (18.26)	63.00 (15.90)
	169 (44.7)	59 (35.8)	89 (39.9)	14 (56.0)	24 (37.5)
	2.00 [0.00, 3.00]	2.00 [1.00, 4.00]	2.00 [1.00, 3.00]	0.00 [0.00, 1.00]	2.00 [1.00, 4.00]
	33 (8.7)	43 (26.2)	20 (9.0)	1 (4.0)	7 (10.9)
	25 (6.6)	14 (8.5)	20 (9.0)	1 (4.0)	6 (9.4)
	96 (25.4)	33 (20.0)	94 (42.2)	10 (40.0)	22 (34.4)
	85 (31.6)	29 (18.2)	56 (25.2)	7 (28.0)	112 (42.1)
	76 (28.3)	45 (28.3)	94 (42.3)	7 (28.0)	93 (35.0)
	108 (40.1)	85 (53.5)	72 (32.4)	11 (44.0)	61 (22.9)
	159 (42.1)	31 (18.8)	129 (57.8)	18 (72.0)	24 (37.5)
	31 (8.2)	13 (7.9)	7 (3.1)	0 (0.0)	9 (14.1)
	93 (24.6)	98 (59.4)	28 (12.6)	0 (0.0)	15 (23.4)
	95 (25.1)	23 (13.9)	59 (26.5)	7 (28.0)	16 (25.0)
	8.00 [4.00, 14.00]	8.00 [4.00, 11.00]	12.00 [6.75, 20.50]	13.00 [9.50, 15.00]	11.00 [6.00, 24.00]
	172 (45.5)	23 (13.9)	21 (9.4)	3 (12.0)	42 (65.6)
	100 (26.5)	41 (24.8)	124 (55.6)	18 (72.0)	16 (25.0)
	207 (54.8)	104 (63.0)	83 (37.2)	6 (24.0)	38 (59.4)
	19 (5.0)	11 (6.7)	6 (2.7)	0 (0.0)	5 (7.8)
	52 (13.8)	9 (5.5)	10 (4.5)	1 (4.0)	5 (7.8)

D. Source control

	Overall	Primary BSI	Urinary tract
n	1954	91	922
Source Control (%)	664 (34.0)	9 (9.9)	166 (18.0)
Days to source control	2.46 (3.00)	2.00 [0.00, 3.00]	1.00 [0.00, 3.00]
Urinary Tract Source Control	169 (8.6)	-	175 (17.4)
Bile duct source control	77 (3.9)	2 (2.2)	4 (0.4)
CVC removal	21 (1.1)	7 (7.7)	1 (0.1)
Surgical source control	403 (20.6)	-	7 (0.7)
Laparotomy	131 (6.7)	-	1 (0.1)
Abscess drainage	130 (6.7)	-	4 (0.4)
Necrotecomy	38 (1.9)	-	1 (0.1)
Misc wound	47 (2.4)	-	2 (0.2)
Amputation	35 (1.8)	-	-
Joint lavage	25 (1.3)	-	-
Pulmonary source control	11 (0.6)	-	-
Other surgical source control	48 (2.5)	-	3 (0.3)

	Abdominal	Respiratory	Skin and soft tissue	Bone and joint	Other
	269	159	222	25	266
	226 (84.0)	16 (10.1)	123 (55.4)	25 (100.0)	99 (37.2)
	0.00 [0.00, 0.00]	1.00 [0.00, 8.50]	0.00 [0.00, 2.75]	0.00 [0.00, 0.25]	0.50 [0.00, 2.25]
	1 (0.3)	-	3 (1.3)	-	1 (1.6)
	76 (20.1)	-	-	-	3 (4.7)
	1 (0.4)	2 (1.3)	1 (0.5)	-	9 (3.4)
	198 (52.4)	15 (9.1)	119 (53.4)	25 (100.0)	20 (31.2)
	121 (32.0)	4 (2.4)	5 (2.2)	-	2 (3.1)
	94 (24.9)	1 (0.6)	24 (10.8)	1 (4.0)	6 (9.4)
	-	1 (0.6)	32 (14.3)	4 (16.0)	0 (0.0)
	8 (2.1)	1 (0.6)	28 (12.6)	8 (32.0)	2 (3.1)
	-	-	34 (15.2)	1 (4.0)	0 (0.0)
	-	-	10 (4.5)	15 (60.0)	0 (0.0)
	-	9 (5.5)	1 (0.4)	-	2 (3.1)
	13 (3.4)	1 (0.6)	11 (4.9)	2 (8.0)	8 (12.5)

E. Pathogens per infection source

	Overall (n=1954)	Primary BSI	Urinary tract
Escherichia coli	997	48	616
MDRO	116 (11.6)	5 (10.4)	75 (12.2)
3GC-R	91 (9.1)	4 (8.3)	57 (9.3)
AGFQ-R	25 (2.5)	1 (2.1)	18 (2.9)
Carba-R	-	-	-
E. cloacae	72	4	25
MDRO	25 (34.7)	-	13 (52.0)
3GC-R	22 (30.6)	-	12 (48.0)
AGFQ-R	2 (2.8)	-	1 (4.0)
Carba-R	1 (1.4)	-	-
K. pneumoniae	144	12	71
MDRO	12 (8.3)	1 (8.3)	5 (7.0)
3GC-R	7 (4.9)	-	3 (4.2)
AGFQ-R	6 (4.2)	1 (8.3)	2 (2.8)
Carba-R	-	-	-
P. mirabilis	99	-	68
MDRO	3 (3.0)	-	1 (1.5)
3GC-R	1 (1.0)	-	-
AGFQ-R	2 (2.0)	-	-
P. aeruginosa	144	5	51
MDRO	9 (6.2)	1 (20.0)	2 (3.9)
Other species	205	14	77
MDRO	22 (10.7)	-	7 (9.1)
3GC-R	21 (10.2)	-	7 (9.1)
AGFQ-R	1 (0.5)	-	-
Multiple species	293	8	100
E.coli	194 (66.2)	7 (87.5)	66 (66.0)
Klebsiella	119 (40.6) 68 (23.2)	5 (62.5)	49 (49.0)
Pseudo	79 (27.0)	2 (25.0)	20 (20.0)
Proteus		1 (12.5)	30 (30.0)

	Abdominal	Respiratory	Skin and soft tissue	Bone and joint	Other
	205	49	45	5	29
	17 (8.3)	10 (20.4)	7 (15.6)	-	2 (6.9)
	16 (7.8)	8 (16.3)	5 (11.1)	-	1 (3.4)
	1 (0.5)	2 (4.1)	2 (4.4)	-	1 (3.4)
	-	-	-	-	-
	12	7	17	3	4
	4 (33.3)	3 (42.9)	5 (29.4)	-	-
	4 (33.3)	2 (28.6)	4 (23.5)	-	-
	-	-	1 (5.9)	-	-
		1 (14.3)	-	-	-
	2-3	17	12	4	5
	1 (4.3)	2 (11.8)	2 (16.7)	1 (25.0)	-
	-	2 (11.8)	2 (16.7)	-	-
	1 (4.3)	-	-	1 (25.0)	-
	-	-	-	-	-
	5	4	18	3	1
	1 (20.0)	-	-	-	-
	1 (20.0)	1 (25.0)	-	-	-
	-	-	-	-	-
	9	35	34	-	10
	-	3 (8.6)	3 (8.8)		1 (10.0)
	31	34	34	6	9
	6 (19.4)	1 (2.9)	6 (17.6)	-	2 (22.2)
	6 (19.4)	1 (2.9)	5 (14.7)	-	2 (22.2)
	-	-	1 (2.9)	-	-
	93	19	63	4	6
	75 (80.6)	7 (36.8)	33 (52.4)	1 (25.0)	5 (83.3)
	40 (43.0)	8 (42.1)	15 (23.8)	-	2 (33.3)
	16 (17.2)	6 (31.6)	21 (33.3)	2 (50.0)	1 (16.7)
	17 (18.3)	4 (21.1)	27 (42.9)	-	-

F. Outcomes per infection source

	Overall	Primary BSI	Urinary tract
n	1954	91	1008
Clinical cure at 2 weeks (%)	1523 (77.9)	72 (79.1)	955 (94.7)
Abscess/drainage	108 (5.5)	-	3 (0.3)
Chronic skin infection	113 (5.8)	-	1 (0.1)
Endovascularinfection	10 (0.5)	3 (3.3)	-
Osteomyelitis	29 (1.5)	1 (1.1)	2 (0.2)
Prostatitis	4 (0.2)	-	4 (0.4)
Arthritis	25 (1.3)	-	-
i.v. antibiotics*	61 (3.1)	1 (1.1)	7 (0.7)
Ongoing symptoms (e.g. fever)	18 (0.9)	-	3 (0.3)
Died	131 (6.7)	15 (16.5)	29 (2.9)
Not cured, alive	300 (15.4)	4 (4.4)	24 (2.4)
Expansion of primary infection	385 (19.7)	4 (4.4)	25 (2.5)
Local abscess (%)	211 (10.8)	-	6 (0.6)
Osteomyelitis (%)	30 (1.5)	-	-
Necrosis (%)	73 (3.7)	-	3 (0.3)
Arthritis (%)	26 (1.3)	-	1 (0.1)
30 day mortality	217 (11.1)	20 (22.0)	66 (6.6)
Length of stay after infection	8.00 [5.00, 14.00]	8.00 [6.00, 13.00]	7.00 [5.00, 10.00]
ICU after index culture	184 (9.4)	11 (12.1)	47 (4.7)

Abdominal	Respiratory	Skin and soft tissue	Bone and joint	Other
378	165	223	25	64
236 (62.4)	120 (72.7)	103 (46.2)	2 (8.0)	35 (54.7)
85 (22.5)	5 (3.0)	7 (3.1)	2 (8.0)	6 (9.4)
15 (4.0)	2 (1.2)	79 (35.4)	9 (36.0)	7 (10.9)
2 (0.5)	-	1 (0.4)	-	4 (6.2)
-	-	16 (7.2)	6 (24.0)	4 (6.2)
-	-	-	-	-
-	-	9 (4.0)	16 (64.0)	-
22 (5.8)	6 (3.6)	16 (7.2)	3 (12.0)	6 (9.4)
9 (2.4)	2 (1.2)	2 (0.9)	1 (4.0)	1 (1.6)
35 (9.3)	30 (18.2)	13 (5.8)	-	9 (14.1)
107 (28.3)	15 (9.1)	107 (48.0)	23 (92.0)	20 (31.2)
167 (44.2)	17 (10.3)	125 (56.1)	24 (96.0)	23 (35.9)
134 (35.4)	3 (1.8)	50 (22.4)	4 (16.0)	14 (21.9)
-	-	22 (9.9)	7 (28.0)	1 (1.6)
8 (2.1)	1 (0.6)	57 (25.6)	4 (16.0)	0 (0.0)
-	-	9 (4.0)	16 (64.0)	0 (0.0)
56 (14.9)	37 (22.4)	23 (10.3)	2 (8.0)	13 (20.3)
9.50 [6.00, 17.00]	10.00 [7.00, 17.00]	13.00 [7.00, 20.00]	25.00 [14.00, 33.00]	10.00 [7.00, 19.50]
65 (17.2)	25 (15.2)	23 (0.3)	4 (16.0)	9 (14.1)

CEPHALOSPORINS CARBAPENEMASE DISEASE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL MORTALITY DISEASE BURDEN GRAM-NEGATIVE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET PROPENSITY SCORE INFECTION COHORT METHODS GRAND-ABC BURDEN CEPHALOSPORINS BACTERIA BLOODSTREAM INFECTION MORTALITY PREDICTION GRAM-NEGATIVE INFECTION PREDICT ANTIBIOTIC CEPHALOSPORINS CARBAPENEMASE DISEASE TRIAL MORTALITY ANTIBIOTIC PREDICT BLOODSTREAM GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL ANTIBIOTIC DISEASE CHAPTER 3 CAUSAL INFERENCE MORTALITY GRAM-NEGATIVE BACTERIA PREDICTION GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM

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

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Summary

Background

Antibiotic resistance in Gram-negative bacteria 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 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. 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 to 1,941 controls (61% caused by *Escherichia coli*, 39% bacteremia). Resistant Gram-negatives (78% third-generation cephalosporin-resistant Enterobacterales; no carbapenem-resistant Enterobacterales) caused 243 infections (12% of all infections). Mortality for resistant infections was increased compared to their non-infected controls (adjusted RR 1·42, 95% CI 0·66-3·09), similarly as was the case for susceptible infections (RR 1·32, 95% CI 1·06-1·65). The RR reflecting attributable mortality of resistance was 1·08 (95% CI 0·48-2·41).

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.

Funding The Netherlands Organisation for Health Research and Development

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 ß-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 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, of which the protocol is available as Supplementary Material (registered at clinicaltrials.gov under number NCT02007343).

Table 1 Definition of Gram-negative highly-resistant micro-organisms (HRMO)

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[9] 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 included *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 extended-spectrum beta-lactamase-positive for this criterion.

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

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

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.

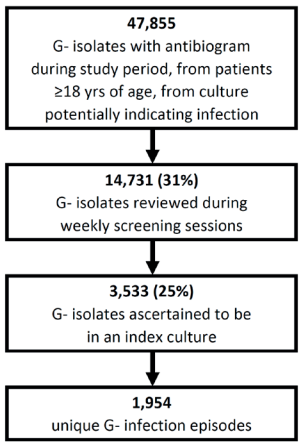

Figure 1 Flow diagram of the screening process. Abbreviations: G-, Gram-negative (defined in Table 1).

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,[9] 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)[10], with the use of packages *Hmisc*[11], *rms*[12], *mice*[13] and *xtable*[14]. 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.[15]

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 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.[16]

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 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.

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	4008 (8·38)	1519 (10·31)	1155 (32·59)
Urine	24323 (50·83)	6845 (46·47)	1160 (32·73)
Lower respiratory tract	8079 (16·88)	2637 (17·90)	251 (7·08)
Fluid, pus, tissue (biopsy)	5505 (11·50)	1962 (13·32)	718 (20·26)
Swab	5186 (10·84)	1549 (10·52)	243 (6·86)
Other	754 (1·58)	219 (1·49)	17 (0·48)
Bacterial isolate			
Escherichia coli	22145 (46·28)	6705 (45·52)	1904 (53·72)
Pseudomonas aeruginosa	5835 (12·19)	1916 (13·01)	337 (9·51)
Klebsiella pneumoniae	4426 (9·25)	1389 (9·43)	346 (9·76)
Proteus mirabilis	3609 (7·54)	1079 (7·32)	232 (6·55)
Enterobacter cloacae cx.	2587 (5·41)	801 (5·44)	177 (4·99)
Other	9253 (19·34)	2841 (19·29)	548 (15·46)
HRMO isolate	6323 (13·21)	1972 (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. Abbreviations: HRMO, highly resistant micro-organism.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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 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 = 1,001, 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 = 190) for non-HRMO infections (RR for HRMO vs non-HRMO 0·92, 95% CI 0·61-1·40; Table 5). Inappropriate antibiotic therapy on the day of infection onset was not associated with higher 30-day mortality (unadjusted RR 0·83, 95% CI 0·62-1·12; adjusted RR 0·79, 95% CI 0·58-1·07).

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).

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	680/1711 (40)	78/243 (32)
- Urinary tract infection	884/1696 (52)	117/240 (49)
- Respiratory tract infection	139/1696 (8)	19/240 (8)
- Intra-abdominal infection (excl biliary tract)	199/1696 (12)	32/240 (13)
- Biliary tract infection	130/1696 (8)	18/240 (8)
- Skin/soft tissue/wound infection (incl mediastinitis)	196/1696 (12)	38/240 (16)
- Other infection source	80/1696 (5)	11/240 (5)
- Postoperative infection	141/1711 (8)	28/243 (12)

Causative pathogen[a]		
- *Escherichia coli*	881/1711 (51)	116/243 (48)
- *Klebsiella pneumoniae*	132/1711 (8)	12/243 (5)
- *Enterobacter cloacae cx.*	49/1711 (3)	27/243 (11)
- *Proteus mirabilis*	96/1711 (6)	3/243 (1)
- *Pseudomonas aeruginosa*	135/1711 (8)	9/243 (4)
- Other species	181/1711 (11)	21/243 (9)
- Multiple species	237/1711 (14)	55/243 (23)
Bacteria other than study pathogens or yeast obtained from index cultures	456/1708 (27)	72/243 (30)
Sepsis severity at infection onset[a]		
- No sepsis	512/1710 (30)	74/243 (30)
- Sepsis	963/1710 (56)	133/243 (55)
- Severe sepsis	115/1710 (7)	21/243 (9)
- Septic shock	120/1710 (7)	15/243 (6)
Antibiotic treatment during the infection episode		
- Receipt of antibiotic therapy prior to hospital admission	167/1710 (10)	30/243 (12)
- Receipt of oral/intravenous antibiotic therapy[b] on the day prior to infection onset	249/1466[c] (17)	46/210[c] (22)
- Receipt of oral/intravenous antibiotic therapy[b] on the day of infection onset	1176/1466[c] (80)	164/210[c] (78)
- Receipt of inappropriate antibiotic therapy[d] on the day of infection onset	567/1466[c] (39)	142/210[c] (68)
Source control performed during the admission after infection onset	570/1711 (33)	94/243 (39)
Status of the infection episode at 14 days after infection onset[a]		
- Patient admitted - infection resolved	183/1711 (11)	38/243 (16)
- Patient admitted - mere completion of antibiotic course	60/1711 (4)	10/243 (4)
- Patient admitted - infection ongoing	150/1711 (9)	30/243 (12)
- Patient discharged - infection resolved at discharge	290/1711 (17)	55/243 (23)
- Patient discharged - mere completion of antibiotic course after discharge	810/1711 (47)	77/243 (32)
- Patient discharged - infection ongoing at discharge	103/1711 (6)	17/243 (7)
- Patient deceased	115/1711 (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.

Abbreviations: HRMO, highly resistant micro-organism.

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	845/1700 (50)	825/1711 (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	361/1700 (21)	137/1446 (9)	57/241 (24)	12/204 (6)
Known colonization with an HRMO	35/1700 (2)	74/1711 (4)	10/241 (4)	68/243 (28)
Gram-negative bacteremia during the year prior to infection onset	14/1700 (1)	77/1711 (5)	6/241 (2)	22/243 (9)
Preceding hospital admission within 3 months prior to infection onset	373/1695 (22)	553/1710 (32)	55/241 (23)	90/243 (37)
Admission from long-term care facility	61/1699 (4)	90/1710 (5)	7/241 (3)	23/243 (9)
Admission type				
- Via emergency ward	1364/1700 (80)	1345/1711 (79)	185/241 (77)	178/243 (73)
- Other form of emergency admission	151/1700 (9)	159/1711 (9)	19/241 (8)	20/243 (8)
- Elective admission	131/1700 (8)	176/1711 (10)	27/241 (11)	36/243 (15)
- Transfer from other hospital	54/1700 (3)	31/1711 (2)	10/241 (4)	9/243 (4)
Origin of infection				
- Community-onset, not healthcare-associated	891/1687 (53)	660/1705 (39)	112/240 (47)	62/242 (26)
- Community-onset, possibly healthcare-associated	14/1687 (1)	59/1705 (3)	0/240 (0)	6/242 (2)
- Community-onset, healthcare-associated	319/1687 (19)	522/1705 (31)	36/240 (15)	84/242 (35)
- Hospital-onset	463/1687 (27)	464/1705 (27)	92/240 (38)	90/242 (37)
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 (7-26)
Hospital ward at infection onset				
- Emergency ward	793/1700 (47)	733/1711 (43)	100/241 (41)	83/243 (34)
- Internal medicine	197/1700 (12)	217/1711 (13)	32/241 (13)	35/243 (14)
- Surgery or gastro-enterology	280/1700 (16)	390/1711 (23)	46/241 (19)	79/243 (33)
- Urology	33/1700 (2)	91/1711 (5)	5/241 (2)	19/243 (8)
- Pulmonary medicine	92/1700 (5)	83/1711 (5)	10/241 (4)	11/243 (5)

- ICU	43/1700 (3)	67/1711 (4)	8/241 (3)	7/243 (3)
- Other ward	262/1700 (15)	130/1711 (8)	40/241 (17)	9/243 (4)
Charlson comorbidity index, median (IQR)	1 (0-3)	2 (0-3)	1 (0-3)	2 (1-4)
Immunodeficiency	145/1700 (9)	206/1710 (12)	23/241 (10)	31/243 (13)
Solid malignancy	335/1700 (20)	507/1711 (30)	44/241 (18)	75/243 (31)
Treatment restriction in place prior to infection onset	438/1699 (26)	423/1711 (25)	57/241 (24)	79/243 (33)
Surgical procedure during the 30 days prior to infection onset	251/1700 (15)	325/1486 (22)	36/241 (15)	57/218 (26)
ICU stay during the 30 days prior to infection onset	113/1700 (7)	133/1452 (9)	22/241 (9)	33/209 (16)
Receipt of prophylactic antibiotic therapy at hospital admission	44/1699 (3)	50/1711 (3)	5/240 (2)	16/243 (7)
ICU stay during the admission from infection onset onwards				
- No	1580/1700 (93)	1476/1711 (86)	227/241 (94)	207/243 (85)
- Already on ICU for >12 hrs at infection onset	33/1700 (2)	42/1711 (2)	5/241 (2)	7/243 (3)
- Already on ICU for 0-12 hrs at infection onset	18/1700 (1)	36/1711 (2)	2/241 (1)	2/243 (1)
- Admission to ICU within 0-12 hrs after infection onset	26/1700 (2)	90/1711 (5)	4/241 (2)	18/243 (7)
- Admission to ICU >12 hrs after infection onset	43/1700 (3)	67/1711 (4)	3/241 (1)	9/243 (4)
Length of hospital stay after infection onset, median (IQR)	5 (3-9)	8 (5-14)	6 (3-12)	9 (6-16)
Discharge destination				
- Home	1156/1700 (68)	993/1711 (58)	152/241 (63)	116/243 (48)
- Home with home healthcare	115/1700 (7)	255/1711 (15)	22/241 (9)	45/243 (19)
- Long-term care facility	259/1700 (15)	263/1711 (15)	46/241 (19)	50/243 (21)
- Terminal care	25/1700 (1)	36/1711 (2)	5/241 (2)	5/243 (2)
- Deceased during admission	81/1700 (5)	138/1711 (8)	6/241 (2)	21/243 (9)
- Other hospital	64/1700 (4)	26/1711 (2)	10/241 (4)	6/243 (2)
Gram-negative bacteremia within 7 to 90 days after infection onset	20/1700 (1)	54/1711 (3)	3/241 (1)	10/243 (4)
All-cause mortality within 30 days after infection onset	145/1695 (9)	190/1709 (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: HRMO, highly resistant micro-organism; ICU, intensive care unit; IQR, interquartile range.

Attributable mortality

After full adjustment for confounding variables, the relative risks for 30-day mortality were 1·42 (95% CI 0·66-3·09) for HRMO infections and their non-infected controls, and 1·32 (95% CI 1·06-1·65) for non-HRMO infections and their non-infected controls (Figure 1). Based on both RRs, the overall RR for 30-day mortality associated with HRMO status was 1·08 (95% CI 0·48-2·41).

When analyzing infected patients only (i.e. without controls) the RR for 30-day mortality for HRMO infections was 0·78 (95% CI 0·50-1·21; Figure 1) after adjustment for patient-related factors, and 0·94 (95% CI 0·60-1·47) 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 0·94 (95% CI 0·62-1·44).

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	116/1239 (9·4)	17/152 (11·2)	133/1391 (9·6)
Hospital-onset infection	73/464 (15·7)	8/90 (8·9)	81/554 (14·6)
All infections	*190/1709 (11·1)*	*25/243 (10·3)*	*215/1952 (11·0)*
	NON-INFECTED CONTROL PATIENTS		
Community-onset infection	95/1220 (7·8)	8/148 (5·4)	103/1368 (7·5)
Hospital-onset infection	50/462 (10·8)	7/92 (7·6)	57/554 (10·3)
All infections	*145/1695 (8·6)*	*15/241 (6·2)*	*160/1936 (8·3)*

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.

Abbreviations: HRMO, highly resistant micro-organism.

Hospital-acquired Gram-negative infections (both HRMO or non-HRMO; n = 554) 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 0·62 (95% CI 0·30-1·27) and an adjusted RR of 0·59 (95% CI 0·28-1·24).

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.[17,18] 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.[19]

A delay in achieving appropriate antibiotic therapy is considered the most important reason for increased mortality in patients infected with antibiotic resistant Gram-negatives.[20] Inappropriate empiric antibiotics have been related to mortality in all forms of sepsis,[21] and specifically in septic shock, for which associations between increasing mortality for every hour that appropriate antibiotics were delayed have been reported.[22] 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.[23] 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.[24]

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 32% 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.[25] 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.[26,27] 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 (72% 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,[28] 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.[29] 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.[30] 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.

References

1. Surveillance Atlas of Infectious Diseases. *European Centre for Disease Prevention and Control*
2. Voets, G. M. *et al.* Population distribution of beta-lactamase conferring resistance to third-generation cephalosporins in human clinical Enterobacteriaceae in the Netherlands. *PLoS One* 7, e52102 (2012).
3. Glasner, C. *et al.* Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveill.* 18, 1–7 (2013).
4. Werkgroep.Infectiepreventie. *Bijzonder resistente micro-organismen (BRMO).* (2013).
5. Dik, J. W. H. *et al.* Positive impact of infection prevention on the management of nosocomial outbreaks at an academic hospital. *Future Microbiol.* 11, 1249–1259 (2016).
6. de Kraker, M. E. A. *et al.* Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant Staphylococcus aureus bloodstream infections. *Antimicrob. Agents Chemother.* 55, 1598–605 (2011).
7. de Kraker, M. E. A. *et al.* Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to Escherichia coli resistant to third-generation cephalosporins. *J. Antimicrob. Chemother.* 66, 398–407 (2011).
8. Horan, T. C., Andrus, M. & Dudeck, M. A. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am. J. Infect. Control* 36, 309–32 (2008).
9. *Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1.* (The European Committee on Antimicrobial Suceptibility Testing, 2018).
10. R Core Team. *R: A language and environment for statistical computing*. (2017).
11. Harrell Jr, F. E. Hmisc: Harrell Miscellaneous (R package version 4.1-1). (2018).
12. Harrell Jr, F. E. rms: Regression Modeling Strategies (R package version 5.1-2). (2018).
13. Buuren, S. van & Groothuis-Oudshoorn, K. mice: multivariate imputation by chained equations in R. *J. Stat. Softw.* 45, (2011).
14. Dahl, D. B. xtable: export tables to LaTeX or HTML (R package version 1.8-2). (2016).
15. Cummings, P. & McKnight, B. Analysis of matched cohort data. *Stata J.* 4, 274–281 (2004).
16. Altman, D. G. & Bland, J. M. Interaction revisited: the difference between two estimates. *BMJ* 326, 219 (2003).
17. Schwaber, M. J. & Carmeli, Y. Mortality and delay in effective therapy associated with extended-spectrum ß-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. *J. Antimicrob. Chemother.* 60, 913–20 (2007).
18. Rottier, W. C., Ammerlaan, H. S. M. & Bonten, M. J. M. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum ß-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. *J. Antimicrob. Chemother.* 67, 1311–20 (2012).
19. Stewardson, A. J. *et al.* The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible Enterobacteriaceae and Staphylococcus aureus in European hospitals, 2010 and 2011: a multicentre retrospective cohort study. *Euro Surveill.* 21, pii=30319 (2016).
20. Friedman, N. D., Temkin, E. & Carmeli, Y. The negative impact of antibiotic resistance. *Clin. Microbiol. Infect.* 22, 416–422 (2016).
21. Paul, M. *et al.* Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob. Agents Chemother.* 54, 4851–4863 (2010).
22. Kumar, A. *et al.* Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med.* 34, 1589–1596 (2006).
23. Klompas, M., Calandra, T. & Singer, M. Antibiotics for sepsis: finding the equilibrium. *JAMA* 320, 1433–1434 (2018).
24. Fitzpatrick, J. M. *et al.* Gram-negative bacteraemia: a multi-centre prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. *Clin. Microbiol. Infect.* 22, 244–51 (2016).
25. Gutiérrez-Gutiérrez, B. *et al.* Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study. *Lancet. Infect. Dis.* 17, 726–734 (2017).

26. Ammerlaan, H. S. M. *et al.* Secular trends in nosocomial bloodstream infections: Antibiotic-resistant bacteria increase the total burden of infection. *Clin. Infect. Dis.* 56, 798–805 (2013).
27. de Kraker, M. E. A. *et al.* The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clin. Microbiol. Infect.* 19, 860–8 (2013).
28. Klein Klouwenberg, P. M. C. *et al.* Interobserver agreement of Centers for Disease Control and Prevention criteria for classifying infections in critically ill patients. *Crit. Care Med.* 41, 2373–8 (2013).
29. Magiorakos, A.-P. *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 18, 268–81 (2012).
30. Cassini, A. *et al.* Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet. Infect. Dis.* 19, 56–66 (2019).

Contributors

WCR, HSMA, and MJMB designed the study. WCR, JWTD, AGMB, JWDZ, JAJWK, PDL, SFTT, BJMV, AJLW, and HSMA collected the data. WCR, JWTD, GC, and MJMB analyzed and interpreted the data. WCR, JWTD and MJMB prepared the manuscript, which all authors reviewed and approved for publication.

Declaration of interest

We declare no competing interests.

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Chapter 3

Supplementary Materials

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 microorganisms 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.*[1] 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 infectionrelated 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 European Committee on

Antimicrobial Suceptibility Testing (EUCAST).[2] For non-fermenters (*Pseudomonas aeruginosa*, *Acinetobacter* spp., *Stenotrophomonas maltophilia*), intrinsic resistance as indicated by EUCAST was additionally incorporated.[3] 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 ß-lactam MICs for Enterobacterales species with chromosomal ß-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 noninfected 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.[4] Imputation was performed separately for the infection-cohort analysis, and for the parallelcohorts 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,[5] 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 ß coefficient for the exposure. This was done to increase precision of the effect estimate, reflected by a narrowing of its confidence interval.[6] Removal of variables started with removing the variable that would result in a new model with the smallest deviance in ß coefficient for the exposure compared to the full model. Subsequently, all variables were evaluated again, and the variable impacting the ß coefficient the least in this round, was removed, always with reference to the ß coefficient of the exposure in the full model. This iterative process was halted if the ß coefficient would deviate >10% from the ß 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 ß coefficient for the exposure would be changed in case of incorporation into the model. The potential confounder with the largest resulting change in ß 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 ß coefficient of the exposure. In each round, one variable could be incorporated into the model, as long as it would change the ß 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 ß 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 ß coefficient. These cycles were repeated until no excluded variable could be found for which inclusion would change the ß coefficient >10%, and no included variable had an impact <10% on the ß 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 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)	238
Universitair Medisch Centrum Utrecht Utrecht	University	1042	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 Hospital.

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

	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	24	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	28	33	55	11	10
Hospital **H**	16	42	27	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.*[1] and other comments
Urinary tract infection (SUTI)	Urine	≥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).
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.

Infection entity	Cultures on which entity can be based	Modifications of original criteria by Horan *et al.*[1] and other comments
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 intraabdominal 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.*[1]: 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.*[1]: not reported if also meningitis.
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.*[1]: 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/ communityonset 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.*[7]
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*.
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.*[8]
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.*[8]

Variable	Definition
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.*[8]
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.*[8]
Hemiplegia	Patients with complete hemiplegia or paraplegia. Adapted from Charlson *et al.*[8]
ICU-acquired weakness or similar	Patients who are bedridden or only mobilizes with help of a wheelchair.
Cerebrovascular disease	Patients with a history of a cerebrovascular accident or transient ischemic attack. Adapted from Charlson *et al.*[8]
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.*[8]
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.*[8]
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.*[8]
Ulcer disease	Paients 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.*[8]
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.*[8]
(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.*[8]
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.*[8]
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.*[8]

Variable	Definition
Dementia	Patients with a diagnosis of a dementia syndrome (e.g. Alzheimer's disease). Adapted from Charlson *et al.*[8]
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 ≤0,5x10^9 or leukocytes ≤1,0x10^9 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[9]
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[9]
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.

Variable	Definition
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, <4x10^9/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.*[10]
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; COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; 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	Model technique	Adjustment variables	Risk ratio (95% CI)	Variables included in final model
Evaluated exposure: HRMO infection				
ICA	Unadjusted	-	0.92 (0.61-1.40)	-
	Backward elimination	Patient-related (small)	0.78 (0.50-1.21)	• Age • Known colonization with an HRMO • Healthcare-associated or hospital-onset infection • Preceding hospital admission • Other bacterial infection at infection onset • Metastasized solid malignancy • Immunodeficiency • Preceding treatment restriction
	Forward addition	Patient-related (large)	0.74 (0.48-1.15)	• Known colonization with an HRMO • Hospital-onset infection • Other bacterial infection at infection onset • Metastasized solid malignancy . Preceding treatment restriction • Charlson comorbidity index ≥3
	Backward elimination	Patient-related[a] Infection-related	0.94 (0.60-1.47)	• Age • Known colonization with an HRMO • Healthcare-associated or hospital-onset infection • Preceding hospital admission • Other bacterial infection at infection onset • Metastasized solid malignancy • Preceding treatment restriction • Bacteremia • Urinary tract infection • Lower respiratory tract infection • Pneumonia • Postoperative infection • Infection with *Pseudomonas aeruginosa* • Infection with other Gram-negative species • Infection with *Enterococcus* spp. • Infection with anaerobic bacteria • Severe sepsis at infection onset • Septic shock at infection onset • Antibiotic therapy prior to hospital admission
	Plus 1[b]	Patient-related[c] Infection-related[c] Therapy-related	1.00 (0.63-1.59)	• As previous model and: • Inappropriate antibiotic therapy on the day of infection onset
	Backward elimination	Admission-related	0.94 (0.62-1.44)	• Admission ward: surgery • Admission ward: urology • Admission ward: ICU • Preceding length of hospital stay
Evaluated exposure: Inappropriate antibiotic therapy on the day of infection onset				
ICA	Unadjusted	-	0.83 (0.62-1.12)	-

Analysis type	Model technique	Adjustment variables	Risk ratio (95% CI)	Variables included in final model
	Backward elimination	HRMO infection Patient-related (small) Infection-related	0.79 (0.58-1.07)	• Bacteremia • Urinary tract infection • Infection with *Pseudomonas aeruginosa*

Analysis type	Model technique	Adjustment variables	Risk ratio (95% CI)	Variables included in final model
	Forward addition	HRMO infection Patient-related (large) Infection-related	0.78 (0.58-1.07)	• Healthcare-associated or hospital-onset infection • Bacteremia • Urinary tract infection • Lower respiratory tract infection • Infection with *Pseudomonas aeruginosa* • Sepsis at infection onset • Septic shock at infection onset • Charlson comorbidity index ≥5
Evaluated exposure: Gram-negative infection				
PCA: non-HRMO	Unadjusted		1.32 (1.06-1.63)	-
	Backward elimination	Patient-related (small)	1.32 (1.06-1.65)	• Other bacterial infection at infection onset • (Par)enteral feeding
	Forward addition	Patient-related (large)	1.23 (0.99-1.54)	• Other bacterial infection at infection onset • (Par)enteral feeding • Charlson comorbidity index ≥3 • Preceding treatment restriction
PCA: HRMO	Unadjusted		1.69 (0.89-3.20)	
	Backward elimination	Patient-related (small)	1.42 (0.66-3.09)	• Sex • Known colonization with an HRMO • Admission from long-term care facility • Other bacterial infection at infection onset • Solid malignancy • Renal disease • Preceding surgical procedure
	Forward addition	Patient-related (large)	1.20 (0.54-2.66)	• Known colonization with an HRMO • Hospital-onset infection • Admission from long-term care facility • Other bacterial infection at infection onset • Solid malignancy • Preceding surgical procedure • Preceding treatment restriction • Peripheral vascular disease

Analysis type	Model technique	Adjustment variables	Risk ratio (95% CI)	Variables included in final model
PCA: HO	Unadjusted		1.45 (1.03-2.04)	-
	Backward elimination	Patient-related (small)	1.58 (1.12-2.22)	• Preceding treatment restriction
Evaluated exposure: HRMO infection				
ICA: bacteremia subgroup	Unadjusted	-	0.62 (0.30-1.27)	-
	Backward elimination	Patient-related (small)	0.59 (0.28-1.24)	• Age • Known colonization with an HRMO • Admission from long-term care facility • Metastasized solid malignancy
	Backward elimination	Patient-related[a] Infection-related	0.82 (0.39-1.74)	• Age • Known colonization with an HRMO • Metastasized solid malignancy • Urinary tract infection • Intra-abdominal infection (excl biliary tract) • Postoperative infection • Infection with *Escherichia coli* • Infection with *Klebsiella pneumoniae* • Infection with *Enterobacter cloacae* • Infection with other Gram-negative species • Severe sepsis at infection onset • Septic shock at infection onset • Antibiotic therapy prior to hospital admission
	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; HRMO, highly resistant micro-organism; ICA, infection-cohort analysis; ICU, intensive care unit; PCA, parallel cohorts analysis.

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 • Hospital-onset infection, healthcare-associated 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 Enterobacteriaceae, 3GC-resistant Enterobacteriaceae, with G- non-fermenters, or with *Pseudomonas* spp. • Preceding bacteremia with Enterobacteriaceae, 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 admission • Receipt of prophylactic antibiotic therapy
Infection-related intermediates	• Causative pathogens, including Gram-negatives and others (16 variables) • Type of infection (10 variables) • Sepsis severity (3 variables) • Antibiotic therapy prior to hospital 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, 3rd generation cephalosporin; G-, Gram-negative.

References

1. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008; 36: 309–32.
2. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1. The European Committee on Antimicrobial Suceptibility Testing, 2018 http://www.eucast.org/clinical_breakpoints/.
3. EUCAST Expert Rules. Version 3.1. The European Committee on Antimicrobial Suceptibility Testing, 2018 http://www.eucast.org/expert_rules.
4. Steyerberg EW. Clinical prediction models: A practical approach to development, validation, and updating. New York, NY, United States of America: Springer Science+Business Media, 2009.
5. Buuren S van, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. *J Stat Softw* 2011; 45. DOI:10.18637/jss.v045.i03.
6. Greenland S, Daniel R, Pearce N. Outcome modelling strategies in epidemiology: traditional methods and basic alternatives. *Int J Epidemiol* 2016; 45: 565–75.
7. Friedman ND, Kaye KS, Stout JE, *et al.* Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. *Ann Intern Med* 2002; 137: 791–7.
8. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373–83.
9. Kotton CN, Kroger AT, Freedman DO. Immunocompromised travelers. CDC Yellow B. 2017. https://wwwnc.cdc.gov/travel/yellowbook/2018/advising-travelers-with-specificneeds/immunocompromised-travelers (accessed Nov 7, 2018).
10. Bone RC, Balk RA, Cerra FB, *et al.* American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864–74.

CEPHALOSPORINS CARBAPENEMASE DISEASE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL MORTALITY DISEASE BURDEN GRAM-NEGATIVE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET PROPENSITY SCORE INFECTION COHORT METHODS GRAND-ABC BURDEN CEPHALOSPORINS BACTERIA BLOODSTREAM INFECTION MORTALITY PREDICTION GRAM-NEGATIVE INFECTION PREDICT ANTIBIOTIC CEPHALOSPORINS CARBAPENEMASE DISEASE TRIAL MORTALITY ANTIBIOTIC PREDICT BLOODSTREAM GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL ANTIBIOTIC DISEASE CHAPTER 4 CAUSAL INFERENCE MORTALITY GRAM-NEGATIVE BACTERIA PREDICTION GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM

The methodology of causal inference in antibiotic resistance research

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Abstract

Introduction

There have been several observational studies on the mortality burden caused by carbapenem resistant bloodstream infections. However, an increase in attention to causal inference from observational data revealed that many causal studies use subpar methods, resulting in uninterpretable effect estimates. We review the methodology of literature on carbapenem resistant bloodstream infections, assess the impact of reported regression models by applying them to two datasets, and describe methodological problems with causal inference and antibiotic resistance in the light of the counterfactual framework.

Methods

We included articles that compared carbapenem resistant bloodstream infections to another group (including non-infected controls), had mortality as outcome, and that did not explicitly have a predictive goal. We investigated methods, bias and causal language. We applied the models reported in the reviewed publications to two datasets and analyze the impact on the effect size (odds ratio) and confidence interval. We related issues found in the review to the counterfactual framework for causal inference, and propose a template that may help with designing studies assessing the burden of carbapenem resistance.

Results

13 studies on the effect of carbapenem resistance on mortality were included. 7 were on Enterobacteriaceae, 5 on *Klebsiella*, 1 on *Pseudomonas* bloodstream infections. 11 out of 13 studies used explicit causal language in describing their findings. Among identified issues with causal inference, backwards selection of variables and unclear reporting of selection and information bias were the most common. 5 out of 13 papers adjusted for inappropriate therapy (an intermediate variables), and three studies adjusted for other variables that occurred after infection onset. The impact of the models on the effect estimates in the studies ranged from -39% to +38% in dataset 1 compared to the crude OR, and -26% to +12% in study two. In two cases, interpretation would have changed to statistically significant in dataset 1.

Conclusion

Methodology of causal inference in studies on the mortality caused by carbapenem resistant bloodstream infections is sub-par, and there is a large impact on reported effect estimates.

Introduction

There have been several studies investigating the effect of carbapenem resistance in Gram-negative bloodstream infections on mortality. Correctly estimating this mortality burden important for allocation of healthcare resources. However, since only observational studies are feasible to answer this causal research question, there is a risk of confounding, information and selection bias.

Many currently published studies use subpar methods for causal inference.[1] This includes adjustment for intermediate variables, confounder selection without employing background knowledge and inadequate study design.[2] Additionally, the language used by researchers often obfuscates causal goals by being implicit about causality.[3] This may result in presenting arbitrary and data-driven models as causal models. These models may be biased (estimate is incorrect) and imprecise (confidence interval too wide), thus resulting in causal estimates that may be false.

In the last decade, there has been an increasing interest in the counterfactual framework (potential outcomes framework) for the estimation of causal effects.[4,5] Three conditions for causal inference from observational studies are outlined in this framework: Exchangeability (conditional independence of exposure and outcome based on measured variables), positivity (exposure in all levels of measured variables) and consistency (a clearly defined causal contrast). These assumptions are operationalized by the target trial, the hypothetical randomized trial that would answer the research question, and the directed acyclic graph, a way to visualize causal relationships between variables, which can help with assessing bias and confounder selection.[6,7] Considering the assumptions of the framework in study design and analysis may be beneficial for improving the reliability of causal estimates.[8]

As of yet, the methods in studies on the burden of carbapenem resistant bloodstream infections have not been investigated. Additionally, antibiotic resistance may pose several particular issues with the aforementioned counterfactual framework assumptions, and describing these in the context of the counterfactual framework may be useful. In this study, we have three goals: 1. to review the study design and statistical methods employed in a selection of literature on carbapenem resistance 2. to apply the statistical models on two datasets to investigate the impact on effect estimates and confidence intervals and 3. To relate the findings from the review to the assumptions of the counterfactual framework.

Methods

Literature review

We searched the literature with the terms **(carbapenemase*) 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*).**

We screened titles and abstracts and included them in the study if they

1. Contrasted infections with carbapenem resistant pathogens to a different kind of infection or no infection
2. Did a multivariable analysis
3. Did not explicitly mention it was for prediction (e.g. a diagnostic model)

For feasibility, we decided to stop at a maximum of 15 publications. We extracted the following data from the articles:

Study design, sample size, effect size, collected variables, statistical analysis, effect estimates and the language used to describe the findings (causal statements). We assessed how the authors reported confounding, missing data, colliders, selection bias and measurement error, and analyzed the studies for adjustment for intermediates and outcome variables. We analyzed the presence of causal statements and language, including notions like "independent risk factor" and any mention of confounding, colliders, directed acyclic graphs and 'causes'.

Application of models from studies to datasets

To show the impact of the different reported models by the authors, we applied the reported multivariable models to two datasets.

The first dataset (Study 1) is a Dutch prospective multicenter cohort study in 8 hospitals on the burden of antibiotic resistance in Gram-negative bacteria in hospitalized adult patients. It contains a random sample of Gram-negative infections collected between 2013 and 2015. From this study, we selected a subset of Enterobacterales bloodstream infections from seven hospitals. The exposure in this dataset was third-generation cephalosporin resistance, the outcome 30-day mortality. Notably, since carbapenem resistance is extremely rare in the Netherlands, we used a different exposure. However, conceptually there is little difference, and we think the same strategy to confounder selection would apply, thus legitimizing this choice of exposure.

The second dataset (Study 2) is a Israeli single center study on the burden of carbapenem resistance in Klebsiella pneumoniae bloodstream infection in hospitalized patients. Data was collected between January and December 2006. Exposure in this dataset was carbapenem resistance, outcome 30-day mortality. Of note, the study reporting from this dataset is included in the review.[9]

We assessed the impact of the different models reported by authors by applying these models to the two datasets. Impact was defined as deviation from the crude outcome measure and size of confidence interval. We assumed that the reported models by the authors reflected the conditions under which the association between carbapenem resistance and mortality can be interpreted as causal. For example, if the final model reported by author included inappropriate therapy, cardiovascular disease and carbapenem resistance, we assumed the authors considered that adjusting for inappropriate therapy and cardiovascular disease would result in a causal estimate of the effect of carbapenem resistance on mortality.

We repeated this process for every study in the review (where possible). In several instances, we had to adapt variables when applying the models in our datasets. For example, one study included the APACHEII disease severity score, which was not part of our two datasets. In that case, we used the Pitt bacteraemia score or sepsis severity score.

We created logistic regression models with the aforementioned exposures and outcome. If anything else was reported (effect modification, analysis with propensity score), we tried to emulate this analysis as described. We also performed a backwards stepwise selection procedure, where we started with a full model with all confounders and select the best fitting model based on the Akaike Information Criterion (AIC).

We report odds ratios (OR's) with 95% confidence intervals.

All analyses were performed in *R* version 3.5.1

Relation with the counterfactual framework

We assessed the different assumptions of the counterfactual framework in relation to antibiotic resistance. We supply this analysis with findings from the literature review, and do suggestions the counterfactual framework in relation to antibiotic resistance and mortality was performed, based on literature and expert knowledge, and supported by findings from the above described literature review.

Results

Review

Thirteen studies were included in the analysis, of which eleven were published in 2012 or later and 6 in 2015 or later. Eleven out of 13 were single center. Over half (7/13) involved resistance in Gram-negative Enterobacterales, followed by Klebsiella pneumoniae (5/13) and one Pseudomonas. The average sample size was 209 +/- 160. Of the thirteen studies, eleven had a causal contrast involving impact of carbapenem resistance versus carbapenem sensitivity on mortality. One study reported an interest in the specific effect of carbapenamase-production compared to non-carbapenemase producing CRE, one was unclear in defining the contrast. Causal claims (e.g. impact, confounding, independent risk factor) were made in 11/13 studies. The most common causal claim was 'independent risk factor' (5/13, 38%) (Table 1).

Table 1: Summary of articles

Descriptives	
Studied population	
Enterobacteriaceae (CRE vs non-CRE)	**7/13**
Pseudomonas BSI	**1/13**
Klebsiella BSI	**5/13**
Control group	
Prospective cohort	**4/13**
Retrospective cohort	**7/13**
Case control	**2/13**
Setting	
One ward	**9/13**
Multiple wards	**4/13**
ICU	**2/13**
Mean sample size	**209 infections +/- 160**
Mean number of carbapenem resistant pathogens (min-max)	**47 [7 – 145]**
Mean effect size (range min-max)	**6.7 (0.46- 9.33**
Clear question of effect CRE from outset	**11/13**
Causal claims	**11/13**
Outcome	
14-day mortality	**2/13**
30-day mortality	**7/13**
In-hospital mortality/ICU mortality	**4/13**
Confounding and modeling	
Explanation of why variables collected	**0/13**
Adjusted for:	
Comorbidities	8/13
Disease severity	6/13
Inappropriate therapy	5/13
Later occurring variables	3/13
Left out carbapenem resistance in final model	2/13
Forced confounders in model	**4/13**
Backwards selection of variables	**10/13**
Other bias and reporting information	
Information on missing data	
Not mentioned	**10/13**
Complete case analysis	**1/13**
Mentioned but unclear	**1/13**
All data complete	**1/13**
Information on timing of exposure/variables	**3/13**
Selection bias	
Information on loss to follow up	**2/13**
Censoring	**1/13**
Discharged patients interpreted as alive	**1/13**
Information on measurement errors	**0/13**
Information of timing of measurements	**3/13**

The inclusion criterion in all studies was a positive blood culture with a respective pathogen. All studies used automated systems (Vitek) for ascertainment, which was sometimes confirmed with disk diffusion methods. Polymicrobial cultures were often a reason for exclusion (6/13).

Occurring exclusion criteria were age <18, polymicrobial blood culture and repeated infection.

Confounding was dealt with by stepwise backward selection methods in ten out of thirteen studies. In one study, a priori confounders were selected and forced in the model. In zero studies it was explicitly explained why certain variables were selected (based on literature references). No directed acyclic graphs were reported. Information bias and selection bias were not explicitly addressed in any of the studies. Missing data were mentioned in 2/13 papers, where a complete case analysis was performed or "no missing data occurred". Timing of confounder measurements (e.g. disease severity) was mentioned in 3 out of 13 papers. Outcomes were 30-day mortality (9/13) 14-day mortality (2/13) and in hospital/ICU mortality (2/13).

Comorbidities were generally studied in groups (cardiovascular disease, malignancy) and further summarized as in a comorbidity score (Charslon comorbidity index). Studies performed in specific wards included more specific candidate confounders, e.g. on hematology wards, variables related to hematology were investigated (e.g. type of malignancy, bone marrow transplant). Adjustment was made for acute disease severity in 8 out of 13 papers, and inappropriate therapy in 5 out of 13. Three studies included variables in the model other than inappropriate therapy that occurred after infection onset, including complications, definitive appropriate therapy and change of definitive therapy.

Application of results to own datasets

The results of the application of the models to the two datasets are shown in table 2.

Study 1 involved 640 patients with a Gram-negative Enterobacterales bloodstream infection from 7 Dutch hospitals. The mortality was 99 out of 640 (15.4%). The crude OR of the effect of third-generation cephalosporin resistance in Enterobacterales (n=61) on 30-day mortality was 0.48 (95% CI 0.16 – 1.11). After adjusting according to several models presented in table 2, the range of odds ratios was 0.30 (95% CI 0.10 – 0.88) to 0.67 (95% CI 0.22 – 1.68), a reduction of 38% and increase of 39% compared to the crude OR, respectively. Width of confidence intervals on the log scale (precision) varied between the analyses, with the largest interval from 0.21 to 2.21 and the smallest from 0.16 to 0.97. Interpretation of the outcome would have changed from 'non-significant' to a statistically significant effect in two of the analyses. In one model (from Nour et al) there was adjustment for infectious complications, a secondary endpoint. When adjusting for a similar secondary endpoint, the OR decreased to 0.31 (95% CI 0.07 – 0.95). Two models could not be applied due to resistance being removed from the analysis in the stepwise regression and no reporting of variables in the model respectively. In a stepwise regression analysis, starting with a full model with demographics, comorbidities, colonization and infection related variables, we come to an OR of 0.56 (95% CI 0.17-1.45).

Table 2: application of causal models to datasets

		OR GRAND-ABC	OR BEN-DAVID	Adjusted variables
Crude OR		0.51 (0.17 - 1.20)	4.96 (2.43 – 11.43)	
Article 1	Andria[26]	0.34 (0.12 – 0.97)	3.82 (1.76 – 8.38)	Propensity score: functional capacity, prior antibiotic, colonization, last chemotherapy treatment, nasogastric tube, appropriate therapy, length of stay
2	Ben-David[27]	0.48 (0.16 -1.19)	5.20 (1.75 –16.45)	Charlson score, Pitt bacteraemia score, ESBL-producing pathogens
3	Biehle[28]	0.48 (0.16 – 1.03)	5.46 (2.13 – 14.76)	Pre-infection LOS, APACHE2-score*
4	Hussein[29]	0.60 (0.20 – 1.42)	3.67 (1.66 – 8.30)	Liver disease, dialysis, bedridden*,Charlson comorbidity score
5	Mouloudi[30]	-	-	Unclear what variables in model
6	Nour[31]	0.31 (0.07 – 0.95)	-	Confounders related to neonatal care, adjustment for secondary outcome variable
7	Peña[32]	0.55 (0.18 – 1.36) Charlson <3: 0.47 (0.06 – 1.67) Charlson >2: 0.52 (0.11 (1.82)	5.14 (1.80- 15.87) Charlson <3: 2.83 (0.53 - 18.17) Charlson >=3: 8.37 (2.08 – 40.84)	Inappropriate therapy, high risk source, pitt bacteraemia score. Effect modification by Charlson comorbidity score.
8	Stoma[33]	0.47 (0.16 – 1.11)	4.34 (1.96 – 9.92)	Inappropriate therapy
9	Tamma[34]	0.43 (0.14 – 1.07])	4.27 (1.59 – 12.14)	Inappropriate empiric and targeted therapy, days of combination ther, colistin, diabetes mellitus, Pitt score*
10	Villegas[35]	0.82 (0.25 – 2.21)	4.85 (1.87 – 13.70)	Change of definitive therapy, definitive therapy with carbapenem, pitt score
11	Xiaopeng[13]	-	-	CR not in final model
12	Schwaber[12]	0.54 (0.18 – 1.31)	5.17 (2.28 – 12.27)	Mechanical ventilation, malignancy
13	Patel[36]	0.50 (0.17-1.19)	3.79 (1.76 – 8.37)	Heart disease, liver disease, ICU-admission
14	Backwards stepwise selection	0.56 (0.17 – 1.49)	5.67 (1.91 – 18.00)	Pitt score, Charlson comorbidity index

Study 2 included 192 patients with a bloodstream infection with *K. pneumoniae*, of which 44 (22.9%) were carbapenemase resistant (KPC producers). There were 74 deaths (38.5%). The crude OR was 4.96 (95% CI 2.43– 11.43). Applying the other models led to a range of 3.79 to 5.67, a 26% reduction to 14% increase in the effect estimate. The largest confidence interval was 1.91 to 18.00 and the smallest 1.66 to 8.30. Adjusting for inappropriate therapy alone decreased the OR from 4.8 to 3.8 [95% CI 1.7 – 8.3). Three models were not applied: the two mentioned above and one (Nour et al) due to neonatal comorbidities and adjustment for a competing event which we did not have data on in study 2.

Table 3: assumptions of the counterfactual framework and antibiotic resistance

Framework part	Definition	What to think about with ABR
Consistency	Clear definition of counterfactual outcome and exposure	How to define resistance? What is the counterfactual? (No infection vs infection with non-resistant pathogen) Role of species Role of resistance genes
Exchangeability	Group of exposed patients would have similar outcome as unexposed if they had not been exposed - conditional on measured variables	What are relevant confounders? Context of confounders? Whole hospital, single ward? Selection of confounders with smaller sample sizes Adjustment for inappropriate therapy and disease severity after infection onset
Positivity	Probability of receiving exposure is >0 for all strata of confounders	Measurement of very specific variables in general hospital population Small sample sizes
Measurement error/bias	Bias caused by incorrect measurement of exposure, outcome and/or other variables.	Timing of measurements (before, after blood culture) Isolation in patients with colonization may lead to fewer measurements Ascertainment of resistance, bias in repeated cultures Definition of confounders (e.g. inappropriate therapy duration?)
Selection bias	Distortion of relation between exposure and outcome by selection into study, loss to follow up or incorrect adjustment (collider bias).	Immortal time (adjustment for therapy after infection onset), loss to follow up (discharge=survival?)

The counterfactual framework and the burden of antibiotic resistance.

The three identifiability conditions, consistency, positivity and exchangeability, form the core of the counterfactual framework. These conditions encompass the different biases, the causal contrast and data problems, and satisfying these conditions (although they are not testable) is necessary for estimating casual effects. Many of the points described in the literature review thus involve 'violations' of one or more conditions. Below, we describe the identifiability conditions and relate them to estimating the mortality burden of antibiotic resistance. To start, we ask readers to think about the hypothetical randomized trial they would conduct to answer the question: what is the attributable mortality of carbapenem resistant infections

compared to carbapenem sensitive infections. We further present our ideas on confounder selection and study design in a directed acyclic graph (figure 1a, Table 3).

Consistency

Consistency involves a clearly defined causal contrast and that this causal contrast is reflected by the data. Although it seems trivial, it is an essential part of causal inference. The causal contrast must be precise enough for the estimate to have relevant implications. As a short illustration of its essence, a trial for pneumonia treatment which would in its protocol describe the exposure as 'intravenous antibiotics' versus 'oral antibiotics', would be frowned upon. Intravenous antibiotics could be anything from penicillin to vancomycin, and thus any outcome of that trial would be impossible to interpret.

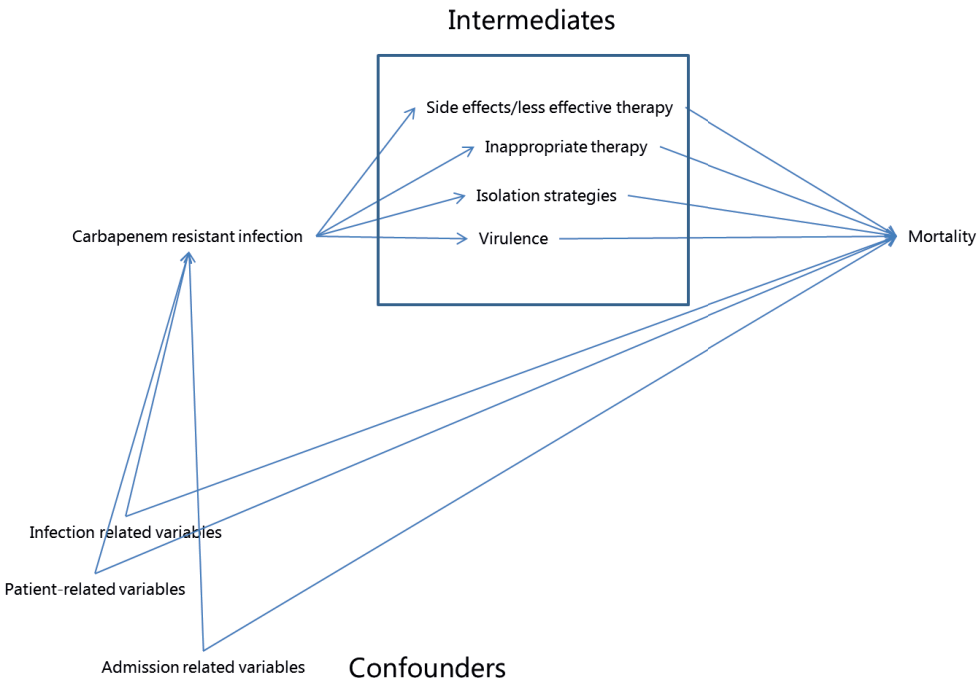

Figure 1a: There are four potential pathways how carbapenem resistant infections cause mortality, the intermediates, and there are three major groups of confounders, as described in the publication, which are important to adjust for. By no means intended to be definitive, or true, we propose this as a template to work with when conducting a study on antimicrobial resistance

We consider three potential points of violation of the consistency assumption.

1. The counterfactual of a resistant infection can be both an infection with a non-resistant pathogen or a control patient without infection, as described by Kaye e al. in 2004.[10] On a grander

scale, this describes the issue of replacement (resistant infections replace sensitive infection) or addition (resistant infections come on top of other infections).[11] The counterfactual of a carbapenem resistant infection thus depends on the chosen perspective and can be a sensitive infection, in the second a non-infected control patient. The implications of this are large: the attributable burden of resistance is much larger when having a non-infected (community dwelling) control than an antibiotic-sensitive infection. In the reviewed studies, 11 out of 13 compare resistant infections to their sensitive infections, and one compares both.[12] It is thus important to explicitly state the control group in the research question and explain the reasons for this choice.

2. Studies involve several pathogens with similar resistance mechanisms but dissimilar virulence. This introduces different versions of the exposure with different causal effects on mortality (for example, a carbapenem resistant *Klebsiella* and *E.coli* are both considered 'exposure' in the same study). Since some resistance mechanisms will be more prevalent in certain species you can (and have to) argue whether this comparison is sensible. In five studies, authors chose to investigate *Klebsiella pneumoniae* infections only. This solves the problem, but may not answer the broader research question of interest.

3. In defining resistance, we can look at resistance phenotypes (based on MIC), resistance genes (which might sometimes have MIC in the sensitive range), intrinsic resistance (e.g. *Stenotrophomonas*), all of which may result in different outcomes. There are also several different mechanisms by which a pathogen can be carbapenem resistant. Similar to the previous points, this results in different versions of the exposure. In designing the target trial, one would specify how resistance would be caused to make sure every patient would get the same version of the treatment (exposure). Although in practice this may be unfeasible, there needs to be consensus about whether an OXA-48 carbapenem resistant pathogen is similar to an NDM carbapenem resistant pathogen when trying to assess the impact of carbapenem resistance. Additionally, over time, definitions of resistance change due to new tests or new guidelines (EUCAST, CSLI), which in hindsight changes the exposure and non-exposure group and creates difficulties in interpreting studies.

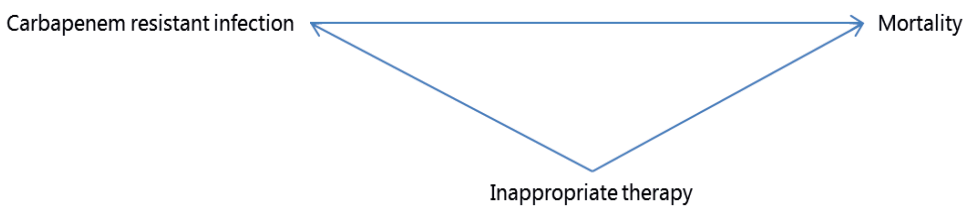

Figure 1b: visualization of what happens when adjusting for inappropriate therapy while interested in the full effect of carbapenem resistance on mortality. By treating it as a confounder, you treat it as a common cause of CR and mortality, which is not particularly logical.

Exchangeability

Exchangeability means that exposure is conditionally independent on the measured variables (implying measurement of all relevant variables). In other words, if the exposed group would have been unexposed, they ought to get the same average outcome as the unexposed group. In observational studies, there are differences between the exposed and unexposed group – which involves both confounding and selection bias. In the target trial, one would randomize to make sure that patients receiving the carbapenem resistant infection are comparable to patients with a sensitive infection in all aspects, also with regards to unmeasured confounding. Since we cannot do that in observational studies, we have to carefully think about what variables are of importance.

Confounding

Based on the review there are two main concerns: choosing adequate confounders and inadequate adjustment. A third issue in observational studies is unmeasured confounding.

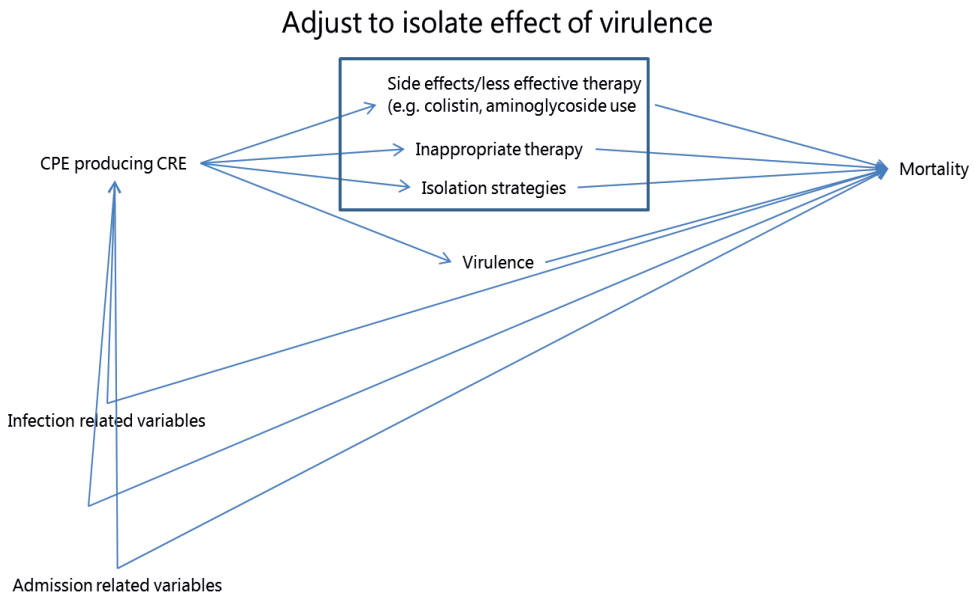

Figure 1c: Based on paper from Tamma et al: when interested in one mediating factor (virulence), adjusting for other intermediates is essential to isolate a single causal pathway, while simultaneously adjusting for the regular confounders that are still present.

Adequate adjustment for confounding preferably involves adjustment for all potential confounders, as this will maximally reduce confounding bias.[5] However, adding more variables to a model comes at a cost of precision: the confidence interval increases and is at odds with constraints in sample size. In the reviewed studies, confounding is primarily treated as a statistical matter by the use of backwards selection of variables. In one study, carbapenem

resistance was dropped from the final model because it was not statistically significant, and thus the research question was not answered.[13]

Background knowledge has been suggested as a more reliable way for variable selection if there is sufficient explanation of this process based on scientific literature.[14] This can also take into account the context-dependency of confounders, since in different settings (and wards), different confounders may be more relevant. Drawing a directed acyclic graph that is specific for the research question and setting can help with clarifying what confounders need to be studied. Additionally, it needs to be kept in mind that a confounder can also have a protective effect on mortality and carbapenem resistance, and that these variables must also be part of the DAG. Further information on variable selection is beyond the scope of this paper, but a good resource for information is a recent publication by Vanderweele et al.[15]

Inadequate adjustment is any adjustment that either removes that specific effect of antibiotic resistance on mortality (an intermediate), or introduces bias by adjusting for a collider.[1617] Adjusting for inappropriate therapy thus cancels out one of the pathways through which antibiotic resistance causes mortality (see figure 1b). The same goes for disease severity (e.g. sepsis, septic shock) measured *after* infection onset. However, with a different research question – in our review the study by Tamma et al, where they want to isolate the effect of a resistance gene – adjusting for these variables can be important (figure 1c).

The last point to address is unmeasured confounding. In observational studies, it can never be completely excluded, and in studies on antibiotic resistance, there may be complex interplays between the microbiome and infections, traveling, family households and immunogenicity that are hard to measure but may be important confounders. Sensitivity analyses can help in showing the potential impact of unmeasured confounding.[18]

Selection bias

Selection bias occurs when there is a systemic difference in the unexposed and exposed group with regards to entering or leaving the study. Selection bias can also result from adjusting for variables that are a common effect of the exposure and the outcome (collider bias). With mortality as an outcome, this is unlikely to happen, but when assessing the effect on morbidity this may be a potential threat. Other forms of selection bias may be selective loss of follow up of patients, where more comorbidity and more severe disease in the antibiotic resistant group might make them more prone to move to a hospice, where follow up stops and it is unknown whether the patient passed away within the study time. If we go back to the trial, and want to estimate the effect of carbapenem resistance in the domain of patients with a bloodstream infection, one hypothetical problem would be that patients with more severe infections caused by resistant pathogens would die before arriving at the hospital, thus not being able to be included in the study and therefore biasing the effect of carbapenem resistant infections on mortality to zero.

Positivity

Positivity is the assumption that there are exposed and non-exposed patients at each level of measured confounders. Violations of positivity are deterministic or random. Deterministic is when a exposed or non-exposed patient cannot be in a certain level of a measured confounder. There do not seem to be any particular deterministic violations of positivity with regards to carbapenem resistance. However, random positivity violation is likely to occur in small sample sizes and many variables to adjust for, and is therefore likely to occur in many studies. For example, in the study by Mouloudi et al, no patients with CRKP had renal disease, while 3 patients in the control group had renal disease. This makes it impossible to adjust for renal disease in this dataset, even if it was an important confounder.[19]

Information bias

Information bias occurs when there is a discrepancy between the measured exposure and/or outcome and the true values. There is independent and dependent, and differential and non-differential measurement bias. Dependency happens if erroneous measurement of outcome and exposure has a similar cause (e.g. a patient recalls both exposure and outcome wrong in an interview). Differential measurement bias occurs when there is a relation between erroneous measurement and the exposure and or outcome.[5] With antibiotic resistance, this latter may occur when in more severely ill patients, more cultures are obtained and the chance of finding a resistance pathogen increases. This form of bias (measurement of exposure depends on precursor of the outcome) can be explored by measuring the number of cultures obtained in different patients may help with understanding the issue.

Further information bias may occur with measurement of confounding variables. There may be discrepancies in patient measurements between isolated (due to colonization with resistant bacteria) patients and non-isolated patients. Differences in timing of confounder measurements introduces bias as well. A disease severity score measured 12 hours before infection onset will be very different than measured at the time of infection onset. If it is measured after infection onset, it is on the pathway of the infection to mortality, and adjusting for this would be inadequate adjustment. This especially causes problems in retrospectively collected data based on chart review, since timing may be imprecise. It would be ideal to standardize measurements of disease severity, but this seems often not feasible. Studies conducted in intensive care units may be able to mitigate some of these concerns by the abundance and precision of (automatically recorded) data points. In the review articles, timing of measurements is not mentioned, and thus we should be careful about interpretation of many adjustments.

Our template

We present our ideas on confounder selection and how carbapenem resistance causes mortality in figure 1a. We suggest to separate potential confounders in three distinct groups: patient, infection and setting-related variables. Furthermore, we assume that there are four pathways how a resistance can lead to mortality:

1. Virulence: genetic mutations leading to increased fitness, increased virulence or propensity to do cellular damage or cause immune reactions, leading to increased acute disease severity

2. Inappropriate treatment: both the carbapenem resistance in itself and the associated co-resistance patterns may lead to an increased chance of inappropriate empiric therapy. Furthermore, co-resistance can severely reduce options of appropriate definitive therapy as well. The risk of inappropriate therapy depends on local practice and guidelines.

3. Isolation precautions may lead to poorer checkups and less involved care for patients, increasing the risk of mortality.

4. Adverse or beneficial effect of treatment. Addition of more antibiotics, with less distinct safety profiles (e.g. aminoglycosides, colistin) might play a role in poorer survival in CR infections.

This can be seen as a basic template for adjustment and is by no means comprehensive, nor does it include all of the addressed points above. It is however a structured approach that may help in streamlining thoughts regarding study design and confounder selection. Examples of inadequate adjustment, and a DAG based on a different review question are shown in figure 1b and 1c.

Discussion

In this study, we reviewed the reporting of causal studies on carbapenem resistance, showed the impact of these models on effect estimates, and describe problems in studying the causal effect of antibiotic resistance on mortality. Most studies use causal language and present their effect estimates as such, while the study design and methods employed lead to sizable variety in effect sizes when applied to the same dataset. Additionally, many relevant aspects of reporting in observational studies were missing.

There has been an increasing interest in causal inference, and aside from methodological developments, more researchers try to apply the principles from a causal framework to improve the quality of their estimates. Last year, journal editors in sleep, critical care and pulmonary medicine published a guide on control of confounding and reporting in observational studies, highlighting problems with regards to confounder selection and reporting, and introducing directed acyclic graphs as a means to clarify causal assumptions.[2] We further specified issues and related them to particular problems with antibiotic resistance research. In studies on the burden of carbapenem resistance, despite many published recently, few of these ideas were applied.

One of the reasons for a lack of clarity in reporting of causal estimates is being vague about the intentions of a study. In short, an epidemiological study can either be descriptive, predictive

(e.g. a prediction model) or causal.[20] In many of the reviewed studies terms like 'independent predictor' are used, which poses itself between predictive ('predictor') and causal (independent, which can be interpreted as causing something), and 'independent factor' and 'independent predictor' are often used interchangeably. Furthermore, terms like 'confounders', a concept only applicable to causal research, are used in the same papers, adding to the confusion.

Interestingly, there have been several publications on methodological issues with antibiotic resistance. Kaye et al. described the importance of choosing the right control group and showed that the impact of MRSA on mortality is larger when comparing to uninfected controls, and that a complete analysis of both the impact of the resistance trait and the public health perspective should include both control groups. The idea of addition and replacement has been further studied by Ammerlaan et al, providing evidence for the concept of addition.[1011] Thom et al report on the impact of adjusting for disease severity at different time points in relation to infection onset, and suggest that measuring the change in disease severity between admission and infection onset is important.[21] Additionally, Rottier et al show that many authors adjust for inappropriate therapy, and the attributive mortality for ESBL BSI increases when not adjusting for this intermediate variable in a meta-analysis on the attributive mortality of ESBL BSI.[22] Schechner et al write about heterogeneity in defining antibiotic resistance and potential consequences in a review from 2013.[23]

The aforementioned studies were important in describing several issues in assessing the burden of antibiotic resistance. However, despite these well-cited papers, many of the ideas have not been implemented in newer studies. We hope that by unifying these points and linking them to the counterfactual framework, we provide a useful basis for new studies on the attributable mortality of antibiotic resistance. We concurrently provide a simple conceptual template to describe our idea of how antibiotic resistance causes mortality, and how to adjust for different types of confounders. The simplicity of the template gives some flexibility to adapt it to a local setting where other patient related variables may be important. For example, when conducting a study in low and middle income countries, hygiene at home (e.g. open defecation) may be an important predictor of both antibiotic resistance and developing worse outcomes.[24]

By applying the models reported by authors to two datasets, we show the variation that occurs by adjusting for several sets of variables. Both the effect estimate and precision (size of the confidence interval) depend heavily on the variables that are added to the model, and effect estimate moves in both directions when adjusting for different variables. Interestingly, adjusting for similar variables in the two datasets does not necessarily change the effect estimate in the same direction. This may partially be explained by a lack of robustness due to smaller sample sizes in the second dataset. Additionally, since the settings between the studies are different, context-dependency of confounders may influence the effect estimates as well. In our view, the value of this part of the study lies in showing the arbitrariness of all the models. Conceptually, it is hard to understand why in one study cardiovascular disease is an important confounder, and liver disease in the other, while both studies are hospital-based

studies on the effect of carbapenem resistance in Klebsiella BSI on mortality. These findings are likely to be the product of randomness, and thus may not reflect a causal association. Implementing background knowledge in the confounder selection process may change this practice, by encouraging researchers to explain these kinds of choices upfront.

An interesting point is that there is no effect of third-generation cephalosporin resistance on mortality in the Dutch study, and the effect estimate is consistently below one in all models, hinting at a protective effect. This was less outspoken in the original cohort, which included all Gram-negative infections (OR of 0.86), but the trend was similar. Reasons for this may be early admission to hospitals and thus mitigating the harm of antibiotic resistance, lower virulence of resistant pathogens, short duration of inappropriate therapy.

The aforementioned issues with reporting, confounder selection impacts the interpretability of effect estimates, and it suggests we do not have any reliable estimates of the effect of carbapenem resistance on mortality. However, estimates from several reviewer papers are currently used in meta-analyses and thus indirectly inform policy and further research.[25] While one may ponder the importance of precisely knowing whether the attributable mortality of carbapenem resistance is 1000 or 10.000 patients per year, scarce healthcare resources and competing public health threats make this an essential endeavor. A further concern is that these numbers also drive further research and pharmacological developments. If antibiotic resistance would not cause harm, developing new antibiotics would be prioritized less, which would free up resources to develop drugs for other diseases.

There are several limitations to consider. First, our study only focuses on carbapenem resistance, and therefore we may have neglected different practices with different pathogens. Second, we decided to interpret the models reported by authors as causal models. It is however questionable whether the authors intend it as such, and whether they would answer 'yes' when asked whether that model is an accurate reflection of reality. However, interpreting the models as causal due to the language used is important in the light of being honest about the intentions of the research, especially since (as mentioned above) these studies are used to estimate the burden of disease. Furthermore, the studies have been performed in different contexts, and this context-dependency per definition does not hold for the two datasets in which we applied the models. However, many studies have been performed in a general hospital population, and thus the underlying confounding structures should at least be similar.

In conclusion, we show that the methodology of causal studies on carbapenem resistance is not ideal, and that there are several issues with reporting. These choices have a major impact on effect estimates. We propose a template which can aid with study design and variable selection.

Acknowledgments

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References

1. Groenwold, R. H. H., Van Deursen, A. M. M., Hoes, A. W. & Hak, E. Poor Quality of Reporting Confounding Bias in Observational Intervention Studies: A Systematic Review. *Ann. Epidemiol.* 18, 746–751 (2008).
2. Maslove, D. M. & Leisman, D. E. Causal Inference From Observational Data: New Guidance From Pulmonary, Critical Care, and Sleep Journals. *Crit. Care Med.* 47, 1–2 (2019).
3. Hernán, M. A. The C-word: Scientific euphemisms do not improve causal inference from observational data. *Am. J. Public Health* 108, 616–619 (2018).
4. Rubin, D. B. Causal inference using potential outcomes: Design, modeling, decisions. *J. Am. Stat. Assoc.* 100, 322–331 (2005).
5. Hernán, M. A. & Robins, J. M. *Causal Inference*. (Chapman & Hall/CRC, forthcoming, 2019).
6. Greenland, S., Pearl, J. & Robins, J. M. Causal diagrams for epidemiologic research. *Epidemiology (Cambridge, Mass.)* 10, 37–48 (1999).
7. Hernán, M. A., Sauer, B. C., Hernández-Díaz, S., Platt, R. & Shrier, I. Specifying a target trial prevents immortal time bias and other self-inflicted injuries in observational analyses. *J. Clin. Epidemiol.* 79, 70–75 (2016).
8. Glass, T. A., Goodman, S. N., Hernán, M. A. & Samet, J. M. Causal Inference in Public Health. *Annu. Rev. Public Health* 34, 61–75 (2013).
9. Ben-David, D. *et al.* Outcome of carbapenem resistant Klebsiella pneumoniae bloodstream infections. *Clin. Microbiol. Infect.* 18, 54–60 (2012).
10. Kaye, K. S., Engemann, J. J., Mozaffari, E. & Carmeli, Y. Reference group choice and antibiotic resistance outcomes. *Emerg. Infect. Dis.* 10, 1125–8 (2004).
11. Ammerlaan, H. S. M. *et al.* Secular trends in nosocomial bloodstream infections: Antibiotic-resistant bacteria increase the total burden of infection. *Clin. Infect. Dis.* 56, 798–805 (2013).
12. Schwaber, M. J. *et al.* Predictors of carbapenem-resistant Klebsiella pneumoniae acquisition anions hospitalized adults and effect of acquisition on mortality. *Antimicrob. Agents Chemother.* 52, 1028–1033 (2008).
13. Li, X. & Ye, H. Clinical and mortality risk factors in bloodstream infections with carbapenem-resistant enterobacteriaceae. *Can. J. Infect. Dis. Med. Microbiol.* 2017, (2017).
14. Hernán, M. a, Hernández-Díaz, S., Werler, M. M. & Mitchell, A. A. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am. J. Epidemiol.* 155, 176–84 (2002).
15. VanderWeele, T. J. Principles of confounder selection. *Eur. J. Epidemiol.* 34, 211–219 (2019).
16. Schisterman, E. F., Cole, S. R. & Platt, R. W. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology* 20, 488–95 (2009).
17. Munafò, M. R., Tilling, K., Taylor, A. E., Evans, D. M. & Smith, G. D. Collider scope: When selection bias can substantially influence observed associations. *Int. J. Epidemiol.* 47, 226–235 (2018).
18. VanderWeele, T. J. & Arah, O. A. Unmeasured Confounding for General Outcomes, Treatments, and Confounders. *Epidemiology* 22, 42–52 (2011).
19. Petersen, M. L., Porter, K. E., Gruber, S., Wang, Y. & van der Laan, M. J. Diagnosing and responding to violations in the positivity assumption. *Stat. Methods Med. Res.* 21, 31–54 (2012).
20. Shmueli, G. To explain or to predict? *Stat. Sci.* 25, 289–310 (2010).
21. Thom, K. A. *et al.* Controlling for Severity of Illness in Outcome Studies Involving Infectious Diseases: Impact of Measurement at Different Time Points. *Infect. Control Hosp. Epidemiol.* 29, 1048–1053 (2008).
22. Rottier, W. C., Ammerlaan, H. S. M. & Bonten, M. J. M. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum ß-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. *J. Antimicrob. Chemother.* 67, 1311–20 (2012).
23. Schechner, V., Temkin, E., Harbarth, S., Carmeli, Y. & Schwaber, M. J. Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. *Clin. Microbiol. Rev.* 26, 289–307 (2013).
24. Stewardson, A. J. *et al.* Effect of carbapenem resistance on outcomes of bloodstream infection caused by Enterobacteriaceae in low-income and middle-income countries (PANORAMA): a multinational prospective cohort study. *Lancet Infect. Dis.* 3099, 1–10 (2019).
25. Martin, A., Fahrbach, K., Zhao, Q. & Lodise, T. Association between carbapenem resistance and mortality among adult, hospitalized patients with serious infections due to enterobacteriaceae: Results of a systematic

literature review and meta-analysis. *Open Forum Infect. Dis.* 5, 1–9 (2018).
26. Andria, N. *et al.* Mortality burden related to infection with carbapenem-resistant Gram-negative bacteria among haematological cancer patients: A retrospective cohort study. *J. Antimicrob. Chemother.* 70, 3146–3153 (2015).
27. Ben-David, D. *et al.* Outcome of carbapenem resistant Klebsiella pneumoniae bloodstream infections. *Clin. Microbiol. Infect.* 18, 54–60 (2012).
28. Biehle, L. R. *et al.* Outcomes and Risk Factors for Mortality among Patients Treated with Carbapenems for Klebsiella spp. Bacteremia. *PLoS One* 10, e0143845 (2015).
29. Hussein, K. *et al.* Impact of carbapenem resistance on the outcome of patients' hospital-acquired bacteraemia caused by Klebsiella pneumoniae. *J. Hosp. Infect.* 83, 307–313 (2013).
30. Mouloudi, E. *et al.* Bloodstream infections caused by carbapenemase-producing klebsiella pneumoniae among intensive care unit patients after orthotopic liver transplantation: Risk factors for infection and impact of resistance on outcomes. *Transplant. Proc.* 46, 3216–3218 (2014).
31. Nour, I. *et al.* Risk factors and clinical outcomes for carbapenem-resistant Gram-negative late-onset sepsis in a neonatal intensive care unit. *J. Hosp. Infect.* 97, 52–58 (2017).
32. Pena, C. *et al.* Prospective multicenter study of the impact of carbapenem resistance on mortality in Pseudomonas aeruginosa bloodstream infections. *Antimicrob. Agents Chemother.* 56, 1265–1272 (2012).
33. Stoma, I., Karpov, I., Milanovich, N., Uss, A. & Iskrov, I. Risk factors for mortality in patients with bloodstream infections during the pre-engraftment period after hematopoietic stem cell transplantation. *Blood Res.* 51, 102–106 (2016).
34. Tamma, P. D. *et al.* Comparing the Outcomes of Patients With Carbapenemase-Producing and Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae Bacteremia. *Clin. Infect. Dis.* 64, 257–264 (2017).
35. Villegas, M. V. *et al.* Characterization and Clinical Impact of Bloodstream Infection Caused by Carbapenemase-Producing Enterobacteriaceae in Seven Latin American Countries. *PLoS One* 11, e0154092 (2016).
36. Patel, G., Huprikar, S., Factor, S. H., Jenkins, S. G. & Calfee, D. P. Outcomes of Carbapenem-Resistant Klebsiella pneumoniae Infection and the Impact of Antimicrobial and Adjunctive Therapies . *Infect. Control Hosp. Epidemiol.* 29, 1099–1106 (2008).

CEPHALOSPORINS CARBAPENEMASE DISEASE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL MORTALITY DISEASE BURDEN GRAM-NEGATIVE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET PROPENSITY SCORE INFECTION COHORT METHODS GRAND-ABC BURDEN CEPHALOSPORINS BACTERIA BLOODSTREAM INFECTION MORTALITY PREDICTION GRAM-NEGATIVE INFECTION PREDICT ANTIBIOTIC CEPHALOSPORINS CARBAPENEMASE DISEASE TRIAL MORTALITY ANTIBIOTIC PREDICT BLOODSTREAM GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL ANTIBIOTIC DISEASE CHAPTER 5 CAUSAL INFERENCE MORTALITY GRAM-NEGATIVE BACTERIA PREDICTION GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM

An international prospective cohort study to validate two prediction rules for infections caused by 3rd-generation cephalosporin-resistant Enterobacterales

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Abstract

Introduction

The possibility of bloodstream infections caused by 3rd-generation cephalosporin-resistant Enterobacterales (3GC-R-BSI) leads to a trade-off between empiric inappropriate treatment (IAT) and unnecessary carbapenem use (UCU). Accurately predicting 3GC-R-BSI could reduce IAT and UCU. We externally validate two previously derived prediction rules for community-onset (CO) and hospital-onset (HO) suspected bloodstream infections.

Methods

In 33 hospitals in 13 countries we prospectively enrolled 200 patients in whom blood cultures were obtained and intravenous antibiotics with coverage for Enterobacterales were empirically started. Cases were defined as 3GC-R-BSI or 3GC-R Gram-negative infection (3GC-R-GNI) (analysis 2), all other outcomes served as comparator. Model discrimination and calibration were assessed. Impact on carbapenem use was assessed at several cut-off points.

Results

4,650 CO infection episodes were included and the prevalence of 3GC-R-BSI was 2.1% (n=97). IAT occurred in 69 of 97 (71.1%) 3GC-R-BSI and UCU in 398 of 4553 non-3GC-R-BSI patients (8.7%). Model calibration was good and the AUC was 0.79 (95% CI: 0.75 – 0.83) for 3GC-R-BSI. The prediction rule potentially reduced IAT to 62% (60/97) while keeping UCU comparable at 8.4% or could reduce UCU to 6.3% (287/4553) while keeping IAT equal. IAT and UCU in *all* 3GC-R-GNI improved at similar percentages.

1,683 HO infection episodes were included and the prevalence of 3GC-R-BSI was 4.9% (n=83). Here model calibration was insufficient.

Conclusion

A prediction rule for community-onset 3GC-R infection was validated in an international cohort and could improve empirical antibiotic use. Validation of the hospital-onset rule yielded suboptimal performance.

Introduction

Choosing empiric antibiotic therapy is increasingly troublesome due to the rise in antibiotic resistance. Inappropriate empiric treatment of infections caused by third-generation cephalosporin resistant Enterobacterales (3GC-R-E) may lead to worse outcomes for individual patients, whilst liberal use of carbapenems increases selective pressure for carbapenem resistance.[1] Therefore, better prediction of a patient's risk of infection with 3GC-R-E would improve clinical decision making in this trade-off between inappropriate empiric antibiotics and unnecessary use of broad-spectrum antibiotics, such as carbapenems.

Previous studies have focused on prediction of bloodstream infections (BSI) caused by third-generation cephalosporin resistant Enterobacterales (3GC-R-BSI).[2,3,4] However, in all studies patients with 3GC-R-BSI were compared to patients with documented BSI, caused by non-3GC-R Gram-negatives, ignoring patients who are empirically treated for Gram-negative infection in whom blood cultures remain or do not yield Gram-negative bacteria. To be useful for selecting empiric antibiotics, a prediction rule must be derived from all patients in that domain, which includes all patients treated for presumed Gram-negative infection, even when the blood culture yields Gram-positive pathogens or no pathogens at all.

Rottier et al. previously developed two prediction rules for 3GC-R-BSI, one for community onset and one for hospital onset suspected bloodstream infections, based on a retrospective nested case-control study in eight Dutch hospitals.[5] Both rules more accurately predicted the risk of 3GC-R-BSI compared to the presence of two risk factors, being prior isolation of 3GC-R-E in microbiological testing or prior fluoroquinolone/cephalosporin use. These risk factors are current recommended in the national guideline for considering coverage of these bacteria in empirical treatment. Yet, before implementation of a new prediction rule, external validation is required. In this study, we validated both prediction rules in an international prospective cohort study.

Methods

Settings and patients

Between February 2017 and June 2019, we performed a prospective cohort study in 33 centers in 13 countries, of which 26 were university hospitals. In every hospital the goal was to collect 200 consecutive infection episodes where a causative role of Gram-negative pathogens was suspected. Such episodes were defined as:

1. The obtainment of a blood culture

AND

2. The start of intravenous antibiotics that cover Gram-negative pathogens within a period of two hours before through 12 hours after blood culture obtainment

AND

3. Patient being 18 years or older

Details on inclusion criteria are specified in supplement A

The outcome of interest was 3GC-R-BSI with all other blood culture results (including negative) serving as comparator. In a second (utility) analysis, 3GC-R-E (which included 3GC-R-BSI) was considered as the outcome. 3GC-R-BSI was defined as an infection episode in which 3GC-R-E was cultured in at least one blood culture bottle (drawn from any site). Multiple sets of blood cultures obtained on the same calendar day were considered part of the same infection episode.

Analysis 2 serves to assess the effect of using the prediction rule use for all 3GC-R infections, when using the prediction rule derived for predicting the risk of 3GC-R-E BSI. To clarify: in analysis 2 we only redefine the outcome to incorporate non-BSI, but do not reassess model performance. The study population (suspected BSI) remains the same.

Infection episodes were categorized as community onset if blood culture obtainment occurred at day 0, 1 or 2 of hospital admission. All other episodes were categorized as hospital onset infections. Species identification and susceptibility testing were based on local standard procedures. All hospitals except one used EUCAST criteria for determination of antibiotic susceptibilities. The other hospital used CSLI criteria.

Data collection

Data were entered in an electronic Case Record Form (eCRF; ResearchOnline) in two steps. First, local investigators screened consecutive blood cultures and determined study eligibility, followed by entering culture and admission dates and relevant predictors for either community or hospital onset infection. Data entry was based on chart review. Second, blood culture results were entered at a later stage in a separate eCRF to avoid information bias. Additional data collection included empiric antimicrobial treatment, the ward on which the blood culture was obtained and the presence of any other clinical cultures yielding 3GC-R E at the day of blood culture obtainment.

Prediction rule and definition

For the two prediction rules, see box 1. For definition of individual predictors, see supplementary table A.

We defined empiric use of carbapenems as appropriate if prescribed in patients with 3GC-R-BSI (analysis 1) and in patients with 3GC-R-GNI (analysis 2). Unnecessary carbapenem use was defined as any carbapenem prescription for infection episodes without 3GC-R-BSI (analysis 1) or without 3GC-R-GNI (analysis 2). Inappropriate therapy was defined as *not* prescribing carbapenems for 3GC-R-BSI (analysis 1) or for 3GC-R-GNI (analysis 2).

Sample size

We used the statistical rule of thumb of 100 cases (3GC-R-BSI) per prediction rule.[6] For

feasibility reasons it was decided to end data collection in June 2019 with 97 and 83 3GC-R-BSI cases of community-onset and hospital-onset infections, respectively.

Box 1: Prediction rules

Community onset rule

Age (number of years)

Prior identification of a 3GC-R EB in the last year (Y/N)

Prior antibiotic use in the last two months (Y/N)

Immunocompromised patient (Y/N)

Suspected infection source: urinary tract (Y/N)

Suspected infection source

Hospital onset rule

Prior identification of a 3GC-R EB in the last year (Y/N)

Prior cephalosporin use in the last two months (Y/N)

Surgery in the last 30 days (Y/N)

Suspected respiratory tract infection (Y/N)

Solid malignancy (Y/N)

Renal disease (Y/N)

Signs of hypoperfusion (Y/N)

Length of stay prior to infection onset (number of days)

Missing data

Three sites that started data collection were excluded: one only included positive blood cultures, one completed data for two patients only and then stopped, and one only entered culture dates without predictor or outcome data.

Included patients without outcome data (culture results) or date of birth were removed from the dataset.

Missing individual predictors were approached pragmatically as to reflect clinical practice, where information about predictors (specifically prior antibiotic use and colonization) is not always available. Thus, whenever a predictor was missing this was considered to be "No". See supplementary for numbers of missing individual predictors.

Statistical analysis

Data was reported as median, mean or percentage where appropriate.

The prediction rules were validated by assessing discrimination and calibration of the prediction models. We calculated c-statistics and corresponding 95% confidence intervals to evaluate the discrimination, which is a measure of how well the model can separate cases and non-cases, and visually assessed the calibration plots. We recalibrated the models to take

into account the higher incidence of 3GC-R-BSI in an international population. To assess the potential impact of the model on "eligibility for carbapenem prescription", we report predictive performance (sensitivity/specificity/positive predictive value/negative predictive value) for different cut-offs, as well as potential changes in carbapenem use with those cut-offs. See supplement B for a more elaborate description of statistical methods.

Ethics

The ethical committees of the University Medical Center Utrecht and all participating hospitals granted a waiver for informed consent because of the observational nature of the study. Patient data was anonymized at the respective study sites.

Results

We included 6,576 infection episodes from 33 hospitals in 13 countries, of which 235 were considered non-eligible (Figure 1). There were 4,650 community-onset and 1,683 hospital-onset infection episodes. Most clinical sites were in Italy (n=7), followed by the Netherlands (n=6) and Spain (n=3). Most 3GC-R-BSI episodes came from Italy (94 out of 180, 52%), followed by Turkey (n=22, 12.2%). In the community onset infections, 55% of episodes had clinical predictors entered within three days or less from blood culture obtainment. In the hospital onset cohort, this held for 49% of episodes (supplementary C).

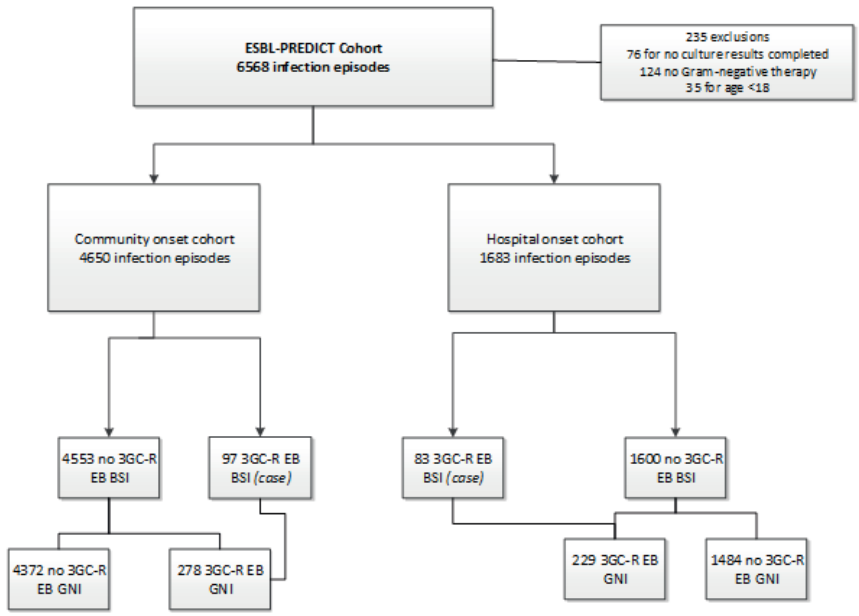

Figure 1: Flow chart of study. There are two separate cohorts, one for the community onset prediction rule and one for the hospital onset prediction rule, where different data was collected. 3GC-R-BSI is third-generation cephalosporin resistant bloodstream infection, 3GC-R GNI is third-generation cephalosporin resistant Gram-negative infection

Community onset cohort

Among the 4,650 community-onset infections, there were 97 3GC-R-BSI episodes (2.05%), of which nine with pathogens were co-resistant to carbapenems (0.2%; 9% of BSI caused by 3GC-R-E). In the remaining 4,553 episodes, blood cultures were either negative (n=3,680) or yielded other pathogens (n=873; Table 1).

Table 1: Predictors and baseline variables of the community onset rule

	3GC-R EB BSI (n=97)	without 3GC-R EB BSI (n=4553)
Predictors		
Age	73.1 ±12.9	66.5 ±17.9
Suspected source of infection		
Urinary tract	52 (53.6%)	933 (20.5%)
Respiratory tract	14 (14.4%)	1615 (35.5%)
Immunocompromised	30 (30.9%)	1095 (24.1%)
Prior culture with 3GC-R EB (<1 year)	27 (27.8%)	255 (5.6%)
Prior antibiotic use (<2 months)	65 (67.0%)	1710 (37.6%)
Other descriptive variables		
Male sex	61 (62.9%)	2611 (57.3%)
Culture obtained where		
Internal medicine	68 (70.1%)	3326 (73.1%)
Surgery	20 (20.6%)	773 (17.0%)
ICU	8 (8.2%)	333 (7.3%)
other	1 (1.0%)	121 (2.6%)
Suspected source of infection		
Intra-abdominal	19 (19.6%)	593 (13.0%)
Other	12 (12.4%)	1615 (35.5%)
Other 3GC-R positive cultures	51 (52.6%)	181 (4.0%)
Of which urine culture	44 (45.4%)	101 (2.2%)
Cultured pathogens		
E.coli	66 (68.0%)	268 (5.9%)
Klebsiella spp.	16 (16.5%)	54 (1.2%)
Other Enterobacterales	15 (15.5%)	39 (0.9%)
No growth	-	3680 (80.8%)
S. aureus	-	98 (2.2%)
Other Gram-positve	-	300 (6.6%)
Other spp	-	95 (2.1%)
Non-fermenters	-	24 (0.5%)
Empiric antibiotics		
Amoxicillin	0 (0.0%)	112 (2.5%)
Co-amoxiclav	7 (7.2%)	767 (16.8%)
1st-gen cephalosporins	0 (0.0%)	13 (0.3%)
2nd -gen cephalosporins	1 (1.0%)	306 (6.7%)
3rd -gen cephalosporins	30 (30.9%)	1736 (38.1%)
4th/5th -gen cephalosporins	2 (2.1%)	28 (0.6%)
Piperacillin/tazobactam	26 (26.8%)	902 (19.8%)
Fluoroquinolones	12 (12.4%)	592 (13.0%)
Aminoglycosides	4 (4.1%)	187 (4.1%)
Carbapenems	28 (28.9%)	398 (8.7%)
Sulfamethoxazol/trimethoprim	0 (0.0%)	35 (0.8%)

Patients with 3GC-R-BSI were older, more frequently colonized with 3GC-R EB in the prior year, more often used antibiotics in two months prior to infection and more often had a clinical suspicion of urinary tract infection and less often had a suspected respiratory tract infection than comparators (Table 1). (See supplement D for a comparison with the derivation study and E for a baseline table per country).

Third-generation cephalosporins were the most frequently prescribed antibiotics (in 30.9% and 38.1% of the patients with and without 3GC-R-BSI, respectively). In total, 426 patients (9.2%) received carbapenems, which included 28 of the 97 patients with 3GC-R-BSI, as did 398 of 4,553 patients without 3GC-R-BSI. This means real life clinical decision making had a 'sensitivity' of 28.9% to treat episodes of 3GC-R-BSI empirically with carbapenems and a specificity of 91.2% to avoid carbapenems in patients not having 3GC-R-BSI. Consequently, undertreatment occurred in 71.1% of the patients with 3GC-R-BSI and overtreatment in 8.7% of the patients without 3GC-R EB BSI.

Among patients that did not have 3GC-R-BSI, 181 (4.0%) had 3GC-R EB isolated from other samples than blood cultures, mainly from urine cultures (n=101; 56%). Therefore, in analysis 2 278 patients were categorized as 3GC-R GNI. In this analysis, carbapenems were prescribed to 65 of 278 patients with (23.4%) and to 361 of 4372 patients (8.2%) without 3GC-R GNI.

Model performance

The community-onset prediction rule had good discrimination, with a c-statistic of 0.79 (95% CI: 0.75 – 0.83). Regression models are shown in Table 3a. The calibration plot of the original model shows structural underprediction (figure 2.a). After recalibration of the intercept, thereby updating the model to reflect the higher incidence of 3GC-R-BSI in the validation cohort, calibration was improved (figure 2.b) See supplement F for further recalibration steps.

Table 3a: Regression models – community onset

Predictors	Original model	Recalibration: updated intercept
Intercept	-7.248	-5.925
Prior identification of 3GC-R EB (prior one year)	1.963	1.963
Suspected source of infection: Urinary tract	1.081	1.081
Immunocompromised	0.491	0.491
Any use of antibiotics (prior two months)	0.314	0.314
Age (per 1-year increment)	0.018	0.018
Suspected source of infection: Lower respiratory tract	-0.896	-0.896

The predicted risk for an individual patient is the logit of the sum of all individual beta coefficients for that patient divided by (1+e^the sum). As an example, a patient with prior identification of 3GC-R, a suspected UTI and 50 years of age has a score of -5.925+1.963+(50*0.018)+1.081=-1.981. The predicted risk is e^-1.981/(1+e^-1.981) =0.12 or 12%.

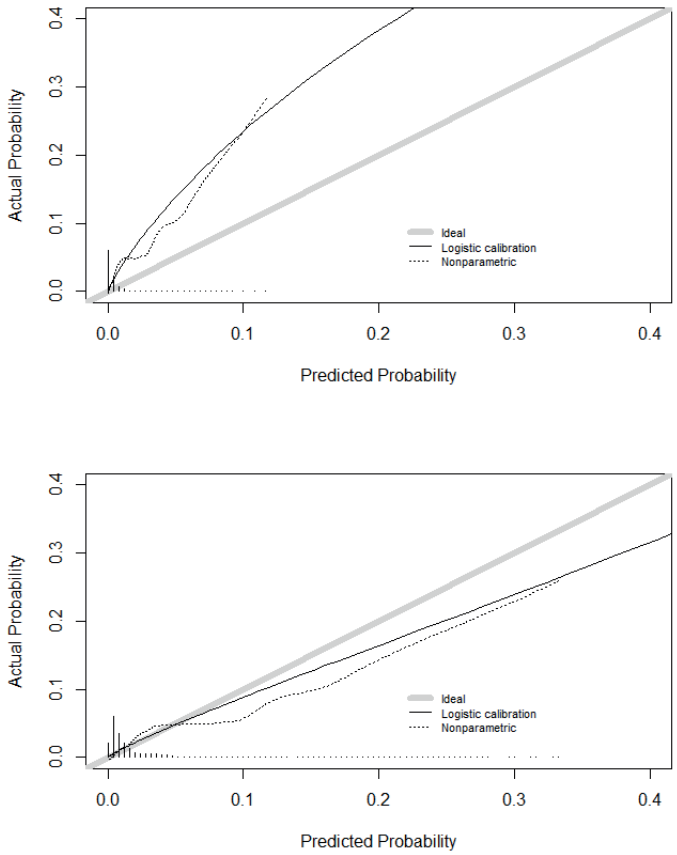

Figure 2: Calibration plots: community onset model .Community onset model. A. Original model. B. Calibration in the large. Systematic underprediction occurs in the original model, which disappears after updating the intercept to account for the higher incidence of 3GC-R BSI in the validation cohort.

Clinical utility

At a 5% cut-off, meaning that patients with a predicted risk of 3GC-R-BSI of 5% and higher, 6.8% of all patients in the cohort would be 'test-positive' for 3GC-R-BSI, and thus eligible for empiric treatment with a carbapenem (table 4). At this cutoff, the prediction rule has a sensitivity of 28.9% and a positive predictive value of 8.9%. If all patients would be treated accordingly, 28 patients with 3GC-R-BSI would be empirically treated with carbapenems, which is similar to what was observed in real life practice in this cohort. Yet, in patients without 3GC-R-BSI, 287 (6.3%) would receive carbapenems, compared to 398 patients observed in real life practice in this cohort, corresponding to a 28% reduction of overtreatment. With a higher cut-off, say 6.6%, overtreatment would reduce by 53% (from 398 to 190) at the expense of the number of patients with 3GC-R-BSI appropriately receiving carbapenems reducing from 28 to 25 patients. For other cut-offs, see supplementary table G.

When using the 5% cut-off from the previous paragraph in analysis 2 (using all 3GC-R GNI episodes as outcome), the proportion of patients with 3GC-R GNI appropriately treated with carbapenems would increase from 24% to 29%, whereas overtreatment would reduce with 35.4% (from 8.2% to 5.3%) (table 4).

Table 4: Clinical impact on carbapenem use, community onset

3GC-R Bloodstream infection as outcome					**3GC-R Gram negative infection as outcome**			
Cutoff	4.3%	5%	6.6%	*Current cohort*	4.3%	5%	6.6%	*Current cohort*
Proportion of cohort (%)	9.1	6.8	4.6		9.1	6.8	4.6	
Sensitivity (%)	38.1	28.9	25.8		35.0	29.2	24.8	
Specificity (%)	91.6	93.7	95.8		92.6	94.7	96.7	
PPV (%)	8.8	8.9	11.6		23.2	26.0	33.4	
NPV (%)	98.6	98.4	98.4		95.6	95.4	95.3	
Appropriate carbapenems	37/97 (38%)	28 / 97 (29%)	25/97 (26%)	*28/97 (29%)*	98/278 (35%)	82/278 (29%)	72/278 (26%)	*65/278 (24%)*
Unnecessary carbapenems	384/4553 (8.4%)	287/4553 (6.3%)	190/4553 (4.2%)	*398 (8.7%)*	323/4372 (7.4%)	233/4372 (5.3%)	143 /4372 (3.2%)	*361 (8.2%)*

The cut-off reflects the predicted 3GC-R-BSI risk for an individual patient. For instance, at the 5% cut-off, 6.8% of all patients in the cohort have a risk of at least 5% for 3GC-R-BSI (proportion of cohort). At this cut-off the prediction rule has a sensitivity of 28.9% and a positive predictive value of 8.9%. If all patients would be treated accordingly 28 patients with 3GC-R-BSI would be empirically treated with carbapenems (which resembles the observed number of patients with 3GC-R-BSI that received carbapenems, thus a 0% change). Another 287 patients without 3GC-R-BSI would also receive carbapenem, which reflects a 28% relative reduction (from 8.7% to 6.3%).

Increasing the cut-off to 6.6% reduces both the number of appropriately treated patients (11% reduction), but also the number with unnecessary carbapenem use (53% reduction).

On the right, the analysis repeated with all 3GC-R Gram-negative infections as outcome. The net carbapenem use is the same, but when considering a carbapenem appropriate for a 3GC-R positive clinical culture, more patients are treated appropriately.

Hospital onset cohort

Among the 1,683 hospital-onset infections there were 83 3GC-R-BSI episodes (4.9%), of which 35 with pathogens co-resistant to carbapenems (2.1%; 42% of BSI caused by Enterobacterales). In the remaining 1,600 episodes blood cultures were either negative (n=1,112) or yielded other pathogens (n=488). 3GC-R-BSI patients more often had renal disease, malignancy, central catheter, signs of hypoperfusion and were more often colonized with 3GC-R EB (43.4% versus 12.5%) than the comparators.

The most frequently prescribed antibiotic in the non-3GC-R-BSI group was piperacillin/tazobactam (31.4%). Carbapenems were the predominantly prescribed class in the 3GC-R-BSI group (n=40, 48.2%), followed by piperacillin/tazobactam (25.3%; Table 2).

Table 2: Predictor and baseline variables of the hospital onset cohort

	3GC-R EB BSI (n=83)	Without 3GC-R-BSI (n=1600)
Predictors		
Suspected source of infection		
Respiratory tract	7 (8.4%)	391 (24.3%)
Central venous catheter	50 (60.2%)	691 (43.0%)
Solid malignancy	26 (31.3%)	417 (26.1%)
Prior culture with 3GC-R EB	36 (43.4%)	200 (12.5%)
Prior cephalosporin use (<2 months)	39 (47.0%)	451 (28.2%)
Surgery in last month	40 (48.2%)	498 (31.1%)
Renal disease	22 (26.5%)	270 (16.9%)
Pre-infection length of stay in days (IQR)	17 (9 – 33.5)	11 (5 – 21)
Hypoperfusion	19 (22.9%)	273 (17.1%)
Other descriptive variables		
Age	63.2 ± 16.0	64.6 ±16.7
Male sex	52 (62.7%)	967 (60.4%)
Suspected source of infection		
Intra-abdominal	43 (51.8%)	278 (17.4%)
Urinary tract	16 (19.3%)	230 (14.3%)
Other	17 (20.5%)	709 (44.1%)
Culture ward		
Internal medicine	38 (45.8%)	969 (60.6%)
Surgery	27 (32.5%)	393 (24.6%)
ICU	17 (20.5%)	251 (15.7%)
Carbapenem resistance	35 (42.2%)	-
Other 3GC-R EB culture	24 (28.9%)	116 (7.3%)
Of which urine	17 (20.5%)	69 (4.3%)
Cultured pathogens		
E.coli	28 (33.7%)	66 (4.1%)
Klebsiella spp.	45 (54.2%)	34 (2.1%)
Other Enterobacterales	10 (12.0%)	33 (2.1%)
No growth	-	1133 (70.8%)
S. aureus	-	75 (4.7%)
Other Gram-positve	-	162 (10.1%)
Other spp	-	52 (3.2%)
Non-fermenters	-	47 (2.9%)
Empiric antibiotics		
Amoxicillin/Penicillin	0 (0.0%)	6 (0.4%)
Co-amoxiclav	3 (3.6%)	143 (8.9%)
1st/2nd -gen cephalosporins	0 (0.0%)	64 (4.0%)
3rd -gen cephalosporins	12 (14.5%)	395 (24.7%)
4th/5th -gen cephalosporins	1 (1.2%)	32 (2.0%)
Piperacillin/tazobactam	21 (25.3%)	502 (31.4%)
Fluoroquinolones	6 (7.2%)	138 (8.6%)
Aminoglycosides	6 (7.2%)	67 (4.2%)
Carbapenems	40 (48.2%)	363 (22.7%)
Sulfamethoxazol/trimethoprim	1 (1.2%)	22 (1.4%)
Colistin, tigeyclin, misc	11 (13.3%)	17 (1.1%)

Table 3b: regression models, hospital onset

Predictors	Original model	Recalibrated intercept
Intercept	-5.807	-4.774
Renal disease	1.372	1.372
Prior identification of 3GC-R EB (prior one year)	1.353	1.353
Any solid malignancy	0.722	0.722
Signs of hypoperfusion (at infection onset)	0.509	0.509
Surgical procedure (prior 30 days)	0.444	0.444
Central vascular catheter (at infection onset)	0.42	0.42
Use of cephalosporins (prior two months)	0.415	0.415
Length of hospital stay prior to infection (per 1-day increase)	0.011	0.011
Suspected source of infection: Lower respiratory tract infection	-1.729	-1.729

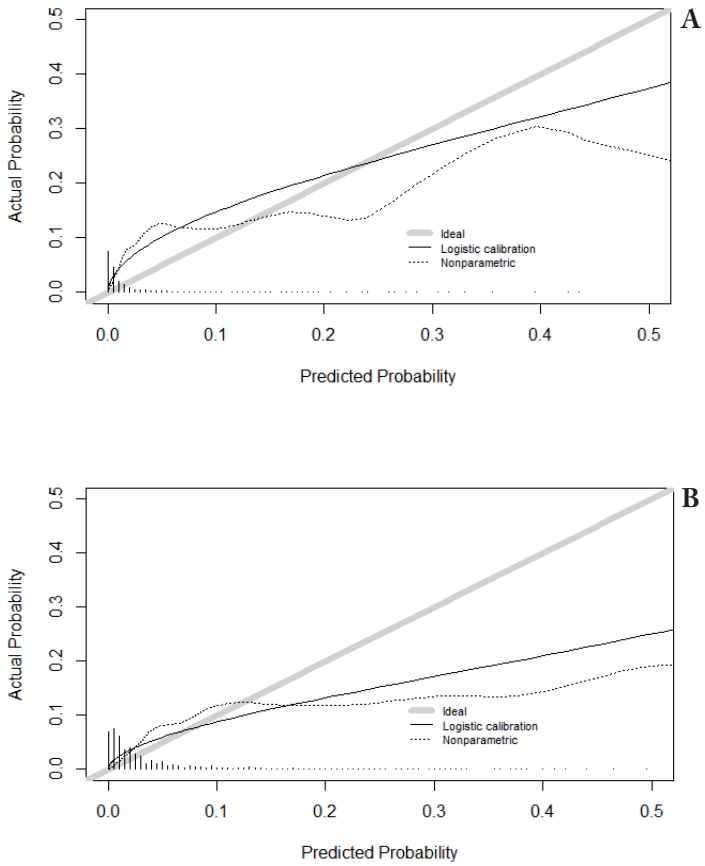

Figure 3. Calibration plots of the hospital onset original model (A), recalibration in the large (B)

Model performance

The c-statistic of the hospital onset prediction rule was 0.75 (95% CI 0.70 – 0.80). Regression models are shown in table 3b. Calibration of the original model was poor (figure 3a), with major underprediction. Recalibration in the large improved the calibration, but still had significant and inconsistent deviations from the ideal line, especially in predicted risks between 0% and 10%, involving the majority of patients (figure 3b). This is reflected in limited clinical utility, with a reduction of 13% in unnecessary carbapenem use while keeping a similar rate of inappropriate therapy. See supplementary table G for cut-off values.

Discussion

We externally validated two prediction rules for 3GC-R infections in patients with a clinical infection in whom empiric antibiotic treatment covering Gram-negative bacteria was initiated. The rule for community onset infections showed good discrimination and calibration and has the potential to safely reduce unnecessary carbapenem use. The hospital onset rule had poor calibration, and its clinical utility was therefore limited.

The prediction rules illustrate the challenging trade-off between inappropriate therapy and overtreatment with broad-spectrum antibiotics. Prediction rules have been considered one of the options to mitigate the increase of broad-spectrum antibiotic prescriptions by aiding risk assessment of patients.[7] While other researchers previously developed prediction tools for ESBL-BSI/3GC-R-BSI [2-4,8-11], these prediction rules have either not been validated, or validation yielded poor performance.[9] Moreover, since these prediction rules (except for Fröding et al.) were derived from comparisons of 3GC-R-BSI to other Gram-negative BSI, they are unsuitable for aiding in initiation of empiric treatment. Most of these studies do not report on calibration, arguably a more important performance metric than discrimination for clinical prediction rules, since accurately predicted risks are the basis for classifying patients in treatment groups.[1011] We consider this international validation an important step forward in using prediction rules to improve antibiotic use.

Several predictors, such as prior isolation of 3GC-R isolates from diagnostic cultures and recent antibiotic use, were already included in guideline recommendations for selecting empiric therapy. However, we observed that 9% of community-onset infections were empirically treated with carbapenems, while only 2.1% of all infection episodes involved 3GC-R-BSI and 6.0% involved 3GC-R infections.[12] Reducing the eligibility for carbapenems therefore seems to offer the biggest advantage of the prediction rule. Importantly, these improvements are extended to other infections caused by 3GC-E (e.g. blood culture negative with positive urine culture) when used in patients with suspected BSI (the study population).

The community-onset prediction rule was generalizable from a low-resistance country (the Netherlands) to a validation cohort with a five times higher incidence of 3GC-R-BSI. This is explained by a similar distribution of individual predictors compared to the derivation

cohort (see supplemental material for direct comparisons and predictor distribution per country). The c-statistic of 0.79 was very similar to that of 0.78 (95% CI: 0.71 - 0.84) in the derivation study. Prior isolation of 3GC-R EB from samples in individual patients was not more prevalent in countries with a higher prevalence of 3GC-R-BSI, implying it serves as a proxy of prior healthcare exposure and a higher risk of infection with resistant bacteria or different screening practices and registration in other countries.

The prediction rule for hospital-onset infections performed markedly worse than the community-onset infection rule, primarily in calibration and impact on eligibility for carbapenem use. There may be several reasons for this difference. First, the predictors among hospitalized patients are less likely to be universal than in community dwelling patients. For example, length of stay depends on organization of healthcare, and median length of stay prior to infection onset per country varied from 6 to 20 days, without a concurrent increase in the incidence of 3GC-R-BSI. Second, renal disease was a major predictor in the derivation study. However, in this international validation cohort, renal disease was much more prevalent among patients without 3GC-R-BSI (being 16% compared to 5% in the derivation study). Although we used the same definitions for the predictors in the current validation cohort, the hospital onset rule had more context dependent predictors and inter-rater variability may have had a larger impact on model performance. Based on this, we question whether a prediction rule for hospital-onset infections can be usable across multiple healthcare systems. In contrast to risk factors for community-onset infections, risk factors for hospitalized patients may reflect poorly understood mechanisms tied to local healthcare practices and nosocomial transmission patterns.

Implementation of the community-onset prediction rule requires consideration of how clinicians will incorporate the rule in clinical practice. It can give an absolute predicted risk to aid decision making or give a treatment recommendation based on pre-defined cut-off values. This could mean different cutoffs for patients with and without septic shock. The rule should be adapted to account for local incidences of 3GC-R-BSI by updating the intercept of the model. Ideally, a prediction rule would be implemented in the electronic patient management system. To what extent real-world use of this prediction rule changes appropriateness of empiric antibiotic therapy requires a diagnostic trial.[13]

Several study limitations are important to mention. First, data collection was intended to be prospective, but this was unfeasible in some centers; around 50% of patients were included retrospectively. Retrospective data entry occurred more frequently in countries with higher prevalence of resistance. Overall, the distribution of predictors was similar among prospectively and retrospectively collected data, and there were no differences in incidences of 3GC-R-BSI between centers with prospective and retrospective data collection from the same region. Finally, time logging allowed us to determine whether the outcome CRF (with culture results) had been completed before the predictor CRF and this occurred in 1% of the cohort. We therefore consider the impact of retrospectively collected data on the study

validity to be limited.

Second, we simplified the clinical problem to 3GC-R in Enterobacterales, while clinicians also may have to consider other potential pathogens, like *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*[14] However, these pathogens were rare in community-onset infections in our cohort.

Third, we only collected susceptibility data of EB isolates for cephalosporins and carbapenems. In the community onset group, 30 patients with 3GC-R-BSI received piperacillin/tazobactam or cephalosporins plus aminoglycosides for empiric treatment. Some of these patients may have received appropriate treatment. However, aminoglycosides may not be an ideal strategy for empiric treatment.[15,] Additionally, co-resistance to aminoglycosides is common in ESBL-producing pathogens, particularly in *Klebsiella* species.[16]

Fourth, we included patients with a respiratory tract infection, where the a priori risk of bacteraemia is low. However, 14% of the 3GC-R-BSI population had a suspected respiratory tract infection according to local physicians, so the risk of 3GC-R-BSI in this group is a legitimate concern. This high percentage may have resulted from the selection of more severe cases in patients with community onset pneumonia. In these patients, obtaining blood cultures is not always standard care and may be associated with higher disease severity, and thus, a higher a priori risk of 3GC-R-BSI.[17]

In conclusion, we externally validated a prediction rule in community onset infections caused by 3GC-R E that may reduce unnecessary broad-spectrum antibiotic prescriptions, which can now be implemented and tested for clinical utility.

References

1. Rottier, W. C., Ammerlaan, H. S. M. & Bonten, M. J. M. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum β-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. *J. Antimicrob. Chemother.* 67, 1311–20 (2012).
2. Tumbarello, M. *et al.* Identifying patients harboring extended-spectrum-β-lactamase-producing Enterobacteriaceae on hospital admission: Derivation and validation of a scoring system. *Antimicrob. Agents Chemother.* 55, 3485–3490 (2011).
3. Kengkla, K., Charoensuk, N., Chaichana, M. & Puangjan, S. Clinical risk scoring system for predicting extended- spectrum b -lactamase-producing Escherichia coli infection in hospitalized patients. *J. Hosp. Infect.* 93, 49–56 (2016).
4. Goodman, K. E. *et al.* A Clinical Decision Tree to Predict Whether a Bacteremic Patient Is Infected With an Extended-Spectrum β-Lactamase-Producing Organism. *Clin. Infect. Dis.* 63, 896–903 (2016).
5. Rottier, W. C. *et al.* Development of diagnostic prediction tools for bacteraemia caused by third-generation cephalosporin-resistant enterobacteria in suspected bacterial infections: a nested case–control study. *Clin. Microbiol. Infect.* 24, 1315–1321 (2018).
6. Vergouwe, Y., Steyerberg, E. W., Eijkemans, M. J. C. & Habbema, J. D. F. Substantial effective sample sizes were required for external validation studies of predictive logistic regression models. *J. Clin. Epidemiol.* 58, 475–83 (2005).
7. Pogue, J. M., Kaye, K. S., Cohen, D. A. & Marchaim, D. Appropriate antimicrobial therapy in the era of multidrug-resistant human pathogens. *Clin. Microbiol. Infect.* 21, 302–312 (2015).
8. Lee, C. H. *et al.* A simple scoring algorithm predicting extendedspectrum b-lactamase producers in adults with community-onset monomicrobial Enterobacteriaceae bacteremia. *Med. (United States)* 96, (2017).
9. Sousa, A. *et al.* Validation of a clinical decision tree to predict if a patient has a bacteraemia due to a β-lactamase producing organism. *Infect. Dis. (Auckl).* 51, 32–37 (2019).
10. Collins, G. S. *et al.* External validation of multivariable prediction models: a systematic review of methodological conduct and reporting. *BMC Med. Res. Methodol.* 14, 40 (2014).
11. Calster, B. Van, Mclernon, D. J., Smeden, M. Van, Wynants, L. & Steyerberg, E. W. Calibration : the Achilles heel of predictive analytics. 1–7 (2019).
12. Rottier, W. C. *et al.* Predictive value of prior colonization and antibiotic use for third-generation cephalosporin-resistant enterobacteriaceae bacteremia in patients with sepsis. *Clin. Infect. Dis.* 60, 1622–1630 (2015).
13. Rodger, M., Ramsay, T. & Fergusson, D. Diagnostic randomized controlled trials: the final frontier. *Trials* 13, 1 (2012).
14. Schechner, V. *et al.* Gram-Negative Bacteremia upon Hospital Admission: When Should Pseudomonas aeruginosa Be Suspected? . *Clin. Infect. Dis.* 48, 580–586 (2009).
15. Paul M, L. L. Cochrane Database of Systematic Reviews Beta lactam antibiotic monotherapy versus beta lactam- aminoglycoside antibiotic combination therapy for sepsis (Review). *Cochrane Database Syst. Rev.* (2014). doi:10.1002/14651858.CD003344.pub3
16. Hawkey, P. M. *et al.* Treatment of infections caused by multidrug-resistant gram-negative bacteria: Report of the British society for antimicrobial chemotherapy/healthcare infection society/british infection association joint working party. *J. Antimicrob. Chemother.* 73, iii2–iii78 (2018).
17. Rodriguez Luna, H. I. & Pankey, G. The utility of blood culture in patients with community-acquired pneumonia. *Ochsner J.* 3, 85–93 (2001).

Chapter 5

Supplementary Materials

A: definition of predictors and antibiotics

1. Any use of antibiotics (prior 2 months): Use of oral, intravenous or intramuscular antibiotics in the last two months as documented in the medical status and if possible – the connected clinical pharmacy. If patients are admitted for an infectious episode, they might have previously been treated by their general practitioner with oral antibiotics and this is hopefully recorded in the medical file. Importantly, one-time prescriptions of for instance surgical prophylaxis should not be scored. Therefore, we deem it unnecessary to screen surgical reports for prophylactic antibiotic use.

2. Cephalosporin use (prior 2 months): Use the same data sources as mentioned previously. All generations should be considered.

3. Central vascular catheter: any form of central vascular access at the time present at the time of blood culture obtainment. This includes central venous lines, arterial lines, peripherally inserted central catheters (PICC), Hickman catheters, central dialysis catheters (TESIO, Sheldon), Swan-Ganz catheter, ports (Port-a-cath) etc. This excludes regular peripheral catheters.

4. Immunocompromised:
   If a patients meets ≥1 of the following criteria:
   1. Patients chronically treated with corticosteroids
   2. Chemotherapeutics in the last month
   3. High-dose corticosteroids in the last month
   4 Other immunosuppressive drugs in the last month
   5. Neutropenia at onset of infection (<0.5x10^9 neutrophils/L or (if not available) <1x10^9 leukocytes/L)

5. Prior identification of 3GC-R EB (prior year): Identification of a 3GC-R EB in any culture the year up to the point of blood culture obtainment. If patients were colonized once, and had negative cultures thereafter, this should still be scored. It should be specified whether this included a bacteremia.

6. Renal disease:
   If a patient meets ≥1 of the following criteria:
   1. Patients on dialysis (hemodialysis or peritoneal dialysis)
   2. Patients who had a renal transplant
   3. Patients with serum creatinines of >265 μmol/L or >3.0 mg/dL (documented as chronic renal disease in medical file).

7. Solid malignancy: Patients with a solid malignancy, fulfilling one of the following criteria:
   1. Have no documented metastases and were treated within the last five years (disregard

any long-term adjuvant therapy, such as hormone blocking therapy in breast cancer)
2. Have metastasized
This includes breast, colon, lung, and a variety of other tumors, including malignant brain tumors and melanoma. The only exception is basal cell carcinoma. Hematological malignancies are not included.

8. Signs of hypoperfusion (previously severe sepsis):
≥1 of the following criteria at onset of infection:
1. Acute oliguria (urine output <0.5 mL/(kg·hr) or 45 mmol/L for at least 2 hrs)
2. Creatinine >175 μmol/L or >2.0 mg/dL
3. Hyperlactatemia (>2 mmol/L or >18.0 mg/dL)
4. Arterial hypotension (systolic blood pressure <90 mmHg, mean arterial pressure <70 mmHg, or decrease in systolic blood pressure >40 mmHg)

9. Surgical procedure (prior 30 days): Any 'major' surgery, which excludes day/outpatient surgery, endo-urological procedures (e.g. cystoscopy, but NOT: TURP/TURT), other forms of endoscopy, interventional cardiology, radiological procedures and remaining small procedures (e.g., incision of abscess, cataract surgery, insertion of central venous catheter).

10. Suspected source of infection: The type of infection that is the suspected cause of illness at the onset of infection. This should be the working diagnosis of the treating clinician at onset of infection and should be obtained from the medical file. The working diagnosis can be based on diagnostics performed earlier (e.g., urinary strip with positive leukocytes, radiographic information). If several sources of infection are deemed equally likely (sometimes reflected by the fact that only a differential diagnosis is presented), 'other' should be selected. It should be categorized into:
1. Urinary tract infection (includes among others cystitis, prostatitis, pyelonephritis, urosepsis)
2. Lower respiratory infection (includes among others pneumonia, bronchitis, empyema, lung abscess, pneumosepsis)
3. Intra-abdominal infection (includes among others cholangitis, cholecystitis, peritonitis, intra-abdominal abscess, diverticulitis, appendicitis)
4. Other (also includes primary infection or unknown source)

Eligible Gram-negative antibiotics

1st Generation Cephalosporins	Cefalotin, Cefazolin
2nd Generation Cephalosporins	Cefaclor, Cefuroxime, Cefamandol
3rd Generation Cephalosporins	Cefotaxime, Ceftibuten, Ceftriaxone, Ceftazidime
4th Generation Cephalosporins	Cefepime
5th Generation Cephalosporins	Ceftolozane
Aminopenicillin	Amoxicillin, Amoxicillin-Clavulanate (Augmentin), temocillin, Piperacillin, Piperacillin-tazobactam
Carbapenems	Meropenem, Imipenem(-Cilastatin), Ertapenem
Aminoglycosides	Gentamicin, Tobramycin, Amikacin
Fluoroquinolones	Ciprofloxacin, Moxifloxacin, Ofloxacin, Levofloxacin
Other	Colistin, Tigeycyclin, Tetracyclin

Patient eligibility

Patients already receiving intravenous antibiotic therapy covering Gram-negative bacteria more than two hours prior to blood culture obtainment were not eligible. Patients already receiving oral antibiotics or systemic antibiotics targeting Gram-positive bacteria only were eligible if intravenous therapy for Gram-negative bacteria was started in the -2 to +12h time frame. Patients could be included in the study multiple times with new infection episodes meeting the eligibility criteria.

Supplementary B: methods

Statistical analysis

Data was reported as median or mean or percentage where appropriate.

The prediction rules were validated by assessing discrimination and calibration of the prediction models. We calculated c-statistics and corresponding 95% confidence intervals to evaluate the discrimination, which is a measure of how well the model can separate cases and non-cases. A c-statistic of 1 represents perfect discrimination, a c-statistic of 0.5 implies no discrimination.

For calibration, we calculated the predicted risk of 3GC-R BSI for every patient by using the beta-coefficients and intercept from the derivation study. Calibration assesses the agreement between this predicted risk and observed risk. In a group of 20 patients with a predicted 3GC-R BSI risk of 5%, you expect one patient with 3GC-R BSI (1/20 = 5%). If there are 10 patients with 3GC-R BSI in that group, the observed risk is 50%, which deviates strongly from the predicted 5%, and thus implies poor calibration. Calibration curves allow visual assessment of this agreement. Poor calibration severely limits the use of a model, since inadequately predicted risks cannot inform decision making.

Based on national epidemiology of 3GC-R EB in participating countries, we expected a higher incidence of 3GC-R BSI compared to the derivation study that was performed in the Netherlands. Thus, recalibration of the model was, a priori, considered necessary. We did this in three steps.7 First we performed a recalibration in the large. This updates the intercept of the model to fit the mean predicted risk in the validation cohort without any change to the original predictors. The second level and third level are re-estimating the slope of the model and re-estimating the individual predictors, respectively.

To assess the potential impact of the model on carbapenem prescriptions, we report predictive performance (sensitivity/specificity/positive predictive value/negative predictive value) for different cut-offs. To clarify, when using the prediction rule, there is a cut-off above which the test is considered 'positive'. This cutoff is a predefined risk of 3GC-R BSI. Thus, with a cut-off of 5%, all patients with a predicted risk of 3GC-R BSI of 5% or higher would be treated with a carbapenem. A low cut-off increases the number of patients eligible for a carbapenem while not having 3GC-R BSI (false positives, i.e., overtreatment). A high cut-off reduces the number of carbapenem prescriptions at the cost of not adequately treating 3GC-R BSI patients with carbapenems (false negatives, i.e., undertreatment). We present several cut-offs and the impact on the number of carbapenem prescriptions, and ultimately choose three cut-offs (low, medium and high) which we consider relevant for clinical practice. For these analyses, we use the model with a recalibrated intercept, since we a priori know that the incidence of 3GC-R-BSI will be higher in the validation cohort. We repeat this analysis considering all 3GC-R GNI, adding non-blood cultures with 3GC-R EB to the outcome (thus 3GC-R non-BSI and BSI) to assess the effect of using the prediction rule on all infections.

Supplementary C. Percentage of prospectively collected data, positive blood cultures and 3GC-R BSI and missings

We considered a prospective inclusion an inclusion where predictor data was filled in three days or more before filling in blood culture results, assuming that in that case, the blood culture result was not known before predictors were filled in to prevent information bias.

Community onset

	n	Prospective data collection (%)	3GC-R-BSI (%)	Positive blood cultures (%)
A01	136	136 (100.0)	0 (0.0)	35 (25.7)
A02	160	131 (81.9)	4 (2.5)	37 (23.1)
A03	116	111 (95.7)	1 (0.9)	22 (19.0)
A04	181	137 (75.7)	0 (0.0)	34 (18.8)
A06	178	0 (0.0)	0 (0.0)	36 (20.2)
A07	51	2 (3.9)	0 (0.0)	7 (13.7)
B02	143	124 (86.7)	1 (0.7)	23 (16.1)
B03	167	166 (99.4)	0 (0.0)	25 (15.0)
B05	125	0 (0.0)	4 (3.2)	29 (23.2)
B06	176	19 (10.8)	10 (5.7)	68 (38.6)
B07	277	2 (0.7)	1 (0.4)	35 (12.6)
C01	151	94 (62.3)	5 (3.3)	37 (24.5)
C02	111	8 (7.2)	9 (8.1)	29 (26.1)
C03	70	16 (22.9)	6 (8.6)	22 (31.4)
C04	140	0 (0.0)	10 (7.1)	59 (42.1)
C05	135	129 (95.6)	1 (0.7)	10 (7.4)
C06	100	0 (0.0)	6 (6.0)	32 (32.0)
C07	158	155 (98.1)	2 (1.3)	24 (15.2)
D01	140	0 (0.0)	2 (1.4)	26 (18.6)
D02	160	0 (0.0)	1 (0.6)	39 (24.4)
E02	185	183 (98.9)	4 (2.2)	46 (24.9)
E03	123	93 (75.6)	0 (0.0)	16 (13.0)
E04	24	2 (8.3)	0 (0.0)	7 (29.2)
F01	165	156 (94.5)	1 (0.6)	24 (14.5)
F02	149	43 (28.9)	2 (1.3)	37 (24.8)
F04	109	106 (97.2)	0 (0.0)	21 (19.3)
G01	43	0 (0.0)	0 (0.0)	11 (25.6)
G02	146	0 (0.0)	2 (1.4)	36 (24.7)
H01	56	53 (94.6)	2 (3.6)	20 (35.7)
I01	22	10 (45.5)	3 (13.6)	7 (31.8)
J01	515	512 (99.4)	17 (3.3)	59 (11.5)
K01	172	126 (73.3)	2 (1.2)	44 (25.6)

L01	66	46 (69.7)	1 (1.5)	18 (27.3)

Hospital onset

	n	Prospective data collection (%)	3GC-R BSI (%)	Positive blood cultures (%)
A01	63	63 (100.0)	0 (0.0)	16 (25.4)
A02	39	32 (82.1)	0 (0.0)	6 (15.4)
A03	86	84 (97.7)	1 (1.2)	31 (36.0)
A04	15	14 (93.3)	0 (0.0)	2 (13.3)
A06	22	0 (0.0)	0 (0.0)	5 (22.7)
A07	0	-	-	-
B02	57	46 (80.7)	1 (1.8)	15 (26.3)
B03	36	36 (100.0)	1 (2.8)	11 (30.6)
B05	27	0 (0.0)	3 (11.1)	7 (25.9)
B06	19	5 (26.3)	2 (10.5)	14 (73.7)
B07	41	0 (0.0)	1 (2.4)	12 (29.3)
C01	47	25 (53.2)	6 (12.8)	23 (48.9)
C02	99	13 (13.1)	20 (20.2)	50 (50.5)
C03	107	24 (22.4)	19 (17.8)	61 (57.0)
C04	58	0 (0.0)	7 (12.1)	34 (58.6)
C05	65	62 (95.4)	0 (0.0)	12 (18.5)
C06	101	2 (2.0)	3 (3.0)	38 (37.6)
C07	40	38 (95.0)	0 (0.0)	15 (37.5)
D01	57	1 (1.8)	1 (1.8)	4 (7.0)
D02	38	0 (0.0)	1 (2.6)	8 (21.1)
E02	61	60 (98.4)	1 (1.6)	25 (41.0)
E03	76	61 (80.3)	1 (1.3)	20 (26.3)
E04	1	0 (0.0)	0 (0.0)	0 (0.0)
F01	28	24 (85.7)	0 (0.0)	9 (32.1)
F02	18	4 (22.2)	0 (0.0)	5 (27.8)
F04	29	27 (93.1)	0 (0.0)	7 (24.1)
G01	135	2 (1.5)	0 (0.0)	29 (21.5)
G02	52	0 (0.0)	0 (0.0)	15 (28.8)
H01	41	36 (87.8)	0 (0.0)	10 (24.4)
I01	52	18 (34.6)	6 (11.5)	19 (36.5)
J01	96	96 (100.0)	5 (5.2)	27 (28.1)
K01	30	21 (70.0)	1 (3.3)	10 (33.3)
L01	47	31 (66.0)	3 (6.4)	13 (27.7)

Missings per variable

As described in the methods, whenever a single predictor was missing (and outcome and age were not missing), it was imputed to "No".

Community onset predictors	**Missing (total n=4650)**
Prior identification of 3GC-R	1 (0.02%)
Immunocompromised	0 (0.0%)
Infection source	0 (0.0%)
Antibiotic use prior 2m	25 (0.53%)

Hospital onset predictors	**Missing (n=1683)**
Prior identification of 3GC-R	1 (0.05%)
Cephalosporin use prior 2m	8 (0.48%)
Infection source	3 (0.18%)
Renal disease	4 (0.24%)
Solid malignancy	5 (0.30%)
Hypoperfusion	8 (0.48%)
Central catheter	4 (0.24%)
Surgery in prior 30days	5 (0.30%)

Inclusions per country

Country	**Number of sites (number of non-university centers)**	**Inclusions (community onset)**
Netherlands	6 (4)	1047 (822)
Spain	3 (1)	716 (620)
Portugal	2 (0)	352 (268)
Italy	7 (0)	1382 (865)
Switzerland	2 (0)	395 (300)
France	3 (0)	470 (332)
Sweden	3 (2)	498 (423)
Germany	2 (0)	376 (189)
Japan	1 (0)	97 (56)
Serbia	1 (0)	74 (22)
Turkey	1 (0)	611 (515)
Belgium	1 (0)	202 (172)
Macedonia	1 (0)	113 (66)

D. Comparison of predictors between validation and derivation study

Table 1: Community onset

Predictor	ESBL-PREDICT: cases	ESBL-PREDICT: controls	Derivation Cases	Derivation: Controls
Age, median (SD, IQR)	73.1 +/- 12.9	66.5 +/- 17.9	69 (61-76)	63 (50-76)
Immunocompromised	30.9%	24.1%	31%	17%
Suspected infection source: UTI	53.6%	20.5%	46%	13%
Suspected infection source: LRTI	14.4%	35.5%	9%	31%
Any use of antibiotics (prior 2 months)	67.0%)	37.6%	60%	40%
Prior identification of 3GC-R EntB (prior 1 year)	27.8%	5.6%	24%	2%

Table 2: Hospital onset

Predictor	ESBL-PREDICT: cases	ESBL-PREDICT: controls	Derivation: cases	Derivation: controls
Length of hospital stay prior to infection (days), median (IQR)	17 (9 – 33.5)	11 (5 – 21)	20 (10-48)	11 (6-19)
Solid malignancy	31%	26%	31%	21%
Renal disease	27%	17%	17%	5%
Surgical procedure (prior 30 days)	48%	31%	45%	36%
Central line (at onset of infection)	60%	43%	61%	36%
Signs of hypoperfusion (at onset of infection)	23%	17%	32%	13%
Suspected infection source: LRTI	8%	24%	5%	26%
Prior use of cephalosporins	47%	28%	60%	35%
Prior identification of 3GC-R EntB (prior 1 year)	43%	13%	35%	5%

E. Baseline per country

Community onset

	Netherlands	Spain and Portugal	Italy	Switzerland	France
n	822	888	865	300	332
Sex (male)	465 (56.6%)	541 (60.9%)	499 (57.7%)	143 (47.7%)	184 (55.4%)
Medical specialty responsible for culture: internal medicine	643 (78.2%)	661 (74.4%)	685 (79.2%)	184 (61.3%)	227 (68.4%)
Surgical ward	152 (18.5%)	123 (13.9%)	110 (12.7%)	68 (22.7%)	54 (16.3%)
ICU	45 (5.5%)	50 (5.6%)	36 (4.2%)	35 (11.7%)	32 (9.6%)
Emergency department	0 (0.0%)	2 (0.2%)	1 (0.1%)	11 (3.7%)	4 (1.2%)
Prior identification of 3GC-R last year	56 (6.8%)	45 (5.1%)	78 (9.0%)	21 (7.0%)	14 (4.2%)
BSI with 3GC-R prior year	5 (0.6%)	5 (0.6%)	24 (2.8%)	6 (2.0%)	1 (0.3%)
Immunocompromised	236 (28.7%)	153 (17.2%)	185 (21.4%)	57 (19.0%)	83 (25.0%)
Infection source					
Intra-abdominal	108 (13.1%)	107 (12.0%)	149 (17.2%)	28 (9.3%)	69 (20.8%)
Other	238 (29.0%)	175 (19.7%)	226 (26.1%)	105 (35.0%)	67 (20.2%)
Respiratory tract	299 (36.4%)	372 (41.9%)	311 (36.0%)	102 (34.0%)	103 (31.0%)
Urinary tract	177 (21.5%)	234 (26.4%)	179 (20.7%)	65 (21.7%)	93 (28.0%)
Antibiotic use prior 2 months	303 (36.9%)	310 (34.9%)	419 (48.4%)	61 (20.3%)	86 (25.9%)
3GC-R BSI (outcome)	5 (0.6%)	16 (1.8%)	39 (4.5%)	3 (1.0%)	4 (1.2%)
Carbapenem use	56 (6.8%)	119 (13.4%)	87 (10.1%)	20 (6.7%)	7 (2.1%)

	Sweden	Germany	Japan	Serbia	Turkey	Belgium	Macedonia
	423	189	56	22	515	172	66
	244 (57.7%)	116 (61.4%)	37 (66.1%)	14 (63.6%)	279 (54.2%)	102 (59.3%)	47 (71.2%)
	254 (60.0%)	98 (51.9%)	31 (55.4%)	10 (45.5%)	422 (81.9%)	133 (77.3%)	46 (69.7%)
	154 (36.4%)	31 (16.4%)	20 (35.7%)	12 (54.5%)	42 (8.2%)	26 (15.1%)	1 (1.5%)
	9 (2.1%)	47 (24.9%)	2 (3.6%)	0 (0.0%)	53 (10.3%)	13 (7.6%)	19 (28.8%)
	0 (0.0%)	6 (3.2%)	3 (5.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	9 (2.1%)	9 (4.8%)	2 (3.6%)	3 (13.6%)	30 (5.8%)	13 (7.6%)	2 (3.0%)
	1 (0.2%)	2 (1.1%)	1 (1.8%)	0 (0.0%)	8 (1.6%)	2 (1.2%)	0 (0.0%)
	98 (23.2%)	49 (25.9%)	23 (41.1%)	9 (40.9%)	164 (31.8%)	63 (36.6%)	5 (7.6%)
	68 (16.1%)	17 (9.0%)	19 (33.9%)	10 (45.5%)	19 (3.7%)	15 (8.7%)	3 (4.5%)
	168 (39.7%)	80 (42.3%)	12 (21.4%)	5 (22.7%)	249 (48.3%)	51 (29.7%)	48 (72.7%)
	98 (23.2%)	53 (28.0%)	15 (26.8%)	3 (13.6%)	190 (36.9%)	75 (43.6%)	8 (12.1%)
	89 (21.0%)	39 (20.6%)	10 (17.9%)	4 (18.2%)	57 (11.1%)	31 (18.0%)	7 (10.6%)
	176 (41.6%)	32 (16.9%)	15 (26.8%)	12 (54.5%)	288 (55.9%)	54 (31.4%)	19 (28.8%)
	3 (0.7%)	2 (1.1%)	2 (3.6%)	3 (13.6%)	17 (3.3%)	2 (1.2%)	1 (1.5%)
	35 (8.3%)	27 (14.3%)	7 (12.5%)	7 (31.8%)	49 (9.5%)	7 (4.1%)	5 (7.6%)

Hospital onset

	Netherlands	Spain and Portugal	Italy	Switzerland	France
n	225	180	517	95	138
Male sex	128 (56.9%)	113 (62.8%)	319 (61.7%)	54 (56.8%)	85 (61.6%)
Prior identification of 3GC-R	20 (8.9%)	14 (7.8%)	117 (22.6%)	21 (22.1%)	13 (9.4%)
Intra-abdominal source	26 (11.6%)	35 (19.4%)	159 (30.8%)	8 (8.4%)	9 (28.3%)
Other source	103 (45.8%)	59 (32.8%)	165 (31.9%)	25 (26.3%)	35 (25.4%)
Respiratory tract	68 (30.2%)	61 (33.9%)	116 (22.4%)	43 (45.3%)	32 (23.2%)
Urinary tract	28 (12.4%)	25 (13.9%)	77 (14.9%)	19 (20.0%)	32 (23.2%)
Cephalosporin use prior 2m	75 (33.3%)	45 (25.0%)	198 (38.3%)	16 (16.8%)	17 (12.3%)
Renal disease	33 (14.7%)	32 (17.8%)	109 (21.1%)	14 (14.7%)	18 (13.0%)
Solid malignancy	49 (21.8%)	44 (24.4%)	165 (31.9%)	21 (22.1%)	51 (37.0%)
Central vascular acatheter	79 (35.1%)	63 (35.0%)	277 (53.6%)	21 (22.1%)	54 (39.1%)
Hypoperfusion	37 (16.4%)	27 (15.0%)	96 (18.6%)	15 (15.8%)	17 (12.3%)
Surgical procedure	56 (24.9%)	46 (25.6%)	178 (34.4%)	20 (21.1%)	30 (21.7%)
Culture ward: internal medicine	23 (10.2%)	16 (8.9%)	67 (13.0%)	6 (6.3%)	11 (8.0%)
Surgical ward	21 (9.3%)	17 (9.4%)	36 (7.0%)	8 (8.4%)	16 (11.6%)
ICU	0 (0.0%)	1 (0.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Length of stay prior (median [IQR])	10.00 [5.00, 18.00]	12.00 [5.00, 21.25]	12.00 [6.00, 26.00]	12.00 [5.00, 24.00]	7.00 [4.00, 15.75]
3GC-R BSI = 1 (%)	1 (0.4%)	8 (4.4%)	55 (10.6%)	2 (2.1%)	2 (1.4%)
Carbapenem use (%)	58 (25.8%)	37 (20.6%)	145 (28.0%)	22 (23.2%)	3 (2.2%)

	Sweden	Germany	Japan	Serbia	Turkey	Belgium	Macedonia
	75	187	41	52	96	30	47
	42 (56.0%)	113 (60.4%)	30 (73.2%)	34 (65.4%)	55 (57.3%)	13 (43.3%)	33 (70.2%)
	2 (2.7%)	26 (13.9%)	4 (9.8%)	5 (9.6%)	8 (8.3%)	2 (6.7%)	4 (8.5%)
	6 (8.0%)	7 (3.7%)	8 (19.5%)	25 (48.1%)	5 (5.2%)	3 (10.0%)	0 (0.0%)
	51 (68.0%)	156 (83.4%)	13 (31.7%)	3 (5.8%)	64 (66.7%)	12 (40.0%)	33 (70.2%)
	12 (16.0%)	16 (8.6%)	10 (24.4%)	2 (3.8%)	22 (22.9%)	10 (33.3%)	6 (12.8%)
	6 (8.0%)	8 (4.3%)	10 (24.4%)	22 (42.3%)	5 (5.2%)	5 (16.7%)	8 (17.0%)
	24 (32.0%)	8 (4.3%)	21 (51.2%)	48 (92.3%)	28 (29.2%)	5 (16.7%)	5 (10.6%)
	4 (5.3%)	46 (24.6%)	6 (14.6%)	6 (11.5%)	15 (15.6%)	2 (6.7%)	7 (14.9%)
	13 (17.3%)	25 (13.4%)	17 (41.5%)	30 (57.7%)	19 (19.8%)	7 (23.3%)	2 (4.3%)
	30 (40.0%)	102 (54.5%)	20 (48.8%)	27 (51.9%)	18 (18.8%)	15 (50.0%)	34 (72.3%)
	15 (20.0%)	34 (18.2%)	5 (12.2%)	5 (9.6%)	9 (9.4%)	8 (26.7%)	24 (51.1%)
	23 (30.7%)	81 (43.3%)	9 (22.0%)	40 (76.9%)	9 (9.4%)	7 (23.3%)	39 (83.0%)
	4 (5.3%)	23 (12.3%)	2 (4.9%)	0 (0.0%)	11 (11.5%)	0 (0.0%)	0 (0.0%)
	11 (14.7%)	11 (5.9%)	4 (9.8%)	2 (3.8%)	14 (14.6%)	2 (6.7%)	0 (0.0%)
	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.1%)	0 (0.0%)	0 (0.0%)
	8.00 [6.00, 21.50]	14.00 [7.00, 25.50]	20.00 [9.00, 31.00]	10.50 [7.00, 19.00]	7.00 [4.00, 19.25]	10.00 [4.25, 19.75]	6.00 [4.00, 10.50]
	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (11.5%)	5 (5.2%)	1 (3.3%)	3 (6.4%)
	10 (13.3%)	79 (42.2%)	8 (19.5%)	19 (36.5%)	14 (14.6%)	3 (10.0%)	5 (10.6%)

F. Extended description of validation process and regression models

Community onset rule

The community-onset prediction rule had good discrimination, with a c-statistic of 0.79 (95% CI: 0.75 – 0.83). In the calibration plot of the original model, there is structural underprediction (predicted risk of 3GC-R-BSI is lower than in reality) (figure 1a). After recalibration in the large, thereby updating the model to reflect the higher incidence of 3GC-R BSI in the validation than in the derivation cohort, model fit was improved. (Likelihood ratio test χ^2 p <0.01) (Figure 2b). The recalibrated slope was 0.87 without improved model fit compared to the model with the updated intercept (LRT p=0.1). Model revision increased the c-statistic to 0.81 (95% CI: 0.77 – 0.85) with no visible improvement of calibration (figure below).

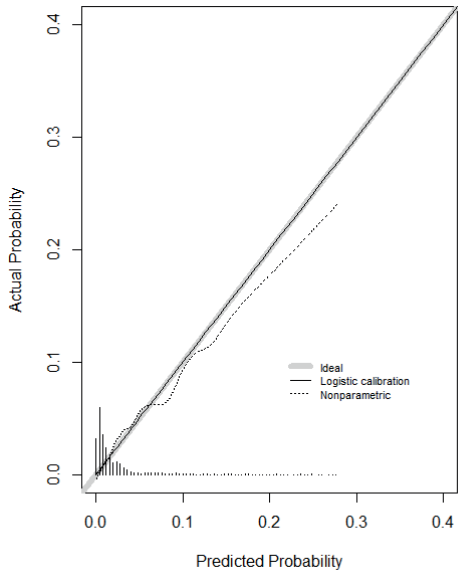

Figure 1a: community onset, model reestimation calibration plot.

Hospital onset

The hospital-onset prediction rule had good discrimination, with a c-statistic of 0.75 (95% CI: 0.70 – 0.80). In the calibration plot of the original model, there is structural underprediction After recalibration in the large, thereby updating the model to reflect the higher incidence of 3GC-R BSI in the validation than in the derivation cohort, model fit was improved, but still poor. (Likelihood ratio test χ^2 p <0.01) (Figure 2b). The recalibrated slope was 0.56 without better model fit compared to the model with the updated intercept (LRT p=<0.01). Model revision increased the c-statistic to 0.77 (95% CI: 0.68 – 0.79), but still had mediocre calibration. (figure below)

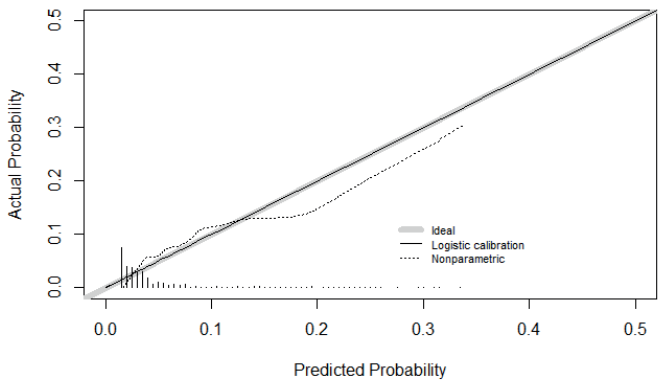

Figure 1b hospital onset, model reestimation calibration plot.

Regression models

Community onset

Predictors	Original model	Recalibraton: updated intercept	Recalibration: updated slope and intercept	Recalculated model
Intercept	-7.248	-5.925	-6.514	-6.82
Slope	-	-	0.87	-
Prior identification of 3GC-R EB (prior one year)	1.963	1.963	-	1.24
Suspected source of infection: Urinary tract infection	1.081	1.081	-	1.086
Immunocompromised	0.491	0.491	-	0.427
Any use of antibiotics (prior two months)	0.314	0.314	-	0.987
Age (per year increase)	0.018	0.018	-	0.028
Suspected source of infection: Lower respiratory tract infection	-0.896	-0.896	-	-0.671

Hospital onset

Predictors	Original model	Recalibrated intercept	Updated slope and intercept	Re-estimated model
Intercept	-5.807	-4.774	-6.321	-3.83
Slope	-	-	0.56	
Renal disease	1.372	1.372	-	0.321
Prior identification of 3GC-R EB (prior one year)	1.353	1.353	-	1.370
Any solid malignancy	0.722	0.722	-	0.119
Signs of hypoperfusion (at infection onset)	0.509	0.509	-	0.079
Surgical procedure (prior 30 days)	0.444	0.444	-	0.476
Central vascular catheter (at infection onset)	0.42	0.42	-	0.229
Use of cephalosporins (prior two months)	0.415	0.415	-	0.551
Length of hospital stay prior to infection (per day increase)	0.011	0.011	-	0.0019
Suspected source of infection: Lower respiratory tract infection	-1.729	-1.729	-	-1.031

Supplementary G: Clinical impact

Table 1: Community onset

Cut-off at predicted risk	1%	3%	4%	4.3%	4.5%
Proportion of cohort (%)	50.1	16.8	10.4	9.1	8.4
Sensitivity (%)	86.6	54.6	38.1	38.1	33
Specificity (%)	50.7	84	90.2	91.6	92.1
PPV (%)	3.6	6.8	7.6	8.8	8.2
NPV (%)	99.4	98.9	98.6	98.6	98.5
Carbapenem prescriptions – 3GC-R BSI					
Appropriately treated 3GC-R-BSI (%)	84 (87%)	53 (54%)	37 (38%)	37 (38%)	32 (33%)
Unnecessary carbapenem use (n)	2246 (49%)	729 (16%)	447 (+10%)	384 (8.4%)	359 (7.8%)
Carbapenem prescriptions: all 3GC-R infections (collateral benefit)					
Appropriately treated 3GC-R (n)	216 (78%)	131 (47%)	100 (36%)	98 (35%)	92 (33%)
Unnecessary carbapenem (n)	2114 (48%)	651 (15%)	384 (8.8%)	323 (7.4%)	299 (6.8%)

Cut-off values chosen to maximize clinical impact in bold.

Table 2: Hospital onset

Cut-off at predicted risk	1%	4.5%	5.2%	5.8%	6%
Proportion of cohort (%)	70.7	27	23.4	21.1	20.7
Sensitivity (%)	96.4	61.4	56.6	48.2	47
Specificity (%)	30.6	74.8	78.3	80.3	80.7
PPV (%)	6.7	11.2	11.9	11.3	11.2
NPV (%)	99.4	97.4	97.2	96.8	96.7
Carbapenem prescriptions – 3GC-R BSI					
Appropriately treated 3GC-R-BSI	80 (96%)	51 (61%)	47 (57%)	40 (48%)	39 (47%)
Unnecessary carbapenem prescriptions	1110 (69%)	404 (25%)	347 (22%)	315 (20%)	309 (19%)
Carbapenem prescriptions – all 3GC-R infections (collateral benefit)					
Appropriate treatment including non-BC	181 (91%)	106 (53%)	97 (49%)	86 (43%)	85 (43%)
Unnecessary carbapenem use including non-BC	1009 (68%)	349 (24%)	297 (20%)	269 (18%)	263 (18%)

Cut-off values chosen to maximize clinical impact in bold.

	5%	5.5%	6%	6.6%	7%	Current cohort data
	6.8	5.7	5.1	4.6	4.4	
	28.9	25.8	25.8	25.8	23.7	
	93.7	94.8	95.3	95.8	96	
	8.9	9.5	10.5	11.6	11.3	
	98.4	98.4	98.4	98.4	98.3	
	28 (29%)	25 (26%)	25 (26%)	25 (26%)	23 (24%)	28 (28.9%)
	287 (6.3%)	239 (5.2%)	213 (4.6%)	190 (4.2%)	180 (3.9%)	398 (8.7%)
	82 (29%)	76 (27%)	75 (27%)	72 (26%)	68 (24%)	65 (23%)
	233 (5.3%)	188 (4.3%)	163 (3.7%)	143 (3.2%)	135 (3.1%)	361 (8.3%)

	6.9%	8%	10%	Current cohort data
	17.5	15.7	11.9	
	44.6	38.6	34.9	
	83.9	85.4	89.3	
	12.5	12.1	14.5	
	96.7	96.4	96.4	
	37 (45%)	32 (39%)	29 (35%)	40 (48%)
	258 (16%)	233 (14.5%)	171 (10.6%)	363 (23%)
	76 (38%)	71 (36%)	57 (29%)	81 (41%)
	219 15%)	194 (13%)	143 (9.6%)	322 (22%)

Appendix

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CEPHALOSPORINS CARBAPENEMASE DISEASE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL MORTALITY DISEASE BURDEN GRAM-NEGATIVE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET PROPENSITY SCORE INFECTION COHORT METHODS GRAND-ABC BURDEN CEPHALOSPORINS BACTERIA BLOODSTREAM INFECTION MORTALITY PREDICTION GRAM-NEGATIVE INFECTION PREDICT ANTIBIOTIC CEPHALOSPORINS CARBAPENEMASE DISEASE TRIAL MORTALITY ANTIBIOTIC PREDICT BLOODSTREAM GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL ANTIBIOTIC DISEASE CHAPTER 6 CAUSAL INFERENCE MORTALITY GRAM-NEGATIVE BACTERIA PREDICTION GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM

Short course aminoglycosides as adjunctive empiric therapy in patients with Gram-negative bloodstream infection, a prospective cohort study

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Abstract

Objective

Short-course aminoglycosides as adjunctive empirical therapy to beta-lactams in patients with a clinical suspicion of sepsis are used to broaden antibiotic susceptibility coverage and to enhance bacterial killing. We aim to quantify the impact of this approach on 30-day mortality in a subset of sepsis patients with a Gram-negative bloodstream infection.

Methods

From a prospective cohort study conducted in 7 hospitals in the Netherlands between June 2013 and November 2015, we selected all patients with Gram-negative bloodstream infection (GN-BSI). Short-course aminoglycoside therapy was defined as tobramycin, gentamicin or amikacin initiated within a 48-hour time window around blood culture obtainment, and prescribed for a maximum of 2 days. The outcome of interest was 30-day all-cause mortality and confounders were selected a priori for adjustment using a propensity score analysis with inverse probability weighting.

Results

626 patients with GN-BSI who received beta-lactams were included. 156 (24.9%) also received aminoglycosides for a median of 1 day. Patients receiving aminoglycosides more often had septic shock (31/156, 19.9% vs 34/470, 7.2%) and had a 8-fold lower risk of inappropriate treatment (3/156, 1.9% vs 69/470, 14.7%). Thirty-day mortality was 17.3% (27/156) and 13.6% (64/470) for patients receiving and not receiving aminoglycosides, yielding a crude and adjusted odds ratio for 30-day mortality for patients treated with aminoglycosides of 1.33 (95% CI 0.80 – 2.15), and 1.57 (0.84 – 2.93), respectively.

Conclusions

Short-course adjunctive aminoglycoside treatment as part of empiric therapy with beta-lactam antibiotics in patients with GN-BSI did not result in improved outcomes, despite better antibiotic coverage of causative pathogens.

Introduction

The global emergence of antibiotic resistance is increasingly complicating the selection of antibiotics for empirical treatment in patients with a clinical suspicion of sepsis. One strategy to reduce the impact of resistance is the addition of empiric aminoglycosides to empiric beta-lactam therapy.

Based on accumulated evidence, combination therapy with aminoglycosides does not provide a benefit for patients with sepsis compared to beta-lactam monotherapy.[1] However, they are recommended in the Surviving Sepsis guidelines and several national guidelines, including Sweden and France.[2,3,4] In the Netherlands, the sepsis guidelines suggest to add a short course (one to two doses) of empiric aminoglycosides when the patient is at increased risk of infection with an extended-spectrum beta-lactamase producing (ESBL) pathogen, defined as prior cephalosporin/fluoroquinolone use and/or colonization with an ESBL-producing pathogen in the last year. In locally adapted guidelines in Dutch hospitals, recommendations range from no aminoglycosides at all to a short course of aminoglycosides in every patient with sepsis. This strategy is thus widely but inconsistently employed, and aside from guidelines, drivers for this heterogeneity are unknown.

In critically ill patients admitted with sepsis in intensive care units, a short course of aminoglycosides as adjunct to beta-lactam antibiotics was associated with an increased risk for kidney injury and a non-significant trend towards increased mortality. It is yet unclear whether this strategy has any benefits in a non-ICU population. We therefore determined in seven Dutch hospitals the effect of short-term empiric aminoglycoside treatment as adjunct to beta-lactam antibiotics on 30-day mortality in patients with Gram-negative bloodstream infection (GN-BSI), a subset of patients with sepsis in which the expected benefits of this strategy would be the largest.

Methods

Study, setting and participants

This study was nested in a prospective cohort in eight hospitals in the Netherlands (seven secondary care hospitals, one tertiary care hospital). In this study, data of 2000 patients with a Gram-negative infection and 2000 non-infected controls were collected to assess the burden of antibiotic resistance in Gram-negative infections in the Netherlands. (ClinicalTrials.gov identifier: NCT02007343, (Rottier WC, Deelen JWT, in preparation)) Patients were included between June 2013 and November 2015. Every week, trained research nurses consecutively screened clinical cultures (excluding screening cultures of rectum or throat) of the previous week and included the first five patients (age >= 18 years) with a positive culture that met all of the following criteria: 1. involved Enterobacterales and/or non-fermenters; 2. constituted a new infection according to the respective CDC-criteria for infection[5]; 3. was the index culture of a new infection episode. Patients already being treated for Gram-negative infection

could not be included as a new infection, and for diagnosis of a new infection treatment of a prior infection had to be completely finished. From this cohort we selected patients with BSI from seven hospitals, as medication data were not available from one secondary care hospital. Patients who died on day 0 and patients who did not receive beta-lactam antibiotics on day 0 or 1 were excluded from the analysis. The Ethics Committee of the University Medical Center Utrecht waived the requirement of informed consent.

Outcome and definitions

The study outcome is 30-day all-cause mortality, which was determined from medical records supplemented with mortality data from the Municipal Personal Records Database, thus, there was no loss to follow up.

Empiric aminoglycosides were defined as the prescription of gentamicin, tobramycin or amikacin on the day before, on and/or after the index culture. Dose per kg was calculated with an average weight of 80kg, which was based on data from a Dutch study conducted in ICU's. Exposure was ascertained by extraction of medication data from the local pharmacy system and confirmed with prescription data in the digital patient records. Appropriate empirical antibiotic therapy was defined as an antibiotic, or a combination of antibiotics, administered on day 0 and/or 1 of which at least one had in-vitro activity based on antibiotic susceptibility testing. Day 0 is the calendar date of blood culture obtainment. Local antibiotic policies for empiric antibiotic treatment in patients with sepsis are listed in supplementary Table 1. First choice treatments included a second-generation cephalosporin plus aminoglycoside in five and monotherapy with a third-generation cephalosporin in two hospitals.

We use the term 'bloodstream infection' (BSI) interchangeably with bacteraemia. Primary BSI is a BSI where no source could be diagnosed, often occurring in neutropenic patients. For further definitions of variables, see supplementary B.

Statistical analysis

All analyses were performed in R version 3.4.3, with use of the packages *jtools* version 2.0.0.[6]

Missing values occurred rarely (<0.1% of all variables in the cohort) and thus no need for imputation or other strategies were deemed necessary; a complete case analysis was performed.

To determine the casual effect of short-course aminoglycosides, we, in short, created a propensity score and used inverse probability weighting of this score to adjust for the pre-selected confounders. For a full description of this process, please see supplementary C.

Sensitivity analyses

We performed four sensitivity analyses to increase robustness of our findings: (1) excluding patients with treatment restriction/DNR; (2) excluding patients in whom blood cultures had been obtained in intensive care unit; (3) excluding ER patients from one hospital in which it was unclear whether all aminoglycoside administrations at the ER were registered; (4) an

analysis without patients with a BSI caused by *Pseudomonas aeruginosa*, since this is sometimes considered as a separate clinical entity for which the standard beta-lactam (+aminoglycoside) is not sufficient.

The study was reported according to the STROBE-guideline for reporting of observational studies.[7]

Results

Among the 1,721 patients in the total cohort with Gram-negative infections, 690 (40.1%) had a BSI, of which six died on day 0 and 58 did not receive beta-lactam antibiotics, leaving 626 patients (figure 1). Of these, 156 received adjunctive aminoglycosides (24.9%) for a median of 1 days (table 1). All received gentamicin or tobramycin.

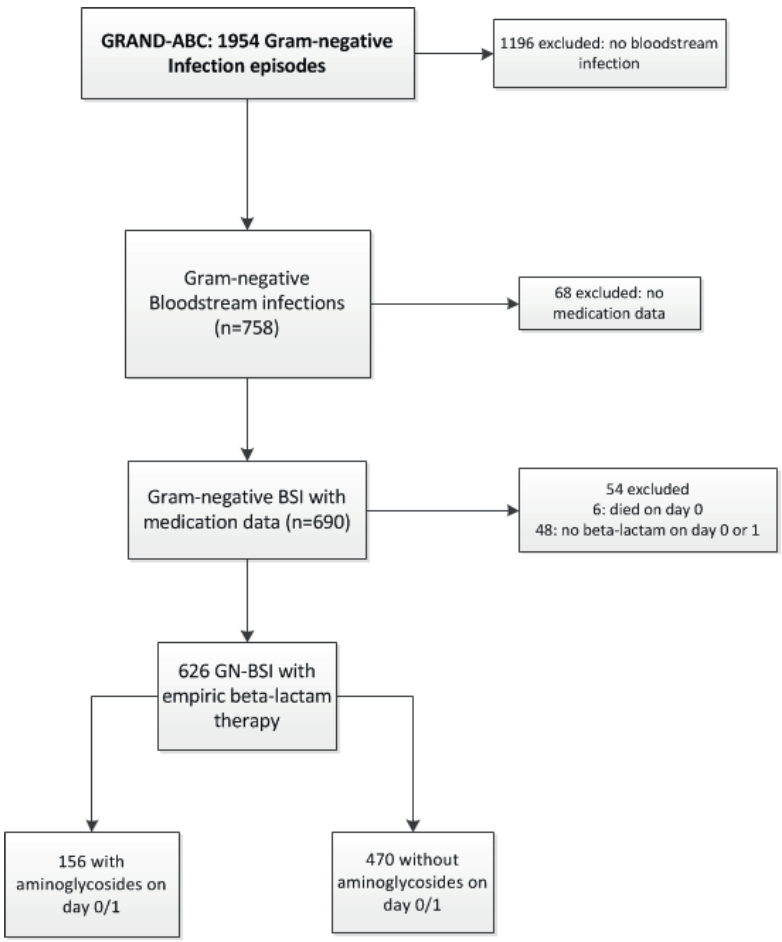

Figure 1. Flowchart.

Table 1: baseline data of patients with and without empiric aminoglycoside therapy

	Patients not treated with aminoglycosides (n=470)	**Patients treated with Aminoglycosides (n=156)**
Age (mean + SD)	72.7 (14.0)	69.6 (14.7)
Female sex (%)	205 (43.6)	78 (50.0)
Charlson comorbidity score (median + IQR))	2 [1 – 4]	2 [0 – 3]
Hospital		
A	56 (11.9)	23 (14.7)
B	41 (8.7)	49 (31.4)
C	109 (23.2)	15 (9.6)
D	100 (21.3)	15 (9.6)
E	68 (14.5)	20 (12.8)
F	56 (11.9)	25 (16.0)
G	40 (8.5)	9 (5.8)
Origin (%)		
Community-onset	193 (41.1)	74 (47.4)
Healthcare-associated	193 (41.1)	56 (35.9)
Hospital-onset	84 (17.9)	26 (16.7)
Chronic kidney disease (%)	29 (6.2)	5 (3.2)
Immunocompromised (%)	54 (11.5)	22 (14.1)
Sepsis severity *(%)*		
Sepsis	381 (81.1)	103 (66.0)
Severe sepsis	55 (11.7)	22 (14.1)
Septic shock	34 (7.2)	31 (19.9)
Infection source *(%)*		
Primary BSI	50 (10.6)	22 (14.1)
Urinary tract	250 (53.2)	88 (56.4)
Abdominal	111 (23.6)	32 (20.5)
Respiratory	14 (3.0)	5 (3.2)
Skin and soft tissue	13 (2.8)	3 (1.9)
Other	32 (6.8)	6 (3.8)
Pathogens (%)		
E.coli	281 (59.8)	92 (59.0)
K. pneumoniae	42 (8.9)	11 (7.1)
Other Enterobacterales	60 (12.7)	22 (14.1)
P. aeruginosa	26 (5.4)	9 (5.8)
Multiple species	61 (13.0)	22 (14.1)
Resistance (%)*		
2nd-gen cephalosporins	128 (23.0)	31 (21.1)
3rd-generation cephalosporins	43 (9.7)	15 (10.2)
Aminoglycosides	36 (7.9)	12 (8.2)
Obtained culture ward (%)		
Surgical	70 (14.9)	14 (9.0)
ICU	23 (4.9)	6 (3.8)
Internal medicine	101 (21.5)	40 (25.6)
Emergency department	276 (58.7)	95 (60.9)
Colonization/infection with 3rd-gen cephalosporin resistant pathogen in prior year (%)	30 (6.4)	9 (5.8)
Treatment restriction (%)	143 (30.4)	30 (19.2)

	Patients not treated with aminoglycosides (n=470)	Patients treated with Aminoglycosides (n=156)
Empiric treatment** (%)		
1st gen Cephalosporin	9 (1.9)	4 (2.6)
2nd gen Cephalosporin	173 (36.8)	95 (60.9)
3rd gen Cephalosporin	169 (36.0)	35 (22.4)
Amoxicillin (+clavulanic acid)	118 (25.1)	36 (23.1)
Piperacillin/tazobactam	30 (6.4)	9 (5.8)
Carbapenem	26 (5.5)	8 (5.1)
Inappropriate day 0 and 1	69 (14.7)	3 (1.9)
Median duration of AG-therapy	-	1 [1-2]
Mean dose of gentamicin***	-	3.9mg/kg
Mean dose of tobramycin	-	4.7mg/kg

Data are given as N (%) unless otherwise indicated * Resistance in Enterobacterales infection episodes (excluding Pseudomonas). Resistance in pseudomonas is 3/35 (8.6%) to ceftazidime and 1/35 (2.9%) to aminoglycosides. **Numbers do not add up to 100% due to escalation/de-escalation on day 1, a patient may start with ceftriaxone on day 0 and escalate to carbapenems on day 1.*** Calculated with an average weight of 80kg.

Among the patients receiving aminoglycosides, 31/156 (19.9%) had septic shock, as compared to 34/470 patients (7.2%) that did not receive aminoglycosides. Treatment restrictions were more prevalent among patients not receiving aminoglycosides (143/470 (30.4%) compared to 30/156 (19.2%) among those without aminoglycosides). Colonization/infection with third-generation-cephalosporin resistant pathogens in the prior year was similar in both groups (5.8% and 6.4% in aminoglycoside and non-aminoglycoside group respectively).

Most episodes of GN-BSI were caused by *Escherichia coli* (n=376/626, 59.6%), followed by *Klebsiella pneumoniae* (n=54/626, 8.5%) and *Pseudomonas aeruginosa* (n=35/626, 5.6%). Multiple Gram-negative species (e.g. *E.coli* AND *K. pneumoniae* from index cultures) were involved in 97/626 BSI (13.3%), of which 62% included *E.coli*.

Proportions of patients with aminoglycosides per hospital ranged from 12% to 54%. (See supplementary Table). Types of beta-lactams used differed between the aminoglycoside-group and no-aminoglycoside group. In the aminoglycoside group, 60.9% (95/156) of prescribed beta-lactams were second-generation cephalosporins, and 22.4% (35/156) third-generation cephalosporins. In the no-aminoglycoside group, 36.8% (173/470) and 36.0% (169/470) of prescribed beta-lactams were second and third-generation cephalosporins, respectively. The proportion of patients receiving carbapanem therapy was similar in the non-aminoglycoside and aminoglycoside group. (5.5% vs 5.1%). Mean aminoglycoside dosages were similar between hospitals and ranged from 3.7mg/kg to 4.3mg/kg for gentamicin and 4.6 mg/kg to 4.7mg/kg for tobramycin. Overall, 72 of 626 patients received inappropriate empirical therapy; 3 patients of 156 (1.9%) that received aminoglycosides and 69 of 470 (14.7%) that did not receive aminoglycosides.

Study site, sepsis severity and Charlson comorbidity were the major predictors of aminoglycoside use in the propensity score model (Table 2). After weighting, all covariates were balanced, with a SD of <0.1. The overall explained variance (McFadden's R^2) of aminoglycoside use

was 15.3%, and study site explained 51.4% of this variance in aminoglycoside use, followed by sepsis severity (12.9%).

Table 2: propensity score model, odds ratio for aminoglycoside use versus no aminoglycoside use

	Odds ratio (95% CI)
Hospital	
A	3.02 (1.40 – 6.68)
B	7.90 (3.90 – 16.77)
C	Reference
D	2.13 (0.88 – 5.22)
E	1.95 (0.89 – 4.33)
F	3.03 (1.43 – 6.61)
G	2.74 (0.99 – 7.41)
Sepsis severity	
Sepsis	Reference
Severe sepsis	1.59 (0.86 – 2.89)
Septic shock	3.17 (1.68 – 6.02)
Treatment restriction	0.88 (0.51 – 1.50)
Second-generation cephalosporin use	2.56 (1.56 – 4.28)
Culture ward	
Surgical	Reference
Internal medicine	1.01 (0.63 – 1.65)
ICU	0.86 (0.34 – 2.06)
Age (per year)	0.98 (0.97 – 1.00)
Sex (female)	1.17 (0.77 – 1.79)
Kidney disease	0.49 (0.15 – 1.36)
Charlson Comorbidity Index	
0	Reference
1	0.48 (0.24 – 0.93)
2	0.99 (0.54 – 1.80)
3-4	0.70 (0.36 – 1.35)
>4	0.78 (0.40 – 1.51)
Origin	
Community onset	Reference
Healthcare associated	0.96 (0.60 – 1.53)
Hospital onset	0.85 (0.46 – 1.55)

Propensity score model. These variables are included in the propensity score, for calculating the chance (propensity) of aminoglycoside use. Propensity score was calculated using a logistic regression analysis.

Outcomes

Overall 30-day mortality was 14.6% (n=91), and 17.3% and 13.6% for those receiving and not receiving aminoglycosides, respectively. The unadjusted odds ratio for 30-day mortality for patients receiving aminoglycosides was 1.33 (95% CI 0.80 – 2.15). The adjusted odds ratio of aminoglycoside use for 30-day mortality was 1.57 (95% CI 0.84 – 2.92). The median time to death was 5 days (IQR 1.5 to 10.5 days) and 7.5 days (IQR 2 to 16 days) for patients receiving and not receiving aminoglycosides, respectively. Length of stay after infection onset was similar in both groups (median 8 days, IQR 6-13).

Seventy-four patients were treated in ICU, of whom 52 were admitted to the ICU within 24 hours. ICU-admission within 24 hours occurred more frequently in the aminoglycoside group (10.2% vs 5.5%). ICU-admission more than 24 hours after obtaining blood cultures occurred in seven patients in the aminoglycoside group (4.4%) and 15 patients in the non-aminoglycoside group (2.7%)

Adjusted odds ratios in the performed sensitivity analyses did not change interpretation (Table 3).

Table 3: Regression analyses – 30-day mortality

	Mortality: no aminoglycosides	**Mortality: aminoglycosides**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
Full analysis (n=626)	64/470 (13.6%)	27/156 (17.3%)	1.33 (0.80 – 2.15)	1.57 (0.84 – 2.92)
Excluding patients with infection onset at ICU (n=597)	57/447 (12.8%)	22/145 (15.3%)	1.24 (0.72 – 2.07)	1.52 (0.76 – 3.05)
Excluding CO/HA cases hospital B (n=558)	58/441 (13.1%)	24/117 (20.5%)	1.70 (0.99 – 2.86)	1.84 (0.96 – 3.55)
Excluding patients with treatment restriction (n=453)	29/327 (8.9%)	19/126 (15.1%)	1.82 (0.97 – 3.37)	1.93 (0.92 – 4.10)
Excluding patients with *Pseudomonas Aeruginosa* BSI (n= 591)	59/444 (13.2%)	23/147 (15.6%)	1.21 (0.71 – 2.02)	1.43 (0.75 – 2.71)

We report the crude and adjusted odds ratios of the impact of short-term adjunctive aminoglycosides on 30-day mortality, along with five sensitivity analyses (further explained in the methods). The adjusted OR was calculated by a logistic regression analysis, using inversed probability weighting to adjust for confounding. The confounders age, sex, culture ward, sepsis severity, Charlson comorbidity score, chronic kidney disease, second generation cephalosporin use, treatment restriction and community onset/healthcare associated/hospital onset were included in the propensity score. Odds ratios reported with 95% confidence interval.

Discussion

In this study of 626 sepsis patients with documented GN-BSI, we were unable to demonstrate an improved clinical outcome for a short-term course of aminoglycosides added to beta-lactams as part of empirical therapy. These results add to an increasing body of evidence regarding the absence of clinical benefits of short-term adjunctive aminoglycosides as part of empirical treatment strategies.

Up to now, three studies determined the effects of short-course aminoglycoside therapy as part of empirical antibiotic treatment in patients with severe sepsis and septic shock. These were however primarily focused on the occurrence of acute kidney injury, not mortality. Two

were retrospective single center studies, with 317 and 341 patients, respectively, and one was a prospective study of 648 patients in two Dutch ICUs.[8–10] In the retrospective studies exposure to aminoglycosides was less than three days in one (ICU-based) study and a single dose in the other study. In both studies aminoglycosides were not associated with either acute kidney failure or clinical benefits. In the prospective ICU-based study, a short-course of aminoglycoside therapy in patients with sepsis was associated with an increased incidence of acute kidney injury, without evidence of clinical benefits. The current study extends this absence of clinical benefits towards a general hospital population with GN-BSI.

Despite similar local antibiotic policies in the participating hospitals, aminoglycoside use varied widely between hospitals. In our propensity score, study site contributed 51% to the explained variance in aminoglycoside use. Our findings also suggest that physicians include the clinical severity of disease and comorbidities in their clinical decision making. Patients that received aminoglycosides were two times more likely to have severe sepsis or septic shock and less frequently had chronic kidney disease or treatment restrictions.

Broadening the antibiotic spectrum of empiric treatment is an important reason for adjunctive use of a short-course of aminoglycosides.[11] In the six non-academic centers, resistance among Enterobacterales to second-generation cephalosporins ranged from 16 to 23%, and resistance to third-generation cephalosporins from 6.4 to 10.3%, whereas resistance to gentamicin ranged from 3.8 to 11.7%. Indeed, adjunctive use of aminoglycosides was associated with an 8-fold lower risk of inappropriate empirical therapy, mainly by mitigating resistance to second-generation cephalosporins. However, despite the lower risk of inappropriate empiric therapy, aminoglycoside use was, also after adjusting for confounding (including use of second-generation cephalosporins) not associated with a higher survival rate at day 30.

The absence of an effect of inappropriate empiric antibiotic therapy on mortality has been reported before in similar patient populations, both in the Netherlands and the United Kingdom.[1213] Faster recognition of sepsis through implementation of sepsis guidelines[2], higher quality of supportive care[14], potentially reduced bacterial virulence due to resistance genes[15] and shorter duration of inappropriate therapy due to faster diagnostic procedures may all contribute to mitigating the effect of inappropriate therapy. Additionally, there might still be an in-vivo effect of beta-lactams in in-vitro non-susceptible bacteria, which may also mitigate the harmful effects of what is considered inappropriate therapy.[16] Another argument might be that a low severity of infections reduces the impact of inappropriate therapy on patient outcome. However, overall 30-day mortality in our study population was 15%, which is comparable to other cohorts of patients with Gram-negative bloodstream infections.[17,18]

Underdosing of aminoglycosides may also contribute to the observed absence of beneficial effects.[1920] The average doses of 3.7mg/kg to 4.3mg/kg for gentamicin and 4.6 mg/kg to 4.7mg/kg for tobramycin, were lower than currently recommended doses (being of 5mg/kg for gentamicin and 5-7mg/kg for tobramycine.[21] Yet, these recommendations are based on

PK/PD principles rather and the consequences of suboptimal dosing on patient outcome are unknown.[21] As short course aminoglycosides often constitutes one single dose of aminoglycosides therapeutic drug monitoring cannot be used to optimize dosing.

The absence of benefit, combined with widespread but heterogeneous use of short-term aminoglycosides calls for a randomized clinical trial, as we discussed before.[22] In such a study we would propose to use higher dosages of gentamycin and tobramycin. The current study was performed in patients with GN-BSI, but this is not a suitable population for an RCT, as the presence of GN-BSI is unknown at initiation of empiric therapy. However, since one of the reasons to use aminoglycosides is to reduce inappropriate therapy in GN-BSI, improvements in outcomes are expected to be the largest in this GN-BSI poplation. For further studies,the more relevant study population would be patients with sepsis, potentially caused by Gram-negative bacteria, and with an a priori risk of 30-day mortality of, for instance, 20%. In such a population we would argue that addition of aminoglycosides should yield an absolute reduction of 30-day mortality of at least 2%. In such a study, the effects on kidney failure should be carefully monitored.

We would like to discuss several study limitations. Naturally, our analysis is susceptible to confounding, even after adjustment. We, therefore, performed four sensitivity analyses, to both account for uncertainties in the data and to better understand several confounders. These analyses support our findings and point at a potentially more harmful effect of aminoglycosides, although the confidence intervals are wide. Furthermore, we adjusted for several important confounders, including sepsis severity. Although existence of an unknown confounder that explains the lack of a beneficial effect of aminoglycosides on mortality seems unlikely, more specific data on disease severity would have increased study validity. Second, average dose of aminoglycosides was lower than guideline recommendations. Although there is no conclusive evidence with regards to effectivity of higher dosages, this may have impacted the effect on mortality. Third, we did not register creatinine levels either before or after infection. Although chronic kidney disease is included as a confounder, we could not explore associations between creatinine levels and mortality. Additionally, the definition of chronic kidney disease in this study precludes milder forms of chronic kidney disease.

In conclusion, we were unable to demonstrate beneficial effects of a short-course of aminoglycosides added to beta-lactam antibiotics on 30-day mortality in patients with GN-BSI. Considering the widespread use of aminoglycosides and uncertainty about its benefits, a randomized trial is warranted.

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Author contributions
JD devised the study, collected data and drafted the manuscript, CHvW and MB aided in devising the study and critically reviewed the manuscript, all other authors aided in collecting data and critically reviewed the manuscript.

Conflicts of interest
All authors state no conflicts of interests.

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References

1. Paul M, L. L. Cochrane Database of Systematic Reviews Beta lactam antibiotic monotherapy versus beta lactam- aminoglycoside antibiotic combination therapy for sepsis (Review). *Cochrane Database Syst. Rev.* (2014). doi:10.1002/14651858.CD003344.pub3
2. Rhodes, A. *et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016*. *Intensive Care Medicine* 43, (Springer Berlin Heidelberg, 2017).
3. Hanberger, H. *et al.* Rational use of aminoglycosides-Review and recommendations by the Swedish Reference Group for Antibiotics (SRGA). *Scand. J. Infect. Dis.* 45, 161–175 (2013).
4. Agence française de sécurité sanitaire des produits de santé. Update on good use of injectable aminoglycosides, gentamycin, tobramycin, netilmycin, amikacin. Pharmacological properties, indications, dosage, and mode of administration, treatment monitoring. *Med. Mal. Infect.* 42, 301–308 (2012).
5. CDC. CDC / NHSN Surveillance Definitions for Specific Types of Infections. *Surveill. Defin.* 2015, 1–24 (2016).
6. Long, J. Package ' jtools ' 2.0.1. *Cran* (2019).
7. Von Elm, E. *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Epidemiology* 18, 800–804 (2007).
8. Picard, W. *et al.* Propensity-Based Study of Aminoglycoside Nephrotoxicity in Patients with Severe Sepsis or Septic Shock. *Antimicrob. Agents Chemother.* 58, 7468–7474 (2014).
9. Ong, D. S. Y. *et al.* Short-Course Adjunctive Gentamicin as Empirical Therapy in Patients With Severe Sepsis and Septic Shock : A Prospective Observational Cohort Study. 64, 1731–1736 (2017).
10. Cobussen, M., De Kort, J. M. L., Dennert, R. M., Lowe, S. H. & Stassen, P. M. No increased risk of acute kidney injury after a single dose of gentamicin in patients with sepsis. *Infect. Dis. (Auckl).* 48, 274–280 (2016).
11. Davis, B. D. Bactericidal synergism between β-Lactams and aminoglycosides: Mechanism and possible therapeutic implications. *Reviews of Infectious Diseases* 4, 237–245 (1982).
12. Fitzpatrick, J. M. *et al.* Gram-negative bacteraemia; a multi-centre prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. *Clin. Microbiol. Infect.* 22, 244–251 (2016).
13. Frakking, F. N. J. *et al.* Appropriateness of empirical treatment and outcome in bacteremia caused by extended-spectrum-β-lactamase-producing bacteria. *Antimicrob. Agents Chemother.* 57, 3092–3099 (2013).
14. Rello, J., Valenzuela-Sánchez, F., Ruiz-Rodriguez, M. & Moyano, S. Sepsis: A Review of Advances in Management. *Adv. Ther.* 34, 2393–2411 (2017).
15. Beceiro, A., Tomás, M. & Bou, G. Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? *Clin. Microbiol. Rev.* 26, 185–230 (2013).
16. Thulin, E., Sundqvist, M. & Andersson, D. I. Amdinocillin (mecillinam) resistance mutations in clinical isolates and laboratory-selected mutants of Escherichia coli. *Antimicrob. Agents Chemother.* 59, 1718–1727 (2015).
17. Holmbom, M. *et al.* 14-Year survey in a Swedish county reveals a pronounced increase in bloodstream infections (BSI). Comorbidity - An independent risk factor for both BSI and mortality. *PLoS One* 11, 1–16 (2016).
18. Battle, S. E. *et al.* Derivation of a quick Pitt bacteremia score to predict mortality in patients with Gram-negative bloodstream infection. *Infection* 0, 0 (2019).
19. Cobussen, M. *et al.* Gentamicin is frequently underdosed in patients with sepsis in the emergency department. *Neth. J. Med.* 73, 443–444 (2015).
20. Moore, R. D., Lietman, P. S. & Smith, C. R. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. *J. Infect. Dis.* 155, 93–9 (1987).
21. Stichting Werkgroep Antibioticabeleid. SWAB guidelines for Antibacterial therapy of adult patients with Sepsis. (2010).
22. Ong, D. S. Y., van Werkhoven, C. H., Cremer, O. L., Thwaites, G. E. & Bonten, M. J. M. Is a randomized trial of a short course of aminoglycoside added to β-lactam antibiotics for empirical treatment in critically ill patients with sepsis justified? *Clin. Microbiol. Infect.* 24, 95–96 (2018).

Chapter 6

Supplementary Materials

A. Local guideline for sepsis with unknown pathogen

Hospital	First line choice	Comments	% of aminoglycosides in GN-BSI patients	% of AG in severe sepsis/septic shock
A	Cefuroxim/ tobramycin 5mg/kg		23/79 (29.1%)	11/21 (52.4%)
B	Cefuroxim/ gentamicin 5mg/kg		49/90 (54%)	15/29 (51.7%)
C	Cefuroxim	Add aminoglycoside when hospital acquired, patient has clinically sepsis or recent treatment with antibiotics	15/124 (12.1%)	3/31 (9.7%)
D	Ceftriaxone	Add gentamicin when clinically unstable	15/115 (13.0%)	5/18 (27.8%)
E	Cefuroxim/ tobramycin		20/88 (22.7%)	5/13 (38.5%)
F	Cefuroxim/ gentamicin		25/81 (30.9%)	12/24 (50.0%)
G	Ceftriaxone	Add gentamicin when "risk factor present for ESBL: prior fluoroquinolone/ cephalosporin use in last month"	9/49 (18.4%)	2/6 (33.3%)

National guideline (from which local guidelines are adapted), suggest second or third-generation cephalosporin for community onset sepsis with unknown pathogen, or co-amoxiclav+aminoglycoside. Adjunctive aminoglycosides are recommended in nosocomial sepsis patients, and when there is an increased risk for an ESBL infection based on cephalosporin/fluoroquinolone use in the last month or colonization with an ESBL-producing pathogen. In the last case, a carbapenem can also be used

B. Definitions of variables

Infections were considered hospital-acquired if the index culture was taken >=48h after hospital admission and healthcare-associated if the index culture was taken <48h after hospital admission and the patient had been hospitalized ≥2 nights in the last three months, was on dialysis, resided in a long term care facility or nursing home, or received intravenous therapy (e.g. chemotherapy) within the last 30 days.[6] All other infections were considered community-acquired.

Antibiotic sensitivity was determined according to local laboratory practices using EUCAST criteria. The type of infection was based on the CDC-criteria for infections.[5] According to definitions of the Dutch Highly Resistant Micro-Organisms (HRMO) third-generation cephalosporin resistance or combined fluoroquinolone and aminoglycoside resistance in Enterobacterales was considered an HRMO, as is carbapenem resistance in Pseudomonas spp.[1]

Comorbidities were scored according to the Charlson Comorbidity Index, assessed two calendar days before infection onset or at the day of admission (for community-acquired infections), and classified in five groups: 0, 1, 2, 3-4 and >4 for the analysis.[2] Patients were considered immunocompromised if they had one or more of the following: 1. chemotherapy in the last 30 days; 2. corticosteroids (≥20 mg prednisone or equivalent per day) for more than two weeks at infection onset; 3. neutropenia before infection onset (neutrophils <0.5*109 cells/L); or 4. Use of other immunosuppressive drugs in the last three months. Treatment restrictions were defined as any policy regarding withholding of treatment and occurs in three levels: Ano cardiac resuscitation, B. no mechanical ventilation and C. no ICU admission. Treatment restriction policies are agreed upon by patients, relatives and doctors and are obligatory recorded in medical charts.

Chronic kidney disease was defined as either a serum creatinine above 265mmol/L (3.0mg/dL), hemodialysis, peritoneal dialysis and/or kidney transplant. Not all patients had all sepsis criteria (e.g. respiratory rate) measured. Since all patients had a GN-BSI and start of empiric antibiotics, we considered the baseline to be sepsis if no symptoms of severe sepsis or septic shock were present. Sepsis severity was based on the most severe clinical state in 24 hours before to 3 hours after culture obtainment, and all patients were classified according to the SEPSIS-II-guidelines (which were the current guidelines when the study initiated) as sepsis, severe sepsis or septic shock.[3]

C. Extended description of statistical methods

To determine the causal effect of added empiric aminoglycosides, we considered several predefined confounders, such as sepsis severity as this is a predictor for both aminoglycoside use and outcome. Chronic kidney disease and other comorbidities may be contra-indications for aminoglycoside use, and also have an impact on mortality, and were, therefore, also included as confounders. Mortality strongly differs between sources of infection and may influence the choice for aminoglycosides. The hospital site impacts the risk of antibiotic resistance, as well as the likelihood of aminoglycoside prescription because of differences between local practices. Additionally, we adjust for use of second-generation cephalosporins, as these are more often used in combination therapy, and we want to isolate the effect of adjunctive aminoglycosides. Finally, treatment restrictions (e.g. do not resuscitate orders, DNR's) are a strong predictor of mortality and may also influence treatment decisions.[4,5]

Because of the study sample size and the proportions of patients that died within 30 days that received aminoglycosides, we used a propensity score analysis to adjust for confounding. The propensity of receiving empiric aminoglycosides was calculated with the aforementioned variables. We performed logistic regression analysis with inverse probability weighting using the propensity score as weight. In short, the weights are calculated as 1/(propensity score) for the treated patients and 1/(1-propensity score) for the untreated patients, thereby creating a

pseudo-population in which the effect of treatment on the outcome is unconditional on the propensity of getting the treatment. We assessed balance of the covariates after weighting using the standardized difference (SD), a difference of SD <0.1 was considered balanced. We used robust standard errors for the 95% confidence interval (CI) to take into account the sampling of the weights.[6]The results were reported as odds ratio with a 95% CI.

To analyze the drivers of aminoglycoside use, we used McFadden's pseudo-R2 to calculate the percentage of explained variance for the variables in the propensity score.

References supplementary material

[1] Werkgroep.Infectiepreventie. Bijzonder resistente micro-organismen (BRMO). 2013.
[2] Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, et al. Updating and validating the charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol 2011;173:676–82. https://doi.org/10.1093/aje/kwq433.
[3] Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. vol. 36. 2008.
[4] Mulder T, van Werkhoven CH, Huijts SM, Bonten MJM, Postma DF, Oosterheert JJ. Treatment restrictions and empirical antibiotic treatment of community-acquired pneumonia in elderly patients. Neth J Med 2016;74:56.
[5] Bradford MA, Lindenauer PK, Wiener RS, Walkey AJ. Do-not-resuscitate status and observational comparative effectiveness research in patients with septic shock*. Crit Care Med 2014;42:2042–7. https://doi.org/10.1097/CCM.0000000000000403.
[6] Robins JM, Hernán MA, Brumback B. Marginal structural models and causal inference in epidemiology. Epidemiology 2000;11:550–60. https://doi.org/10.1039/C29690000418.

CEPHALOSPORINS CARBAPENEMASE DISEASE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL MORTALITY DISEASE BURDEN GRAM-NEGATIVE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET PROPENSITY SCORE INFECTION COHORT METHODS GRAND-ABC BURDEN CEPHALOSPORINS BACTERIA BLOODSTREAM INFECTION MORTALITY PREDICTION GRAM-NEGATIVE INFECTION PREDICT ANTIBIOTIC CEPHALOSPORINS CARBAPENEMASE DISEASE TRIAL MORTALITY ANTIBIOTIC PREDICT BLOODSTREAM GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL ANTIBIOTIC DISEASE CHAPTER 7 CAUSAL INFERENCE MORTALITY GRAM-NEGATIVE BACTERIA PREDICTION GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM

General discussion

J.W. T. Deelen

Gram-negative infections, and antibiotic resistance in particular, are a growing public health concern. In this thesis, several issues related to the size of the burden of resistance, methodology and empiric treatment were discussed.

The first part of the discussion will focus on the findings in chapter 2, 3 and 4, all related to the national disease burden, and these are further put into perspective by comparisons with other burden studies and future prospects. The second part of the discussion will focus on empiric treatment strategies, with an emphasis on implementation of the findings from chapter 5. The third part of the discussion wraps it up, along with some views on future research.

The attributable burden of resistance in Gram-negative bacteria

In chapter 2, we estimated the national incidence and mortality of Gram-negative infections, based on a combination of prospective cohort data (GRAND-ABC) and the national surveillance database (ISIS-AR). We found that aside from the regularly studied Gram-negative bloodstream infections, a third of the 30-day all-cause mortality after infection onset occurs after a non-bacteremic Gram-negative infection.

In chapter 3, we present the main analysis of the GRAND-ABC study and estimated the attributive mortality of antibiotic resistance in Gram-negative infections in the Netherlands. This was found to be zero, meaning that in the Netherlands, antibiotic resistance in GNI does not cause additional mortality compared to non-resistant infections. If anything, the analyses pointed at a potentially protective effect, which was even more emphasized in the sub-group analysis with Gram-negative bloodstream infections (GN-BSI).

Together, these two findings provide insight in the current situation regarding antibiotic resistance in Gram-negative bacteria in the Netherlands. Historically, the levels of antibiotic resistance in the Netherlands are low, with ESBL infection rates in suspected bloodstream infections of 0.9-1.4%[1], colonization of approximately 5.0% in the general population[2], and low rates of MRSA.[3] This level of resistance has marginally increased over the last few years, but can be considered stable (EARS-NET), and the resistant infections themselves do not cause additional mortality. On the other hand, there is a large number of Gram-negative infections which can be considered a major public health concern.

Other large-scale studies on the mortality of resistance in Gram-negative infections paint an ambivalent image. These studies were mostly performed with ESBL *E.coli* bloodstream infections in multiple European countries, and report OR's for (30-day) mortality of third-generation cephalosporin resistant *E.coli* BSI versus sensitive of 1.63 (95% CI 1.13 – 2.25) (general population) and a HR of 1.3 (95% CI 0.8 – 2.2) in an ICU population.[4,5] The third study, by de Kraker et al. employed parallel matched cohort design, similar to ours. In the parallel matched cohort study, HRMO and non-HRMO infected patients are compared to non-infected controls to adjust for confounding. Despite this similarity in methods, they reported an OR for (30-day) mortality of 2.5 (95% CI 0.9 – 6.8).[6]

Next to these large-scale studies, there are many smaller studies on the mortality of antibiotic resistant Gram-negative infections. In a review from 2012 on the mortality of ESBL-BSI, Rottier et al. reported an adjusted OR of 1.52 (95% CI 1.15 – 2.01).[7] Comparing this to a sub-analysis of HRMO BSI in the GRAND-ABC cohort (which is ~75% third-generation cephalosporin resistant (3GC-R) *E.coli* and *Klebsiella* BSI), the odds ratio was 0.59 (95% CI: 0.30 – 1.27), indicating no excess mortality. Furthermore, in this paper, it was described that studies reported a high OR when they did not adjust for any confounders, and how in many studies that did adjust for confounders, inappropriate therapy was erroneously included in the model, leading to an underestimation of the actual mortality. This practice of adjusting for inappropriate therapy is still prevalent in studies on the mortality of carbapenem resistance (chapter 4). The pooled OR of studies not adjusting for inappropriate therapy was 2.77 (95% CI 2.13 – 3.60), signifying an even stronger difference between these results and our study.

The attributable mortality of antibiotic resistance, together with an estimate of the number of resistant infections, is used to calculate the (mortality) burden of antibiotic resistance. One of the most important studies to date on this topic is the 2018 review by Cassini et al.[8] In this impressive study, they estimate the disease burden (mortality and disability adjusted life years (DALY's)) of several resistant pathogens for European countries. However, when comparing their findings to ours there are similarities and discrepancies. In the Netherlands, they report 491 3GC-R *E.coli* BSI (95% CI: 457 – 530), an incidence of 2.9 (95% CI 2.7 – 3.1)/100.000 inhabitants (using the total Dutch population of 16.9 million). The numbers for 3GC-R *Klebsiella* BSI are 116 (109 – 123), and incidence 0.69 (0.65 – 0.73). We calculated the number of GN-BSI and HRMO GN-BSI (mostly 3GC-R *E.coli* and HRMO *Klebsiella*) in **chapter 2** and estimated the number of HRMO BSI to be 1012 (690 – 1340) per year. Using our own estimates of 3GC-R *E.coli (*45% of all HRMO infections) we come to an estimate of 450 3GC-R *E.coli* per year, which is very similar. Considering that Cassini et al calculated the incidence for the whole population and we included only adults, our actual number would be (marginally) larger if we also included children. Of note, the source data for these estimations, the Dutch national surveillance database, is the same.

The discrepancies arise when calculating the attributable deaths. To calculate the number of attributable deaths, they use pathogen-specific mortality estimates derived from meta-analyses of earlier studies. They report the attributable deaths in the Netherlands caused by 3GC-R *E.coli* and *Klebsiella* in 2015 to be 85 (75-95) and 19 (18-20), respectively. However, the attributable mortality of resistance in GNI in the Netherlands was estimated to be 0 in chapter 3, and the attributable burden of antibiotic resistance in Gram-negative bacteria in the Netherlands is thus zero.

Why all the discrepancies?

There are numerous differences between outcomes reported in this thesis and burden and mortality estimates reported in other studies. We hypothesize in chapter 3 that the absence of attributable mortality in the Netherlands is due to clinically involved microbiologists,

quick turn-around times in the lab that reduce the duration of inappropriate therapy, and the availability of antibiotics for infections we consider highly resistant (e.g. ESBL). This however does not explain why there are different outcomes reported for the same resistant mechanism. There may be two possible (not mutually exclusive) explanations. Estimates of burden studies have reliability issues or the effect of antibiotic resistance is different across different settings.

In Chapter 4, I summarize the evidence on mortality caused by carbapenem resistance. The analysis reveals serious problems with the quality of methodology and reporting, that may lead to different estimates for mortality resulting from infections caused by carbapenem-resistant Gram-negative bacteria. The effects of antibiotic resistance on mortality are calculated by using different confounders sets for adjustment, and selection and information bias are not taken into account. The different estimates may thus be an underestimation of the actual differences, since due the low reporting quality, it is hard to assess selection and information bias in the individual studies.

The more worrying aspect is that these individual studies become part of meta-analyses, and that these analyses, by the sole virtue of being meta-analyses, often reach truth-status. This may currently be the weakest link in studies on the burden of antibiotic resistance, since most published estimates are biased. Additionally, the tools used for grading the level of evidence (e.g. GRADE) are not by themselves enough to cover all the limitations and biases and, upon discovering that there are significant flaws in a certain study, it is often still included in the analysis.

Research on antibiotic resistance is not an exception, despite a clear reporting guideline for observational studies.[9,10] However, as the science of causal inference is becoming more known, the use of directed acyclic graphs, concepts like the Target Trial and more honest communication about causal goals will hopefully improve causal estimates of mortality caused by antibiotic resistance, and in the end estimates of the burden of antibiotic resistance.[11–13]

In our own study on the attributable mortality of resistance, we tried to mitigate these issues by conducting a prospective study, having a random sample of infections by including the first 5 infections per week, and extensively adjusting for confounding, including several sensitivity analyses, to see the effect of different sets of confounders. These methodological choices may partly explain the lack of effect of antibiotic resistance on mortality in this study, since mortality can be explained by confounding.

The alternative explanation is that the effect of antibiotic resistance differs across different settings. For this to occur, the underlying mechanisms by which antibiotic resistance causes mortality must per definition also be different. In chapter 4, I postulate that there are four ways how antibiotic resistance impacts mortality: inappropriate therapy (due to the resistant pathogen not being covered by empiric antibiotics), higher virulence, isolation strategies for patients or side effects of antibiotics, several of which were previously described by Friedman

et al in 2016.[14] Of these four ways, inappropriate therapy seems the most likely way by which antibiotic resistance in Gram-negative bacteria causes more mortality, since there is, as of yet, no evidence for higher virulence, no evidence for an impact of isolation on mortality (though it may cause harm in other ways) and side effects of antibiotics are well known, and would not be responsible for a large increase in mortality.[14] In our study however, inappropriate empiric therapy was not associated with worse outcomes. Furthermore, in chapter 6 on aminoglycoside use, we found that despite an 8-fold reduction in inappropriate therapy in the aminoglycoside group in GN-BSI patients, (1.2% vs 14.9%), there was no difference in outcome between the two groups after adjusting for confounders. Additionally, a study from Fitzpatrick et al on GN-BSI performed in the United Kingdom also showed no effect of inappropriate therapy on mortality in these patients.[15] On the other hand, a systematic review of the impact of inappropriate therapy shows an impact of inappropriate therapy on mortality in sepsis of OR 1.6 (95% CI: 1.37 – 1.86), with effect sizes from individual studies ranging from 0.93 to 15.5.[16]

Thus, there is evidence that inappropriate therapy is also different across different settings (although these individual studies most likely suffer from the same methodological shortcomings as the studies on carbapenem resistance in chapter 4). At this point, one may get the impression that it is all chaos and misery regarding the reliability of burden estimates. In the next paragraph, I will discuss potential reasons for different outcomes for similar pathogens, and do suggestions for further burden estimates of antibiotic resistance.

Missing puzzle pieces

One thing that may explain the different results regarding the mortality of antibiotic resistance is the context in which the study has been conducted. In an average baseline table, you can assess the patient population up to a certain degree (acute disease severity, comorbidities) and in some (multicenter studies), additional information is provided about the centers and wards on which the study is conducted. Despite this, much context regarding the patients and hospitals is not reported. Indeed, there are major differences between countries in hospital care, pre-hospital care, prevention, and hygiene practices that may affect the impact of antibiotic resistance, potentially functioning as effect modifiers and setting-specific confounders.

For example, the impact of inappropriate therapy and thus antibiotic resistance may be modified by the time it takes for a patient to arrive at the hospital. If a patient with septic shock is seen by a GP, it would take a maximum 15 minutes (from the moment the GP calls) for the ambulance to arrive, and treatment with i.v. fluids is started a few minutes after, underway to the hospital. Since we know that time to appropriate therapy is most vital in the sickest patients, a delay of initiation of supportive care (fluid resuscitation) pre-admission may modify the potential impact of inappropriate therapy in a negative way.[17] To clarify, if the infection was caused by a resistant pathogen, the impact of inappropriate therapy may be less severe if fluid resuscitation was started a few hours earlier. Thus, if in different countries this pre-admission delay is the norm (e.g. not enough ambulances, longer distance to hospitals),

the attributive mortality of antibiotic resistance will be larger than in the Netherlands. Of note, early treatment of sepsis by intravenous administration of ceftriaxone in the ambulance did not improve outcomes in patients in the Netherlands.[18]

Another example is the different practices around culture obtainment. In **chapter 5** we show that in hospitals in Europe, blood culture positivity (in patients in whom i.v. Gram-negative therapy is started) ranges from 15 to 60%. Worse outcomes are to be expected if there is a tendency not to obtain cultures from less severely ill patients. In this population, the impact of inappropriate therapy will be larger, and thus resistant pathogens (which are not necessarily themselves associated with more severe infections) will have a larger (visible) effect on mortality. Although far from conclusive evidence for this hypothesis, it is supported by the wide range of reported mortality from GN-BSI with, for example, 10% in a Swedish population study[19] to 26% for non-MDR and 54% for MDR Gram-negative pathogens in a hospital in Brazil.[20] Most studies on infections include only positive cultures, and information on positivity rates and culture habits are not regularly reported in studies on the burden of antibiotic resistance, while this may be vital to understand the aforementioned differences in outcomes.

Even in Europe, these differences may be major. Gutierrez-Gutierrez et al published a study on combination treatment in patients with carbapenem-resistant BSI, and described that in some hospitals, mortality was inexplicably higher than in others, despite other measured confounders, and included "high-mortality hospital" as a major predictor for mortality in the final model.[21] This hints at different quality of care or regional differences between patients that are not captured by the regularly measured variables. Other contextual factors that may influence the effect of antibiotic resistance, are differences in supportive care, whether some patients receive iv treatment at home and are thus not admitted, the role of treatment restrictions/DNR's and policy regarding discharge of patients. Treatment restrictions in particular reflect cultural and religious differences, and are at the same time a strong predictor of mortality.[22,2324,25] In **chapter 3 and 6,** we found that 30% and 27% of patients had a treatment restriction, which in Chapter 3 was associated with HRMO GNI (33% vs 25% in HRMO versus non-HRMO GNI patients) and with not receiving aminoglycosides in chapter 6 (30% vs 19.1%).

The aforementioned examples illustrate that there are many potential explanations for reported differences in mortality caused by antibiotic resistance. To increase our understanding of the impact of antibiotic resistance, we must consider these mechanisms before conducting a study. My first recommendation in providing clarity in this matter would be to start with a more extensive background description of how healthcare is organized in the country/hospital that is being studied. Furthermore, visits to hospitals in different countries might help to understand the context of your own hospital. ESCMID observerships provide an opportunity to do so.

The future of burden estimations

In the previous paragraphs, I described the absence of attributable mortality in the Netherlands, tried to explain differences in outcomes between studies and described methodological limitations in studies assessing the attributable mortality of antibiotic resistance. There is a lot of uncertainty in burden estimates, and our estimate shows that the attributable burden in the Netherlands is limited. However, a major component of the burden caused by antibiotic resistance is the potential future burden, since resistance in Gram-negative bacteria has 'progressed' over time from resistance to co-amoxiclav through cephalosporins and now carbapenem resistance. Future projections must take into account the consequences of pan-resistance, which were not included in our assessment.

Future projections however face even more difficulties (and criticism) than estimates of the current burden. One of the more notorious projections of the burden of antibiotic resistances comes from the AMR review. This review was commissioned by the British government to investigate the scope and opportunities to tackle antibiotic resistance on an international level, and was led by Jim O'Neill, a former Goldman-Sachs economist.[26] It is responsible for the often-quoted '10 million deaths due to antibiotic resistance by 2050', which comes from its first publication, written by KPMG, a global consulting firm, and the RAND institute, a global economic policy institute. This number was based on the assumption that resistance would increase by 40% in the next 15 years, and the number of infections would double due to longer periods of being infected and higher rates of transmission.

These findings were criticized for several reasons.[27] First, the estimated total number of BSI's were based on non-representative (non-population based) data, and extrapolations to other countries were crude. Next, estimation of resistant infections was problematic due to bias introduced by different blood culture obtainment practices, and extrapolation to other types of infections was based on unclear evidence. Third, calculations of attributable mortality were biased due to reliance on old studies with unclear methodology, and lastly, there was no scientific scrutiny due to the absence of confidence intervals and of peer review.

This is a bit of a conundrum. On the one hand, these criticisms are scientifically valid. On the other hand, the report was not intended as a scientific paper. As a policy document it fulfills its goal by providing several scenarios including a worst-case scenario based on legitimate, and arguably not unrealistic assumptions. The report answered the question whether antibiotic resistance poses a potentially big threat in the future and should be part of governmental policies, a question that to most microbiologists/infectious diseases specialists and scientists in this field, including the authors of the criticism of the report[28], already would answer with 'yes'. Therefore, the report can be considered successful and important by putting antibiotic resistance on the agenda of for example the UN and national governments, despite scientific shortcomings. Additionally, long-term predictions that would satisfy scientific scrutiny have not been made.

To move forward with assessing the future burden, it seems we need 1. an accurate assessment of the burden and attributable mortality now, including *how* resistance would cause that mortality. 2. a localized approach that takes into account this knowledge, to accurately determine the burden per geographical area (where **chapter** 3 can form the basis for the Netherlands and similar countries) and 3. insight into development of resistance over the next years.

Empiric treatment and infections

In chapter 5, I describe the international validation study of two prediction rules for third generation cephalosporin resistant Enterobacterales bloodstream infections (3GC-R EB BSI) in patients with suspected bloodstream infection. This resulted in a positive validation of the community-onset prediction rule (performance comparable to derivation study and potential reductions in carbapenem use) and a negative validation of the hospital-onset model (calibration off compared to original study and little to no reduction in unnecessary carbapenem use).

The goal of these prediction rules is to improve empiric treatment of infections. This includes reducing the risk of inappropriate treatment – inadequate treatment of a resistant pathogen – and the risk of overtreatment: unnecessary broad-spectrum antibiotics for a non-resistant infection. In this tragedy of the commons situation, in which the interests of the individual patient are at odds with the interests of society at large, clinicians have to navigate the choice of empiric treatment, and prediction rules can help with stratifying risks and choosing an antibiotic. In the Netherlands, the strategy suggested by the current guideline is to take into account prior cultures/colonization with 3GC-R-EB and cephalosporin/quinolone use in the last 30 days.[29] Both predictors had low positive predictive values based on a Dutch retrospective cohort study, and guideline adherence to these two rules was only 27%, but higher adherence would not lead to more appropriate treatment, since the predictive value of these predictors by themselves was limited.[30]

Previously published prediction rules have several drawbacks.[31–33] One of the most important methodological issues in diagnostic research is choosing the right control group.[34] If you compare ESBL+ vs ESBL- GN BSI, the diagnostic rule only tells you something about when you already know there is a Gram-negative infection, thus after the initial results of the blood culture. Such prediction rules may be useful for early de-escalation, if initial treatment was with carbapenems or other broad-spectrum antibiotics. However, for choosing empiric therapy, the control group of the prediction rule should involve all patients with a *suspected* Gram-negative infection, since these all receive empiric therapy, and you want to be informed for all of them.[1]

We confirmed in an international study the predictive capabilities of the community onset rule. However, the performance of the hospital onset rule was limited, and its potential for improving antibiotic use seems low. Although I already discuss the reasons in chapter 5,

expanding on this issue, in the light of what I have written before, seems useful.

The hospital onset rule depends, more than the community onset rule, on variables that are less 'universal'. Similar to what I described earlier in this discussion regarding a lack of contextual knowledge regarding the burden of antibiotic resistance, the association between measured variables and mortality or the presence of 3GC-R-BSI heavily depends on context. If prior surgery in one hospital only involves orthopedic surgery (clean, generally low rates of infection), this probably does not confer an increased risk of 3GC-R BSI (or BSI in general), while in a hospital that specializes in complicated colo-rectal surgery, it may be a strong predictor. Renal disease was a very strong predictor of 3GC-R BSI in the derivation study, but had less impact in the validation study. This might be due to different immunosuppressants in transplant patients, the number of hospital visits that is higher in the Netherlands, how we deal with dialysis, or perhaps that people with kidney disease in the Netherlands have a lower socio-economic status and are more prone to have dirty houses with ESBL in the kitchen. These examples are to explain the relativity of measurements and illustrate the relevance of context: due to the absence of contextual understanding, the hospital onset prediction rule is unusable outside the Netherlands.

On a more positive note, the community onset prediction rule does not seem to suffer from these issues and generalizes to an international population, despite up to 20-fold different incidences of 3GC-R BSI in the participating centers. The risk factors are, compared to the hospital onset rule, more 'generic', and reflect a combination of risk factors for a BSI compared to non-BSI (UTI vs pneumonia, age) and risk factors for a 3GC-R EB (colonization, prior antibiotic use).

From validation to implementation

In epidemiologic research, there is an abundance of published prediction rules that will never be used in clinical practice. For example, a recent systematic review of diagnostic rules for cardiovascular risk in a general population showed 363 prediction rules for the same clinical question.[35] Another study among British GP's, showed low awareness of presence of prediction rules for several clinical problems.[36] With 3GC-R EB, this is not as bad (there are around 4 or 5), but as of yet, there does not seem to be a widespread implementation of any of these rules.

With validating two previously derived prediction rules, we took the first step to implementation. Many prediction rules fail in validation, due to overfitting, low sample sizes or poor statistical modeling choices in the original study.[37] But now that we successfully validated the community onset rule in European hospitals, how do we go from validation to implementation?

For successfully implementing a prediction rule, there are three requisites.[38] One, there must be a clinical user case in which the prediction rule can be effectively used. Two: it

must be practical (e.g. a really good prediction rule with 100 predictors that requires manual completion will not be used since it takes a long time to be filled in). Three, the information it provides must be helpful in making a clinical decision.

Although we can safely say that the first two criteria have been satisfied, since the trade-off between broad-spectrum antibiotic use and inappropriate therapy is a well-known problem, and the prediction rule consists of only 5 easily attainable variables, the third criterion requires some reflection. To illustrate some potential problems, it is interesting to compare the ESBL-PREDICT rule to an implemented prediction rule like the Wells-score.[39] There seem to be three key differences.

First, in the Wells-score there is a clear algorithm that takes disease severity into account. If a patient has severe symptoms of pulmonary embolism, he or she will get the diagnostic workup. If there's a high risk of PE, a CT will also happen, if there is low risk, PE can reliably be excluded (to a risk of 1-2%) by use of a D-Dimer. On the contrary, ESBL-PREDICT, at this point provides an absolute risk for 3GC-R BSI, and whether a certain risk is acceptable or not needs to be decided by the treating physician and is most likely based on severity of infection. Research literature on use of prediction rules shows that clinicians struggle with interpreting absolute risks.[40,41], and the close cut-offs in the community onset rule (4.3, 5.0 and 6.2%) compound this problem. Therefore, an algorithm that incorporates disease severity, like the Wells, might increase the usefulness of the prediction rule.

Second, the burden of inappropriate treatment is carried by the patient (who in the worst-case dies), while the burden of unnecessary carbapenem use is carried by society. Incorporation of societal benefit in this decision-making process is difficult, since for an individual doctor and patient there is no burden of receiving carbapenems compared to cephalosporins, while in the Wells-score a workup leads to potentially invasive procedures and a radiation risk. The absence of individual downsides to carbapenem use incentivizes the use, and the prediction rule should therefore be especially useful to reduce unnecessary carbapenem use by having a high negative predictive value.

Third, even in an ideal situation, there will be a major risk of inappropriate therapy. While the Wells score reliably excludes low-risk patients, choosing a cut-off that maximizes appropriate treatment still misses 61% of 3GC-R BSI patients.

Implementation thus requires careful thought and consideration of the aforementioned issues. Clinicians have to be involved to help illustrating how they think, and what kind of presentation of information would be useful to them. At the same time, the discrepancy between unnecessary carbapenem use (8% of patients in the community onset cohort) and appropriate therapy (29%) of 3GC-R BSI patients) is significant, and the differences between patients receiving a carbapenem and not receiving a carbapenem are small with regards to ward type and most other variables (unpublished data), which gives the idea that

carbapenem prescription does not depend on the measured variables in our study (aside from immunocompromised and haematological patients). This is promising, because this more or less random variation in antibiotic use may be more sensitive to improvement by a prediction rule.

The next steps should therefore focus on the how the information the prediction rule provides can be best used by clinicians, and whether an algorithm is more useful than providing an absolute risk. Preferably, introduction of the prediction rule on emergency wards is done in a research project (implementation trial), so we can carefully assess the impact of the prediction rule, and optimize the use where needed.

Adjunctive aminoglycosides in empiric treatment

In chapter 6, we reported an absence of beneficial effect of adjunctive short-course aminoglycosides on mortality in patients with Gram-negative bacteremia. Considering aminoglycosides are widely used in the Netherlands for the supposed reduction of mortality, a randomized trial seems in order.

Interestingly enough, there is already quite a bit of evidence regarding the usefulness of adjunctive aminoglycosides. A systematic review from 2014 concluded that there are no beneficial effects, and a study on two Dutch ICU's in patients with septic shock demonstrated an increased risk of kidney injury and no mortality benefits in the aminoglycoside group.[42,43] The reason why it is still applied in the Netherlands, is most likely because short term aminoglycosides (1-2 days) have never been studied in a trial. While the national sepsis guidelines suggest the use of aminoglycosides when there is a higher risk of infection with ESBL, locally adapted guidelines differ widely In some locally adapted guidelines gentamicin is *always* added in septic patients, while in another it is only used in hemodynamically unstable patients and yet other hospitals never recommend it for empirical treatment.[29] This variation means that in one hospital, you would get potentially harmful or beneficial aminoglycosides, and in another you would never, all based on unclear evidence. It also becomes apparent that there are different views on aminoglycosides: does it broaden the antibiotic spectrum (and would carbapenems here be preferred due to their safety profile), or does it do 'something extra' by the means of synergistic effects with beta-lactam antibiotics. From studying the history of the Dutch sepsis guideline, it seems that aminoglycoside use is more of a historical choice than an evidence-based decision. In the 1999 guidelines, it is stated that despite the lack of evidence for beneficial effects of aminoglycosides, the biological principles (synergy) and increased coverage are so important that they should simply be prescribed, especially in more ill patients.[44] In the 2010 (current) guidelines, it is stated that aminoglycosides can be prescribed to broaden the coverage. However, in local guidelines, aminoglycosides are sometimes recommended in more severely ill patients, and sepsis severity, aside from site, is the most important driver of aminoglycoside use, and not prior colonization with resistant pathogens.

The other major difference in this regard is the choice between a second and third-generation cephalosporin. Aminoglycosides are more often prescribed with second-generation cephalosporins, and this is indeed important, since resistance for 2G-cephalosporins is around 20% in participating hospitals, while resistance to third-generation cephalosporins hovers between 4 and 10% in Enterobacterales, similar to resistance to aminoglycosides. Thus, the issue of less than ideal coverage of 2G-cephalosporins can be solved by prescribing 3G-cephalosporins in sepsis patients, without introducing a potential risk for kidney damage. Additionally, most clinicians tend to prescribe carbapenems in patients with actual risk factors for ESBL, thus bypassing aminoglycosides there as well. Some objections to prescription of 3G-cephalosporins are less adequate treatment of *S. aureus* infections, and the potential of 3G-cephalosporins to more quickly induce resistance. For both ideas there is no conclusive clinical evidence.

There is thus a lot of variation between (and in) hospitals, and the use of aminoglycosides is not based on solid evidence. A well-designed clinical trial should end this kind of discussions, but there are – as usual – logistic constraints. If there is an effect (that is not expected based on this study and prior studies), it may not be large. The highest risk ratio from an individual trial reported in the systematic review from Paul et al. is 2.43 in favor of combination therapy, and the lowest 0.33, while all the trials together lead to an RR of 0.85 (95% CI 0.75 – 1.01) in favor of monotherapy, although in this trial aminoglycoside therapy was as a rule given longer than the short-course adjunctive therapy studied in chapter 6. As a consequence, the sample size will be large. Additionally, since doctors more or less prescribe aminoglycosides ad-lib, there are some expected problems with adherence by doctors, especially in 'aminoglycoside-believer-centers'. On the other hand, it wouldn't be the first time that a trial shows different results compared to observational studies. The MERINO-trial, on definitive treatment of ESBL-BSI with either carbapenems or piperacillin/tazobactam, showed a surprising increase of mortality in patients treated with piperacillin/tazobactam, while observational studies showed that they were non-inferior.[45,46]

Putting it all together

Above, I touched upon the several topics discussed in this thesis, and expressed some concerns on the future, antibiotic resistance, and then dug deep into the use of prediction rules in clinical practice. Readers who made it this far may wonder about a coherent message in this thesis.

Bringing the individual conclusions together there are six statements:

1. There is a large burden of Gram-negative infections in the Netherlands.
2. There is no attributable mortality of antibiotic resistance in Gram-negative bacteria in the Netherlands, no impact of inappropriate (empiric therapy), and therefore the burden of antibiotic resistance in Gram-negative bacteria is negligible.

3. We can optimize empiric therapy in patients with suspected BSI by implementing the ESBL-PREDICT rule on emergency wards, although there are some important caveats for implementation.

4. There does not seem to be a beneficial effect of short-course adjunctive aminoglycosides on 30-day mortality of patients with Gram-negative bloodstream infections.

5. We need a trial on the benefits and harms of short-course empiric aminoglycosides to end this debate.

6. Causal research on the burden of antibiotic resistance is biased and chosen methods have a large impact on reported effect estimates.

The two most important takeaways are that with regards to antibiotic resistance, the Netherlands is doing well. It appears that other factors than antibiotic treatment are more important for survival, and we could in theory safely reduce unnecessary broad-spectrum antibiotic use in this country. The other takeaway is that we can successfully predict 3GC-R BSI in an international population, and that the prediction rule should be implemented for its potential to reduce unnecessary carbapenem use.

The future

Considering the current limited threat of antibiotic resistance in the Netherlands, it may be convenient to sit back and relax. Much like the climate crisis, the incentive to not do anything because 'we're such a small country' and 'France, the USA and China are much worse', and "it won't be a problem here" could be large. It is therefore promising that microbiologists, infectious disease specialists and antibiotic steward teams are all taking their responsibility, together with the ministry of health to combat and prevent antibiotic resistance in the Netherlands.

However, considering the small threat of AMR in the Netherlands and the major burden in other countries[47], we should ask ourselves whether spending millions of research and healthcare funding on reducing unnecessary antibiotic use in a country where AMR is a small problem is a wise thing to do. Especially since the burden of all Gram-negative infections is more of a concern than antibiotic resistance alone. Furthermore, since the drivers of antibiotic resistance are more and more known and point to economic factors (poverty[48], sanitation[49], socio-economic factors[50], corruption[51]) spending funding on lavish trials almost seems a little decadent. Therefore, collaborations with researchers and policy makers in LMIC's, trying to learn how to prevent the spread of antibiotic resistance in areas struck by poverty and creating awareness of antibiotic resistance amongst health care professionals and patients globally seems the way to go forward, and may also teach us a thing or two about infection prevention in high-income countries. Meanwhile, keeping the situation here as it is, with strong infrastructure, infection prevention and surveillance, should let us sleep soundly with

regards to antibiotic resistant infections in the Netherlands for the years to come.

References

1 Rottier WC, van Werkhoven CH, Bamberg YRP, *et al.* Development of diagnostic prediction tools for bacteraemia caused by third-generation cephalosporin-resistant enterobacteria in suspected bacterial infections: a nested case–control study. *Clin Microbiol Infect* 2018; 24: 1315–21.

2 van den Bunt G, van Pelt W, Hidalgo L, *et al.* Prevalence, risk factors and genetic characterisation of extended-spectrum beta-lactamase and carbapenemase-producing Enterobacteriaceae (ESBL-E and CPE): a community-based cross-sectional study, the Netherlands, 2014 to 2016. *Euro Surveill* 2019; 24. DOI:10.2807/1560-7917.ES.2019.24.41.1800594.

3 Wertheim HFL, Vos MC, Boelens HAM, *et al.* Low prevalence of methicillin-resistant Staphylococcus aureus (MRSA) at hospital admission in the Netherlands: The value of search and destroy and restrictive antibiotic use. *J Hosp Infect* 2004; 56: 321–5.

4 Lambert ML, Suetens C, Savey A, *et al.* Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: A cohort study. *Lancet Infect Dis* 2011; 11: 30–8.

5 Stewardson AJ, Allignol A, Beyersmann J, *et al.* The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible Enterobacteriaceae and Staphylococcus aureus in European hospitals, 2010 and 2011: A multicentre retrospective cohort study. *Eurosurveillance* 2016; 21. DOI:10.2807/1560-7917.ES.2016.21.33.30319.

6 de Kraker MEA, Davey PG, Grundmann H, group on behalf of the B study. Mortality and Hospital Stay Associated with Resistant Staphylococcus aureus and Escherichia coli Bacteremia: Estimating the Burden of Antibiotic Resistance in Europe. *PLoS Med* 2011; 8: e1001104.

7 Rottier WC, Ammerlaan HSM, Bonten MJM. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum ß-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. *J Antimicrob Chemother* 2012; 67: 1311–20.

8 Cassini A, Högberg LD, Plachouras D, *et al.* Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis* 2019; 19: 56–66.

9 Groenwold RHH, Van Deursen AMM, Hoes AW, Hak E. Poor Quality of Reporting Confounding Bias in Observational Intervention Studies: A Systematic Review. *Ann Epidemiol* 2008; 18: 746–51.

10 Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Epidemiology* 2007; 18: 800–4.

11 Hernán MA. The C-word: Scientific euphemisms do not improve causal inference from observational data. *Am J Public Health* 2018; 108: 616–9.

12 Shrier I, Platt RW, Rothman K, *et al.* Reducing bias through directed acyclic graphs. *BMC Med Res Methodol* 2008; 8: 70.

13 Hernán MA, Sauer BC, Hernández-Díaz S, Platt R, Shrier I. Specifying a target trial prevents immortal time bias and other self-inflicted injuries in observational analyses. *J Clin Epidemiol* 2016; 79: 70–5.

14 Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. *Clin Microbiol Infect* 2016; 22: 416–22.

15 Fitzpatrick JM, Biswas JS, Edgeworth JD, *et al.* Gram-negative bacteraemia; a multi-centre prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals. *Clin Microbiol Infect* 2016; 22: 244–51.

16 Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob Agents Chemother* 2010; 54: 4851–63.

17 Kumar A, Roberts D, Wood KE, *et al.* Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; 34: 1589–96.

18 Alam N, Oskam E, Stassen PM, *et al.* Prehospital antibiotics in the ambulance for sepsis: a multicentre, open label, randomised trial. *Lancet Respir Med* 2018; 6: 40–50.

19 Holmbom M, Giske CG, Fredrikson M, *et al.* 14-Year survey in a Swedish county reveals a pronounced in-

crease in bloodstream infections (BSI). Comorbidity - An independent risk factor for both BSI and mortality. *PLoS One* 2016; 11: 1–16.
20 Leal HF, Azevedo J, Silva GEO, *et al.* Bloodstream infections caused by multidrug-resistant gram-negative bacteria: Epidemiological, clinical and microbiological features. *BMC Infect Dis* 2019; 19: 1–11.
21 Gutiérrez-Gutiérrez B, Salamanca E, de Cueto M, *et al.* Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study. *Lancet Infect Dis* 2017; 17: 726–34.
22 Bradford MA, Lindenauer PK, Wiener RS, Walkey AJ. Do-not-resuscitate status and observational comparative effectiveness research in patients with septic shock*. *Crit Care Med* 2014; 42: 2042–7.
23 Mulder T, van Werkhoven CH, Huijts SM, Bonten MJM, Postma DF, Oosterheert JJ. Treatment restrictions and empirical antibiotic treatment of community-acquired pneumonia in elderly patients. *Neth J Med* 2016; 74: 56.
24 Lin KH, Chen YS, Chou NK, Huang SJ, Wu CC, Chen YY. The associations between the religious background, social supports, and do-not-resuscitate orders in Taiwan: An observational study. *Med (United States)* 2016; 95: 1–6.
25 Saeed F, Kousar N, Aleem S, *et al.* End-of-Life Care Beliefs Among Muslim Physicians. *Am J Hosp Palliat Med* 2015; 32: 388–92.
26 Neill JO'. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations The Review on Antimicrobial Resistance Chaired. 2014.
27 de Kraker MEA, Stewardson AJ, Harbarth S. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? *PLoS Med* 2016; 13: 1–6.
28 Harbarth S, Balkhy HH, Goossens H, *et al.* Antimicrobial resistance: One world, one fight! *Antimicrob Resist Infect Control* 2015; 4: 1–15.
29 Stichting Werkgroep Antibioticabeleid. SWAB guidelines for Antibacterial therapy of adult patients with Sepsis. 2010.
30 Rottier WC, Bamberg YRP, Dorigo-Zetsma JW, Van Der Linden PD, Ammerlaan HSM, Bonten MJM. Predictive value of prior colonization and antibiotic use for third-generation cephalosporin-resistant enterobacteriaceae bacteremia in patients with sepsis. *Clin Infect Dis* 2015; 60: 1622–30.
31 Goodman KE, Lessler J, Cosgrove SE, *et al.* A Clinical Decision Tree to Predict Whether a Bacteremic Patient Is Infected With an Extended-Spectrum ß-Lactamase-Producing Organism. *Clin Infect Dis* 2016; 63: 896–903.
32 Tumbarello M, Trecarichi EM, Bassetti M, *et al.* Identifying patients harboring extended-spectrum-ß-lactamase-producing Enterobacteriaceae on hospital admission: Derivation and validation of a scoring system. *Antimicrob Agents Chemother* 2011; 55: 3485–90.
33 Sousa A, Pérez-Rodríguez MT, Suarez M, *et al.* Validation of a clinical decision tree to predict if a patient has a bacteraemia due to a ß-lactamase producing organism. *Infect Dis (Auckl)* 2019; 51: 32–7.
34 Lijmer JG, Mol BW, Heisterkamp S, *et al.* Empirical evidence of design-related bias in studies of diagnostic tests. *JAMA* 1999; 282: 1061–6.
35 Damen JAAG, Hooft L, Schuit E, *et al.* Prediction models for cardiovascular disease risk in the general population: Systematic review. *BMJ* 2016; 353. DOI:10.1136/bmj.i2416.
36 Plüddemann A, Wallace E, Bankhead C, *et al.* Clinical prediction rules in practice: Review of clinical guidelines and survey of GPs. *Br J Gen Pract* 2014; 64: 233–42.
37 Collins GS, de Groot J a, Dutton S, *et al.* External validation of multivariable prediction models: a systematic review of methodological conduct and reporting. *BMC Med Res Methodol* 2014; 14: 40.
38 Reilly BM, Evans AT. Translating clinical research into clinical practice: impact of using prediction rules to make decisions. *Ann Intern Med* 2006; 144: 201–9.
39 Wells PS, Anderson DR, Rodger M, *et al.* Derivation of a simple clinical model to categorize patients probability of pulmonary embolism: Increasing the models utility with the SimpliRED D-dimer. *Thromb Haemost* 2000; 83: 416–20.
40 Hembroff LA, Holmes-Rovner M, Wills CE. Treatment decision-making and the form of risk communication: Results of a factorial survey. *BMC Med Inform Decis Mak* 2004; 4: 1–9.
41 Freeman ALJ. How to communicate evidence to patients. *Drug Ther Bull* 2019; 57: 119–24.

42 Paul M LL. Cochrane Database of Systematic Reviews Beta lactam antibiotic monotherapy versus beta lactam- aminoglycoside antibiotic combination therapy for sepsis (Review). *Cochrane Database Syst Rev* 2014. DOI:10.1002/14651858.CD003344.pub3.

43 Ong DSY, Frencken JF, Klouwenberg PMCK, *et al.* Short-Course Adjunctive Gentamicin as Empirical Therapy in Patients With Severe Sepsis and Septic Shock : A Prospective Observational Cohort Study. 2017; 64: 1731–6.

44 van Kasteren ME, Stobberingh EE, Janknegt R, Wijnands WJ, van der Meer JW. [Optimizing the antibiotics policy in the Netherlands. IV. SWAB- guidelines for antimicrobial therapy of adults with sepsis in hospitals. Foundation Antibiotics Policy Work Group]. *Ned Tijdschr Geneeskd* 1999; 143: 611–7.

45 Harris PNA, Tambyah PA, Lye DC, *et al.* Effect of Piperacillin-Tazobactam vs Meropenem on 30-Day Mortality for Patients With E coli or Klebsiella pneumoniae Bloodstream Infection and Ceftriaxone Resistance: A Randomized Clinical Trial. *JAMA* 2018; 320: 984–94.

46 Gutiérrez-Gutiérrez B, Pérez-Galera S, Salamanca E, *et al.* A Multinational, Preregistered Cohort Study of ß-Lactam/ß-Lactamase Inhibitor Combinations for Treatment of Bloodstream Infections Due to Extended-Spectrum-ß-Lactamase-Producing Enterobacteriaceae. *Antimicrob Agents Chemother* 2016; 60: 4159–69.

47 Gandra S, Tseng KK, Arora A, *et al.* The Mortality Burden of Multidrug-resistant Pathogens in India: A Retrospective, Observational Study. *Clin Infect Dis* 2019; 69: 563–70.

48 Planta MB. The role of poverty in antimicrobial resistance. *J Am Board Fam Med* 2007; 20: 533–9.

49 Bürgmann H, Frigon D, Gaze WH, *et al.* Water and sanitation: An essential battlefront in the war on antimicrobial resistance. *FEMS Microbiol Ecol* 2018; 94: 1–14.

50 Collignon P, Beggs JJ, Walsh TR, Gandra S, Laxminarayan R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planet Heal* 2018; 2: e398–405.

51 Collignon P, Athukorala PC, Senanayake S, Khan F. Antimicrobial resistance: The major contribution of poor governance and corruption to this growing problem. *PLoS One* 2015; 10: 1–13.

CEPHALOSPORINS CARBAPENEMASE DISEASE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL MORTALITY DISEASE BURDEN GRAM-NEGATIVE ESBL GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET PROPENSITY SCORE INFECTION COHORT METHODS GRAND-ABC BURDEN CEPHALOSPORINS BACTERIA BLOODSTREAM INFECTION MORTALITY PREDICTION GRAM-NEGATIVE INFECTION PREDICT ANTIBIOTIC CEPHALOSPORINS CARBAPENEMASE DISEASE TRIAL MORTALITY ANTIBIOTIC PREDICT BLOODSTREAM GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM-NEGATIVE ANTIBIOTIC RESISTANCE MORTALITY ESBL ANTIBIOTIC DISEASE APPENDIX CAUSAL INFERENCE MORTALITY GRAM-NEGATIVE BACTERIA PREDICTION GRAND-ABC INFECTION COHORT STUDY GRAND-ABC BACTERIA PREDICTION CAUSAL INFERENCE TARGET BLOODSTREAM INFECTION DISEASE BURDEN GRAM

Nederlandse samenvatting

J.W. T. Deelen

Gram-negatieve infecties – infecties veroorzaakt door Gram-negatieve bacteriën, zijn een groeiende bron van zorg. Deze bacteriën zijn doorgaans onderdeel van de normale darmflora, maar kunnen in vatbare patiënten tot infecties leiden. Bij patiënten in ziekenhuizen hebben Gram-negatieve infecties een grote verscheidenheid aan uitingsvormen. Het kan gaan om een simpele urineweginfectie – zoals vele vrouwen jaarlijks ook buiten het ziekenhuis meemaken, tot zeer ernstige infecties met bacteriën in het bloed (bacteriëmie) waarbij er een reële kans op overlijden is. In principe kan ieder orgaansysteem getroffen worden door een dergelijke infectie.

Zowel de toename van het aantal infecties in een ouder wordende populatie met een toenemende hoeveelheid chronische aandoeningen, als de toename van antibioticaresistentie in deze pathogenen zijn problematisch. Antibioticaresistentie is de laatste jaar veelvuldig in het nieuws geweest, met nieuws over superbugs en onbehandelbare infecties. In de praktijk lijkt het in Nederland gelukkig niet zo ver, maar de groei en verspreiding van resistente Gram-negatieve bacteriën zou een slechte uitwerking kunnen hebben op patiënten. Antibioticaresistentie is niet een binair gegeven. Er zijn verschillende soorten antibiotica, en bacteriën kunnen gevoelig of resistent zijn tegen verscheidene soorten. In Nederland hebben wij de gewoonte om Gram-negatieve bacteriën te beschouwen als Bijzonder Resistent Microorganisme (BRMO) op het moment dat een bacterie, zoals bijvoorbeeld de *Escherichia coli,* resistent is voor een van onze meest gebruikte antibioticasoorten die we hebben: de cefalosporines.

Omtrent Gram-negatieve infecties in opgenomen patiënten zijn nog veel open vragen. In dit proefschrift spelen er twee grote thema's. De eerste vraag betreft de ziektelast: hoeveel van deze infecties vinden er plaats, en hoeveel mensen overlijden hierdoor? Deze vraag is essentieel: informatie hierover geeft ons (zorgverleners, beleidsmakers, patiënten) een idee van hoe belangrijk het is. Als zeer concreet voorbeeld: deze samenvatting is door de auteur in zijn pyjama geschreven temidden de Coronavirus-lockdown. Op het moment dat dit virus qua ziekte-ernst vergelijkbaar zou zijn geweest met een gewone verkoudheid zouden deze drastische maatregelen onnodig zijn geweest: informatie over de omvang van het probleem drijft dus beleidskeuzes. In hoofdstuk 2 proberen we een antwoord te geven op hoeveel infecties er plaatsvinden door Gram-negatieve infecties en hoeveel sterfgevallen er op jaarbasis optreden. Deze vraag over ziektelast speelt eveneens voor antibioticaresistente infecties, het onderwerp van hoofdstuk 3. In dit hoofdstuk probeerden we vast te stellen hoeveel extra sterfte er in Nederland optreedt door de hiervoor genoemde Gram-negatieve BRMO-infecties. Omdat er voor antibioticaresistentie interventies mogelijk zijn om dit te voorkomen – zoals isolatie van patiënten, contactbescherming van zorgverleners, ander antibioticabeleid – is kennis over de sterfte belangrijk om een inschatting te maken welke van deze interventies nodig zijn.

Hoewel onderzoek naar sterfgevallen vrij eenvoudig klinkt – je kunt immers gewoon tellen – is de praktijk weerbarstiger. Een van de problemen omtrent het vaststellen van het aantal

doden door resistente infecties betreft 'confounding'. Er kunnen andere factoren een rol spelen die zowel de kans groter maken dat iemand een resistente infectie krijgt en de kans verhoogt op sterfte. Als je hier geen rekening mee houdt, schrijf je een dergelijk sterfgeval onterecht aan de resistentie toe. Naast confounding zijn er nog vele andere methodologische problemen bij het vaststellen een effect van antibioticaresistentie op sterfte. In hoofdstuk 4 vatte ik recente literatuur over sterfgevallen door antibioticaresistentie samen en analyseerde ik waar dit qua methodes goed ging en waar er ruimte was voor verbetering.

Het tweede deel van dit proefschrift draait om de behandeling van Gram-negatieve infecties. Een van de belangrijkste problemen is dat wanneer een zieke patiënt behandeld moet worden vanwege een verdenking op een infectie, het onbekend is door welke bacterie hij of zij ziek is geworden. Dit komt omdat kweekuitslagen en resistentiebepalingen 24 tot 48 uur duren. In Nederland – waar antibioticaresistentie relatief zeldzaam is – worden resistente bacteriën niet gedekt door het antibioticum dat doorgaans gegeven wordt. Het risico van iedereen het middel geven dat wèl effectief is, is dat er resistentie tegen dat (laatste red)middel ontstaat, waardoor behandeling van dergelijke infecties helemaal onmogelijk wordt. Dit betekent dat patiënten met een resistente infectie de eerste twee dagen niet adequaat behandeld worden door de gegeven antibiotica – met een mogelijk hoger risico op overlijden tot gevolg. Een manier om dit probleem op te lossen is om te kijken of we op basis van bepaalde risicofactoren kunnen voorspelen welke patiënten er ziek worden door een resistente bacterie. In hoofdstuk 5 kijken we naar de effectiviteit en bruikbaarheid van twee voorspelregels voor resistentie tegen derde generatie cefalosporines (in de praktijk wordt dit resistentiemechanisme ESBL genoemd, al is niet ieder geval van dergelijke resistentie een ESBL) in Gram-negatieve bacteriën.

In hoofdstuk 6 proberen we antwoord te geven op weer een andere vraag omtrent de behandeling van Gram-negatieve bacteriëmieën. In de Nederlandse sepsis-richtlijnen wordt geadviseerd bij risico op een ESBL-infectie kortdurend een aminoglycoside bij te starten. Er zijn echter tekenen dat deze strategie mogelijk nierschade en slechtere overleving geeft bij patiënten op de IC. In hoofdstuk 6 kijken we in hoeverre dit speelt bij een algemene ziekenhuispopulatie met een Gram-negatieve bacteriëmie.

In **Hoofdstuk 2** hebben we proberen vast te stellen hoeveel Gram-negatieve infecties er jaarlijks in Nederland optreden, en hoeveel mensen er overlijden in de dertig dagen na het optreden van een infectie. Dit hebben we gedaan door middel van de GRAND-ABC-studie, een onderzoek waar in 8 ziekenhuizen in Nederland gedetailleerde informatie is verzameld over 1954 opgenomen patiënten met een Gram-negatieve infectie. Deze informatie is vervolgens gecombineerd met de nationale microbiologische surveillance-database van het RIVM – ISIS-AR. In deze database zijn zo'n 60 tot 75% van de microbiologische laboratoria aangesloten, wat betekent dat tussen 60 en 75% van alle afgenomen kweken in Nederland in deze database staat. Omdat niet elke kweek een nieuwe infectie vertegenwoordigt, hebben we met de GRAND-ABC-database gekeken wat de verhouding is tussen aantal kweken en nieuwe infecties. Verdere gegevens over sterfte en resistentie konden we ook uit de GRAND-

ABC-studie halen. Uiteindelijk vonden we dat er op jaarbasis in 2016 in Nederland 19544 Gram-negatieve infecties zijn in opgenomen patiënten, waarvan er in 9645 gevallen sprake is van een bacteriëmie. Bij deze 19544 infecties overlijden er naar schatting 2198 mensen in de dertig dagen na het optreden van de infectie (wat iets anders is dan het moeilijker vast te stellen overlijden dóór de infectie). Dit getal is vergelijkbaar met het aantal mensen dat jaarlijks in Nederland overlijdt aan suikerziekte. Deze getallen maken dat Gram-negatieve infecties een belangrijk probleem zijn in de Nederlandse gezondheidszorg, en dat strategieën om het aantal infecties te verminderen nuttig kunnen zijn om de ziektelast omlaag te brengen.

In **Hoofdstuk 3** hebben we dezelfde GRAND-ABC-studie gebruikt om antwoord te geven op de vraag of infecties met resistente Gram-negatieve bacteriën zorgen voor een hogere sterfte dan die met niet-resistente Gram-negatieve bacteriën. Dit hebben we gedaan door van 2000 patiënten met een Gram-negatieve infectie en 2000 patiënten zonder een infectie gegevens te verzamelen in 8 ziekenhuizen in Nederland. Van de 2000 patiënten met een Gram-negatieve infectie hadden er 254 (12%) een resistente infectie met een BRMO, waarvan 75% bestond uit derde-generatiecefalosporineresistente bacteriën. Door de BRMO-infecties te vergelijken met de niet-resistente infecties, en alle infecties te vergelijken met niet-geïnfecteerde patiënten (een zogeheten parallel matched cohort design), hebben we een schatting kunnen maken van het sterfgevallen dat te wijten valt aan antibioticaresistentie. Hierbij hebben we ook zoveel mogelijk rekening gehouden met andere mogelijke factoren (confounders), die de resultaten zouden kunnen vertekenen. Tegen alle verwachtingen in bleek antibioticaresistentie niet tot extra sterfte te zorgen in Nederland, ondanks een hoger risico op verkeerde initiële behandeling. Andere studies over dit onderwerp laten veelal wèl een hogere sterfte zien door antibioticaresistente infecties van dit type. Mogelijke verklaringen hiervoor zijn het minder dan ideale onderzoeksdesign dat is gebruikt in andere onderzoeken en de snelle turn-aroundtijd voor kweekresultaten in Nederland (in vergelijking met andere landen).

De methodologische aspecten van het vaststellen van de sterfte door antibioticaresistentie is verder uitgediept in **Hoofdstuk 4**. In deze studie hebben we de recente literatuur over sterfte door carbapenemresistente Gram-negatieve bacteriën samengevat en daarbij specifiek gelet op methodologische aspecten. Hierbij waren in meerdere gevallen problemen met het onderzoeksdesign, statistische analyse en de rapportage van de bevindingen. Qua onderzoeksdesign was er in veel gevallen sprake van een kleine studie, waarbij niet werd uitgelegd waarom bepaalde variabelen van belang zouden zijn om te meten. Wat betreft statistische analyse, werd in 5/13 onderzoeken gecorrigeerd voor verkeerde initiële therapie. Door hiervoor te corrigeren beschouw je dit als iets dat onafhankelijk van antibioticaresistentie sterfte kan veroorzaken, terwijl het juist een direct gevolg is. Hiervoor corrigeren is dus per definitie onjuist. Andere problemen waren gebrekkige informatie over missende patiënten, geen beschrijving van mogelijke informatiebias en selectiebias en onduidelijkheid over wanneer bepaalde variabelen (bijvoorbeeld ziekte-ernst) gemeten waren. Dit alles kan leiden

tot een sterke vertekening van de resultaten.

Bij het toepassen van de statistische modellen die andere onderzoekers hadden gemaakt op twee datasets (waaronder de GRAND-ABC-dataset uit hoofdstuk 2 en 3), vonden we dat het effect van resistentie op sterfte kon verschillen met 38% minder tot 39% meer sterfte in de GRAND-ABC-dataset en 26% minder tot 14% meer in de andere dataset, afhankelijk van het gebruikte statistische model. Dit staat nog los van alle bias die veroorzaakt zou kunnen zijn door incorrect om te gaan met missende gegevens en patiënten waarvan de uitkomst niet bekend is.

In **Hoofdstuk 5** gaan we naar deel 2 van het proefschrift, en focussen we op het voorspellen van resistente infecties. In deze studie hebben we twee voorspelregels – een voor patiënten uit de community die opgenomen worden in het ziekenhuis (de community-onsetregel), een voor mensen die al opgenomen liggen in het ziekenhuis en dan een infectie krijgen (hospital-onsetregel) gevalideerd in een internationale patiëntpopulatie. Deze regels waren eerder ontworpen op basis van een Nederlandse patiëntpopulatie – en deze validatie zou duidelijk maken of deze echt zou werken. Bij dit project waren 33 ziekenhuizen in 13 landen betrokken, en in al deze ziekenhuizen zijn data verzameld over patiënten bij wie er een verdenking was op een bacteriëmie en die intraveneus met antibiotica behandeld moesten worden. In totaal waren ruim 6500 patiënten geïncludeerd in deze studie. De belangrijkste vondst was dat de community-onsetregel goed kan voorspellen of iemand ziek is geworden door een resistente bacterie, en vooral goed kan voorspellen wanneer iemand juist niet ziek is door een resistente bacterie. Met deze voorspelregel kan onnodig carbapenemgebruik – een antibioticum dat we het liefst zo min mogelijk gebruiken – worden gereduceerd met ongeveer 28%. Interessant hieraan is dat patiënten in de community internationaal dusdanig weinig verschillen dat deze Nederlandse voorspelregel goed te gebruiken is. De hospital-onsetregel werkte helaas minder goed, hier spelen waarschijnlijk internationaal grote verschillen tussen ziekenhuizen een grote rol. De volgende stap is de implementatie van de community-onsetregel zodat deze vermindering in onnodig antibioticagebruik daadwerkelijk bereikt kan worden.

In het laatste hoofdstuk, **Hoofdstuk 6**, analyseren we de gevolgen van een veelgebruikte behandelstrategie voor infecties in Nederland. In de behandelrichtlijnen voor sepsis staat al lang de optie om eenmalig aminoglycosides toe te voegen aan de al gegeven cefalosporines als er een verhoogd risico is op een infectie met een resistente bacterie (in het specifiek ESBL). In de praktijk wordt in sommige ziekenhuizen aan alle patiënten met verdenking op sepsis een aminoglycoside gegeven, en in andere ziekenhuizen weer helemaal niet. Dit is een probleem, omdat aminoglycosides antibiotica zijn met mogelijk aanzienlijke bijwerkingen: het kan voor oor- en nierschade zorgen. Dit beleid van eenmalig aminoglycosides is niet eerder uitgebreid onderzocht. Wij vonden dat het toevoegen van aminoglycosides mogelijk leidt tot een wat verhoogd risico op sterfte bij patiënten met een Gram-negatieve bacteriëmie, ondanks dat het het risico op inadequate empirische therapie verlaagt. Het feit dat deze strategie nog veel gebruikt is en er veel 'believers' zijn maakt dat het belangrijk is om in een gerandomiseerde

trial uit te zoeken wat de beste behandeling is.

Conclusies

In dit proefschrift zijn verschillende aspecten van Gram-negatieve infecties besproken. Gram-negatieve infecties zijn een belangrijke bron van sterfte, en het is daarmee relevant om in te zetten op preventie. Deze ziektelast bestaat ondanks het feit dat er in Nederland weinig resistentie is, waarbij de resistentie zelf ook geen extra sterfte veroorzaakt. Het gebruik van voorspelregels voor patiënten met verdenking op een bacteriëmie zou het zou onnodig carbapenemgebruik kunnen reduceren. Kortdurend aminoglycosidegebruik bij patiënten met sespis heeft mogelijke negatieve gevolgen voor patiënten, en we moeten dit op basis van de kennis uit dit proefschrift uitzoeken door middel van een gerandomiseerde trial.

Alhoewel het gunstig is dat in Nederland de ziektelast van resistentie laag is, moeten we ons best doen dat zo te houden. Tegelijkertijd bestaat er een dreiging vanuit het buitenland, waar resistentie tegen carbapenems – hier nog beschouwd als laatste redmiddel – schering en inslag is. In een toenemend globaliserende wereld is het zaak om hier de resistentie zo beperkt mogelijk te houden, en samen te blijven werken met andere landen om dit probleem internationaal te bestrijden.

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Dankwoord

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