KEEPING ANTIBIOTIC RESISTANCE AT BAY

Hospital-based surveillance of antibiotic-resistant Gram-negative bacteria

Denise van Hout

Keeping antibiotic resistance at bay:

Hospital-based surveillance of

antibiotic-resistant Gram-negative bacteria

Denise van Hout

Keeping antibiotic resistance at bay: hospital-based surveillance of antibiotic-resistant Gram-negative bacteria
PhD dissertation, Utrecht University, the Netherlands

ISBN:	9789463807678
Cover image	Graphic 211X by Mareike Böhmer
Layout:	Denise van Hout
Printing:	ProefschriftMaken | www.proefschriftmaken.nl

Financial support by the Julius Center for Health Sciences and Primary Care for the printing of this thesis is gratefully acknowledged.

Keeping antibiotic resistance at bay

Hospital-based surveillance of antibiotic-resistant Gram-negative bacteria

Antibioticaresistentie tegengaan: ziekenhuissurveillance van antibioticaresistente Gram-negatieve bacteriën

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op

dinsdag 19 mei 2020
des middags te 12:30 uur

door

Denise van Hout

geboren op 1 december 1989
te Nijmegen

Promotoren:	Prof. dr. M.J.M. Bonten Prof. dr. J.A.J.W. Kluytmans
Copromotor:	Dr. P.C.J.L. Bruijning-Verhagen

hakuna matata

INDEX

1

General introduction

Denise van Hout

ANTIBIOTIC RESISTANCE

The human body is inhabited by trillions of micro-organisms, consisting of numerous different bacterial species. [1] These bacteria live at different places in and on the human body, including the skin, nose, mouth and intestinal tract. We commonly refer to this innocent bacterial residency as 'colonisation'. During evolution, the human host and its colonising bacteria have developed complex relationships with each other and both rely on these interactions for growth and fitness. The large majority of these bacteria reside in the human intestinal tract and there, along with other micro-organisms, constitute the gut microbiota. The gut microbiota not only protects the human body from invasion by pathogenic bacteria but also serves important metabolic processes, including nutrient absorption, fermentation of complex carbohydrates and the production of vitamins. [2] However, this symbiosis and intricate ecosystem of the human host and its colonising bacteria is sometimes disrupted. When that happens, bacteria that usually reside innocently in the gut can become invasive and cause infections. The underlying mechanism is often multi-factorial and can include a disturbed balance of the gut microbiota composition (for example after a course of broad-spectrum antibiotics), decreased functioning of the host immune system or breach of physical barriers as a result of a surgical procedure. There are differences in the potential of different bacterial species to cause infection, the so called pathogenic potential. In the large intestine, anaerobic bacteria – bacteria that grow in the absence of oxygen – by far outnumber other bacteria, including the facultative anaerobic bacterial family of Enterobacterales. Still, infections by these anaerobic bacteria, even though they do occur and can have severe consequences, are rare. Certain Enterobacterales species however, like *Escherichia coli* or *Klebsiella pneumoniae*, are frequently encountered pathogens in human infections. This pathogenic potential of colonizing bacteria is why certain preventive antibiotic regimens were developed with the aim to eradicate colonisation with these bacteria in vulnerable patients. Two examples are selective decontamination of the digestive tract (SDD) and selective oropharyngeal decontamination (SOD). These regimens contain topical antibiotics to decolonise the oropharynx (SOD and SDD) and/or gut (SDD only) from Enterobacterales and other Gram-negative bacteria (GNB), *Staphylococcus aureus* and yeasts, in order to prevent infections and thereby mortality in patients that are critically ill. [3–5] There is accumulating evidence that in Dutch intensive care unit (ICU) patients SDD is associated with better patient outcome than SOD. However, the SDD regimen is also more elaborate and expensive, causing uncertainty about which regimen should be preferred in terms of balance between costs and effects. [3–7]

Acquired antibiotic resistance is the ability of a bacterium to survive and multiply in the presence of an antibiotic agent that would normally inhibit growth or kill this type of bacterium. Antibiotic resistance can develop spontaneously, as a result of random mutations in the genetic material. If then selective pressure is applied by use of antibiotics, only the fittest bacteria will survive and

multiply, leading to growth and multiplication of the antibiotic-resistant clones. During bacterial reproduction the antibiotic resistance genes can be transferred directly to the bacterium's 'offspring', called vertical gene transfer. Another mechanism in bacteria to acquire antibiotic resistance is through horizontal gene transfer. Many antibiotic resistance genes are carried on plasmids. Plasmids are mobile genetic elements that can be exchanged between different bacteria, even between bacteria of different species. An important example of plasmid-mediated resistance is the exchange of genes encoding extended-spectrum beta-lactamases (ESBLs), enzymes that are able to hydrolyse commonly used antibiotics such as penicillins and third-generation cephalosporins (i.e. cefotaxime, ceftriaxone and ceftazidime). [8]

For an individual person it is not immediately dangerous if bacteria that colonise the gut become resistant to a certain antibiotic. However, an ongoing increase in the spread and occurrence of bacteria that are resistant to multiple antibiotics, so called multidrug-resistant micro-organisms (MDRO), is a worrisome trend from a public-health perspective for two main reasons. First, dissemination of MDRO threatens the effectiveness of empirical antibiotic therapy; antibiotics that are given to patients suspected of infection before causative pathogen and antimicrobial susceptibility is known. Over time, this can lead to broadening of empirical antibiotic therapy regimens – which may drive the use of last-resort antibiotics such as carbapenems – in turn further stimulating resistance development. Secondly and perhaps most importantly, antibiotic treatment options for patients that are infected by MDRO are limited, jeopardizing cure of infectious diseases that cause considerable morbidity and mortality.

HOSPITAL-BASED SURVEILLANCE OF ANTIBIOTIC RESISTANCE

An important tool to monitor and control antibiotic resistance – and to keep resistance at bay – is surveillance. Surveillance is defined as the ongoing systematic collection, analysis and interpretation of health data, closely integrated with the timely dissemination of these data to those who need to know. [9] Surveillance can track temporal changes in bacterial populations, allows the early detection of relevant antibiotic-resistant strains and supports timely notification and investigation of outbreaks. [10] It also supports prompt and effective implementation of control measures and at the same time can measure their effect. Surveillance from the hospital perspective is of particular importance because the hospital is an important reservoir for MDRO. In this setting, antibiotic pressure (hence, selective pressure) is continuously high and cross-transmission of antibiotic-resistant strains between patients may occur. Furthermore, patients that require admission to the hospital comprise a vulnerable population compared to the overall general population, and are therefore at risk for poor health outcomes.

Hospital surveillance data can be applied to direct medical care, for example when designing regional guidelines on empirical therapy regimens. In the Netherlands, national surveillance data were used to evaluate the coverage of different antibiotic treatments for complicated urinary tract infections in hospitalised patients, and supported new recommendations for empirical treatment. [11] As mentioned before, surveillance findings can also be used to measure the impact of different infection prevention and control programs and can inform policy makers when allocating resources for preventive interventions. For example, local surveillance data were successfully used in a Dutch before-after single-centre study that investigated the effect of implementing routine pre-operative oral antibiotics on the incidence of surgical site infections after colorectal surgery. [12] On a national scale, routine antibiotic susceptibility testing data are collected for the Dutch surveillance system called the 'Infectious Diseases Surveillance Information System for Antimicrobial Resistance' (ISIS-AR). [13] Among others, these surveillance data have been used to investigate time trends in antibiotic resistance levels in hospitals where selective decontamination was routinely applied versus hospitals where this was not the case [14,15], to observe national trends in extended-spectrum cephalosporin-resistant *E. coli* and *K. pneumoniae* [16] and to track the epidemiology of carbapenemase-producing Enterobacterales. [17]

There are many different methods by which hospital-based surveillance can be approached. These can be broadly categorized into the following: (a) prospective versus retrospective surveillance, (b) detection of colonisation versus detection of (healthcare-associated) infections and (c) patient-based versus laboratory-based surveillance. [18] In this thesis, a collection of projects was performed that aimed to improve prospective patient-based detection of MDRO colonisation, prospective laboratory detection of MDRO and surveillance of circulating bacterial populations in colonisation and infection. We also investigated the cost-effectiveness of two decolonisation strategies in the ICU.

DETECTION OF COLONISATION WITH MULTIDRUG-RESISTANT ORGANISMS (MDRO)

Detection of colistin resistance during selective digestive decontamination (SDD)

When antibiotics are routinely administered to patients for therapeutic purposes or as part of an infection prevention protocol, monitoring antibiotic resistance development is crucial. SDD is an example of such a preventive antibiotic regimen. The SDD regimen consists of, among others, a gastro-intestinal suspension of the topical antibiotics tobramycin and colistin and is recommended in the Netherlands for all ICU patients with an expected length of stay in the ICU of >48 hours. [19] To monitor the occurrence of antibiotic resistance in ICU patients

treated with SDD, respiratory and rectal swabs are obtained upon ICU admission and twice weekly thereafter until ICU discharge. These swabs are cultured on non-selective media and resistance patterns of all bacterial isolates that are found are determined. Monitoring resistance to colistin is of particular importance, as colistin is increasingly regarded as a potential last-resort antibiotic against infections with multidrug-resistant GNB. [20] Unfortunately, laboratory testing for phenotypic colistin resistance is problematic. [21–23] Underlying reasons are diverse. These include, among others, poor diffusion of polymyxins (i.e. colistin) into agar and frequent occurrence of heteroresistance to polymyxins in certain Gram-negative species – presence of sub populations with different antimicrobial susceptibilities within a single bacterial isolate. [24–27] At present, the only method considered appropriate for susceptibility testing is manual broth microdilution (BMD). [21,23] However, BMD is laborious, prone to manual errors and therefore not suitable for routine implementation in most clinical microbiology laboratories. In particular as part of the SDD surveillance protocol, a large number of samples is processed daily by the medical microbiology laboratory and these samples are currently not subjected to colistin BMD. The use of a selective medium, such as the SuperPolymyxin™ medium (ELITech Group, Puteaux, France), has been suggested as a screening tool for colistin resistance in case of large numbers of patient samples. [28–32] In principle, only colistin-resistant bacteria will be able to grow on this medium, facilitating reliable and easier detection of phenotypic colistin-resistant isolates. Nonetheless, a recent study reported a relatively high proportion of colistin-susceptible isolates growing on the SuperPolymyxin™ medium. [33] It is currently unknown whether this method should be added to the laboratory pipeline of microbiological SDD surveillance, for example as a screening medium, in order to facilitate better detection of rectal carriage with colistin-resistant GNB.

Risk assessment for MDRO carriage upon hospital admission

SDD surveillance, as described above, is a suitable surveillance method to monitor longitudinal antibiotic resistance levels in the ICU and to screen for MDRO acquisition during ICU stay. For the detection of MDRO carriers that enter the hospital, universal or risk-based screening upon admission can be applied, a strategy that has been proposed to decrease nosocomial cross-transmission of MDRO. Universal screening applies to all patients hospitalised to the hospital or a certain ward, whereas risk-based screening is specifically aimed at identifying patients at high risk of colonisation with MDRO. If based on screening the patient is identified to be at high risk of MDRO carriage, pre-emptive contact precautions are installed and microbiological culturing is performed. The underlying rationale of this strategy is that an important part of MDRO carriage among hospitalised patients may be missed, or detection delayed, if relying on routine (i.e. clinical) cultures during hospital stay only. [34–38] In the Netherlands, screening upon admission originated in 1988 to control the emergence of methicillin-resistant *S. aureus* (MRSA). [39] It was part of the Dutch 'search and destroy' strategy for MRSA. This effective and internationally well-known strategy also included other strict infection control measures, along with a nationwide policy of restrictive antibiotic use. [40–44] In more recent years, risk-based screening upon admission

was extended and became recommended in national guidelines to also control other MDRO, such as multidrug-resistant GNB. [45,46] This infection control policy is currently mandatory for every Dutch hospital and adherence is audited by the Dutch Healthcare Inspectorate (in Dutch: 'IGZ'). Still, there is controversy around the efficiency of universal MDRO risk assessment upon hospitalisation. [47–53] Important factors are the number of patients that need to be screened to detect an MDRO carrier, the number of admissions in which pre-emptive isolation is started but discontinued after negative screening (as well as time to test results) and the proportion of detected carriers relative to the total number of hospitalised MDRO carriers. Also, its cost-effectiveness not only depends on the costs and effectiveness of the infection control policy, but also on how antibiotic-resistant bacteria impact the total burden of infections and the estimated (prevented) attributable mortality due to infections with MDRO. [54]

In our large tertiary care centre the University Medical Center Utrecht (UMCU), the risk-assessment for MDRO carriage is operationalised in a 6-point MDRO questionnaire in the electronic medical record. The questionnaire is taken from each patient that goes through pre-operative screening, visits the emergency department or is admitted to a hospital ward. A positive MDRO risk assessment (i.e. presence of ≥1 risk factor) is followed by obtainment of screening cultures (i.e. nose, rectal and/or throat swab) and instalment of pre-emptive contact precautions until culture results are known. Yet, it is currently unknown how many new MDRO carriers are detected by this strategy, that would otherwise have remained unnoticed.

SURVEILLANCE OF THE MOLECULAR EPIDEMIOLOGY OF *E. COLI*

E. coli is found in the gut of almost all humans as an intestinal commensal, but it is also the most common cause of urinary tract infections and one of the most frequently found bacteria in bloodstream infections. [55–61] Bacteraemia caused by E. *coli* (ECB) is a severe infectious disease, illustrated by a high 30-day mortality up to 18%. [62,63] In the Netherlands, less than 10% of all ECB episodes is caused by multidrug-resistant strains, but this number is increasing over time. [61,64] Another worrisome finding is that the overall annual incidence of ECB episodes is increasing across Europe, which highlights the need for novel preventive approaches such as the development of *E. coli* vaccines. [64–68] Recently, a first *E. coli* vaccine entered the clinical phase of development with promising results in a phase Ib study. [69,70]

To inform future preventive strategies as well as optimization of treatment, it is important to have a thorough insight in the current molecular epidemiology (i.e. serotypes) of bacteraemic *E. coli* strains. However, thus far, studies that investigated the molecular epidemiology of ECB

in the Netherlands were performed as relatively small single-centre studies [71] or focussed on ESBL-positive strains only. [72] Furthermore, there is limited Dutch data on the extent to which bacteraemic *E. coli* strains from patients with different epidemiological characteristics are genomically different. This combination of clinical and molecular characteristics of ECB episodes is increasingly important, because it can be used in further clinical studies investigating *E. coli* vaccines and may help to differentiate between different target populations for the prevention of infections by specific (high-risk) strains.

Molecular characterization studies have shown that *E. coli* strains mainly become resistant by the exchange of mobile genetic elements carrying resistance genes, such as genes encoding for ESBLs. These strains are often co-resistant to other classes of antibiotics. [73–75] The observed increase in antibiotic-resistant *E. coli* infections seems to be largely driven by an increase in community onset infections. [67,68] Therefore, ESBL-producing *E. coli* in the community currently serves as an important antibiotic resistance surveillance marker in the Netherlands. From 2014 to 2016, a large-scale open population study that included 8,788 participants (of which 4,177 submitted a faecal sample) was performed in the Netherlands and found a prevalence of 5% in faecal carriage of ESBL-producing *E. coli*. [76] All resistant isolates were characterised, and sequence types as well as resistance genes were described. Such studies provide very valuable insights in the current molecular epidemiology of community faecal carriage of ESBL-producing *E. coli.* Unfortunately, such studies are resource intensive and usually not regularly performed. In most Dutch hospitals, ESBL-producing *E. coli* isolates causing invasive infection are routinely stored in the microbiology laboratory. These clinical samples could potentially be a useful tool for surveillance of the molecular epidemiology of ESBL-producing *E. coli* in the community of the Netherlands. However, it is currently unknown to what extent, and on what levels, the molecular epidemiology of ESBL-producing *E. coli* isolates from routine clinical samples from primary or secondary care are comparable to those from faecal samples of the Dutch general population.

AIMS AND OUTLINE OF THIS THESIS

Continuous evaluation and optimization is a key part of hospital-based surveillance for antibiotic resistance. An important part is the assessment of balance between investigated resources and clinical yield (i.e. the cost-effectiveness) as well as the efficiency of different infection prevention strategies. This information can help clinicians and policy makers to allocate or redirect healthcare resources to where it is most beneficial or most needed, and will thereby result in optimal patient care.

Currently, in all Dutch intensive care units (ICUs) it is recommended to perform selective digestive decontamination (SDD) in patients with an expected ICU stay longer than 48 hours. However, there is uncertainty about whether the preference of SDD over selective oropharyngeal decontamination (SOD) is justified, as the SDD regimen is more extensive in terms of medication and microbiological culturing and therefore per patient-day more expensive than the SOD regimen. In **Chapter 2**, we determined the cost-effectiveness of SDD versus SOD in ICUs with low levels of antimicrobial resistance in an individual patient data meta-analysis. An important part of the SDD regimen is surveillance of antibiotic resistance. As colistin is one of the topical components of SDD, part of this surveillance is aimed at colistin resistance development. In **Chapter 3**, we determined the value of adding the selective SuperPolymyxin™ medium to the current laboratory pipeline in the detection of rectal carriage of Gram-negative bacteria with acquired colistin resistance in ICU patients receiving SDD. **Chapter 4** describes an observational study that evaluated the yield and efficiency of the current risk assessment upon hospital admission for the detection of MDRO carriage.

Chapter 5 describes the rationale and design of the EPIGENEC study (EPIdemiology and GENetics of *E. coli*), which was set up as a collaboration between the UMCU and the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) to optimize the current molecular surveillance of *E. coli* in the Netherlands. **Chapter 6** then focusses on the current clinical and molecular epidemiology of ESBL-negative and ESBL-positive *E. coli* causing bacteraemia in the Netherlands in patients with different clinical characteristics. In **Chapter 7**, we explored the potential value of clinical samples in future molecular surveillance of the human ESBL-positive *E. coli* reservoir, by assessing to what extent molecular characteristics of ESBL-positive *E. coli* from extra-intestinal infection are comparable to ESBL-positive *E. coli* from faecal carriage in the Dutch general population.

Finally, the most important conclusions of this thesis are summarised and discussed in **Chapter 8**, along with future perspectives.

REFERENCES

1. Gilbert, J. A. *et al.* Current understanding of the human microbiome. *Nat. Med.* **24**, 392–400 (2018).
2. Jandhyala, S. M. *et al.* Role of the normal gut microbiota. *World J. Gastroenterol.* **21**, 8787–8803 (2015).
3. 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).
4. Oostdijk, E. A. N. *et al.* Notice of Retraction and Replacement: Oostdijk *et al.* Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. JAMA. 2014;312(14):1429-1437. *JAMA* **317**, 1583–1584 (2017).
5. Plantinga, N. L. *et al.* Selective digestive and oropharyngeal decontamination in medical and surgical ICU patients: individual patient data meta-analysis. *Clin Microbiol Infect* **24**, 505–513 (2018).
6. Oostdijk EAN, de Wit GA, Bakker M, *et al.* Selective decontamination of the digestive tract and selective oropharyngeal decontamination in intensive care unit patients: a cost-effectiveness analysis. *BMJ Open* **3**, e002529 (2013).
7. Price, R., Maclennan, G. & Glen, J. Selective digestive or oropharyngeal decontamination and topical oropharyngeal chlorhexidine for prevention of death in general intensive care: systematic review and network meta-analysis. *BMJ* **348**, g2197 (2014).
8. Paterson, D. L. & Bonomo, R. A. Extended-spectrum beta-lactamases: a clinical update. *Clin. Microbiol. Rev.* **18**, 657–686 (2005).
9. Teutsch SM, Thacker SB. Planning a public health surveillance system. *Epidemiological Bulletin: Pan American Health Organization*. **16**, 1–6. (1995).
10. WHO. Antimicrobial resistance surveillance. Available at: https://www.who.int/medicines/areas/rational_use/AMR_Surveillance/en/. Accessed 1 December 2019.
11. Koningstein, M. *et al.* Recommendations for the empirical treatment of complicated urinary tract infections using surveillance data on antimicrobial resistance in the Netherlands. *PLoS One* **9**, e86634 (2014).
12. 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.* **69**, 93–99 (2019).
13. 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**, (2017).
14. Houben, A. J. M. *et al.* Selective decontamination of the oropharynx and the digestive tract, and antimicrobial resistance: a 4 year ecological study in 38 intensive care units in the Netherlands. *J. Antimicrob. Chemother.* **69**, 797–804 (2014).
15. van der Bij, A. K., Frentz, D. & Bonten, M. J. M. Gram-positive cocci in Dutch ICUs with and without selective decontamination of the oropharyngeal and digestive tract: a retrospective database analysis. *J. Antimicrob. Chemother.* **71**, 816–820 (2016).
16. Van Der Steen, M., Leenstra, T., Kluytmans, J. A. J. W. & Van Der Bij, A. K. Trends in expanded-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* among Dutch clinical isolates, from 2008 to 2012. *PLoS One* **10**, e0138088 (2015).
17. Vlek, A. L. M. *et al.* Detection and epidemiology of carbapenemase producing Enterobacteriaceae in the Netherlands in 2013-2014. *Eur. J. Clin. Microbiol. Infect. Dis.* **35**, 1089–1096 (2016).
18. CDC. CDC / NHSN Surveillance Definitions for Specific Types of Infections. *Surveill. Defin.* 2015, 1–24 (2016).
19. Stichting Werkgroep Antibioticabeleid (SWAB). SWAB Richtlijn: selectieve decontaminatie bij patiënten op de intensive care. 1–29 (2018).
20. Mendelson, M. *et al.* The One Health stewardship of colistin as an antibiotic of last resort for human health in South Africa. *Lancet Infect Dis* **18**, e288-94 (2018).
21. Poirel, L., Jayol, A. & Nordmann, P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance

Mechanisms Encoded by Plasmids or Chromosomes. *Clin. Microbiol. Rev.* **30**, 557–596 (2017).
22. Tan, T. Y. & Ng, S. Y. Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin. *Clin. Microbiol. Infect.* **13**, 541–544 (2007).
23. The European Committee on Antimicrobial Susceptibility Testing. Joint EUCAST and CLSI recommendation - Recommendations for colistin (polymyxin E) MIC testing. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf (2016). Accessed 1 December 2019.
24. Li, J. *et al.* Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **50**, 2946–2950 (2006).
25. Lo-Ten-Foe, J. R., de Smet, A. M. G. A., Diederen, B. M. W., Kluytmans, J. A. J. W. & van Keulen, P. H. J. Comparative evaluation of the VITEK 2, disk diffusion, etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* strains. *Antimicrob. Agents Chemother.* **51**, 3726–3730 (2007).
26. Jayol, A., Nordmann, P., Brink, A. & Poirel, L. Heteroresistance to colistin in *Klebsiella pneumoniae* associated with alterations in the PhoPQ regulatory system. *Antimicrob. Agents Chemother.* **59**, 2780–2784 (2015).
27. Gales, A. C., Reis, A. O. & Jones, R. N. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. *J. Clin. Microbiol.* **39**, 183–190 (2001).
28. Germ, J. *et al.* Evaluation of a novel epidemiological screening approach for detection of colistin resistant human Enterobacteriaceae isolates using a selective SuperPolymyxin medium. *J. Microbiol. Methods* **160**, 117–123 (2019).
29. Girlich, D., Naas, T. & Dortet, L. Comparison of the Superpolymyxin and ChromID Colistin R Screening Media for the Detection of Colistin-Resistant Enterobacteriaceae from Spiked Rectal Swabs. *Antimicrob. Agents Chemother.* **63**, pii: e01618-18 (2018).
30. Nordmann, P., Jayol, A. & Poirel, L. A Universal Culture Medium for Screening Polymyxin-Resistant Gram-Negative Isolates. *J. Clin. Microbiol.* **54**, 1395–1399 (2016).
31. Jayol, A., Poirel, L., Andre, C., Dubois, V. & Nordmann, P. Detection of colistin-resistant Gram-negative rods by using the SuperPolymyxin medium. *Diagn. Microbiol. Infect. Dis.* **92**, 95–101 (2018).
32. Caniaux, I., van Belkum, A., Zambardi, G., Poirel, L. & Gros, M. F. MCR: modern colistin resistance. *Eur. J. Clin. Microbiol. Infect. Dis.* **36**, 415–420 (2017).
33. Przybysz, S. M., Correa-Martinez, C., Kock, R., Becker, K. & Schaumburg, F. SuperPolymyxin medium for the screening of colistin-resistant Gram-Negative bacteria in stool samples. *Front. Microbiol.* **9**, 2809 (2018).
34. Harris, A. D. *et al.* Co-carriage rates of vancomycin-resistant Enterococcus and extended-spectrum beta-lactamase-producing bacteria among a cohort of intensive care unit patients: implications for an active surveillance program. *Infect. Control Hosp. Epidemiol.* **25**, 105–108 (2004).
35. Maragakis, L. L., Tucker, M. G., Miller, R. G., Carroll, K. C. & Perl, T. M. Incidence and prevalence of multidrug-resistant acinetobacter using targeted active surveillance cultures. *JAMA* **299**, 2513–2514 (2008).
36. Salgado, C. D. & Farr, B. M. What proportion of hospital patients colonized with methicillin-resistant *Staphylococcus aureus* are identified by clinical microbiological cultures? *Infect. Control Hosp. Epidemiol.* **27**, 116–121 (2006).
37. Ostrowsky, B. E. *et al.* Vancomycin-resistant enterococci in intensive care units: high frequency of stool carriage during a non-outbreak period. *Arch. Intern. Med.* **159**, 1467–1472 (1999).
38. Warren, D. K., Nitin, A., Hill, C., Fraser, V. J. & Kollef, M. H. Occurrence of co-colonization or co-infection with vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* in a medical intensive care unit. I*nfect. Control Hosp. Epidemiol.* **25**, 99–104 (2004).

39. Werkgroep Infectiepreventie. Beleid bij meticilline-resistente *Staphylococcus aureus*. (1988).
40. Werkgroep Infectiepreventie. WIP Richtlijn Meticilline-resistente *Staphylococcus aureus* (MRSA). (2012). Available from: https://www.rivm.nl/sites/default/files/2018-11/121205%20MRSA%20v1a%20def.pdf.
41. Vos, M. C., Ott, A. & Verbrugh, H. A. Successful search-and-destroy policy for methicillin-resistant *Staphylococcus aureus* in The Netherlands. *Journal of Clinical Microbiology* **43**, author reply 2034-5 (2005).
42. Vos, M. C. *et al.* 5 years of experience implementing a methicillin-resistant *Staphylococcus aureus* search and destroy policy at the largest university medical center in the Netherlands. *Infect. Control Hosp. Epidemiol.* **30**, 977–984 (2009).
43. van Rijen, M. M. L., Bosch, T., Heck, M. E. O. C. & Kluytmans, J. A. J. W. Methicillin-resistant *Staphylococcus aureus* epidemiology and transmission in a Dutch hospital. *J. Hosp. Infect.* **72**, 299–306 (2009).
44. Vandenbroucke-Grauls, C. M. Methicillin-resistant *Staphylococcus aureus* control in hospitals: the Dutch experience. *Infect. Control Hosp. Epidemiol.* **17**, 512–513 (1996).
45. Werkgroep Infectiepreventie. WIP Richtlijn Maatregelen tegen overdracht van bijzonder resistente micro-organismen (BRMO). (2005).
46. Werkgroep Infectiepreventie. WIP Richtlijn Bijzonder Resistente Micro-organismen (BRMO). (2013) Available from: https://www.rivm.nl/sites/default/files/2018-11/130424 BRMO.pdf.
47. Fatkenheuer, G., Hirschel, B. & Harbarth, S. Screening and isolation to control meticillin-resistant *Staphylococcus aureus*: sense, nonsense, and evidence. *Lancet* **385**, 1146–1149 (2015).
48. Kavanagh, K. T., Calderon, L. E. & Saman, D. M. Viewpoint: a response to 'Screening and isolation to control methicillin-resistant *Staphylococcus aureus*: sense, nonsense, and evidence'. *Antimicrob. Resist. Infect. Control* **4**, (2015).
49. Otter, J. A., Mutters, N. T., Tacconelli, E., Gikas, A. & Holmes, A. H. Controversies in guidelines for the control of multidrug-resistant Gram-negative bacteria in EU countries. *Clin. Microbiol. Infect.* **21**, 1057–1066 (2015).
50. Tacconelli, E. *et al.* ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. *Clin. Microbiol. Infect.* **20** Suppl 1, 1–55 (2014).
51. Harris, A. D., McGregor, J. C. & Furuno, J. P. What infection control interventions should be undertaken to control multidrug-resistant gram-negative bacteria? *Clin. Infect. Dis.* **43** Suppl 2, S57-61 (2006).
52. Cooper, B. S. *et al.* Isolation measures in the hospital management of methicillin resistant *Staphylococcus aureus* (MRSA): systematic review of the literature. *BMJ* **329**, 533 (2004).
53. Wassenberg, M. W. M. & Bonten, M. J. M. The Dutch MRSA policy can and should be different - In Dutch: Het Nederlandse MRSA-beleid kan en moet anders. *Ned. Tijdschr. Geneeskd.* **154**, A2575–A2575 (2010).
54. Wassenberg, M. W. M., de Wit, G. A., van Hout, B. A. & Bonten, M. J. M. Quantifying cost-effectiveness of controlling nosocomial spread of antibiotic-resistant bacteria: the case of MRSA. *PLoS One* **5**, e11562–e11562 (2010).
55. Foxman, B. & Brown, P. Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. *Infect. Dis. Clin. North Am.* **17**, 227–241 (2003).
56. Laupland, K. B., Gregson, D. B., Church, D. L., Ross, T. & Pitout, J. D. D. Incidence, risk factors and outcomes of *Escherichia coli* bloodstream infections in a large Canadian region. *Clin. Microbiol. Infect.* **14**, 1041–1047 (2008).
57. den Heijer, C. D. J., Donker, G. A., Maes, J. & Stobberingh, E. E. Antibiotic susceptibility of unselected uropathogenic *Escherichia coli* from female Dutch general practice patients: a comparison of two surveys with a 5 year interval. *J. Antimicrob. Chemother.* **65**, 2128–2133 (2010).
58. Buetti, N., Atkinson, A., Marschall, J. & Kronenberg, A. Incidence of bloodstream infections: a nationwide surveillance of acute care hospitals in Switzerland 2008-2014. *BMJ Open* **7**, e013665 (2017).

59. 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).
60. Campbell, N. & Reece, J. Biology. (San Francisco: Pearson Education Inc, 2002).
61. de Greeff, S. C. & Mouton, J. W. Nethmap 2019. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. (2019).
62. Abernethy, J. *et al.* Epidemiology of *Escherichia coli* bacteraemia in England: results of an enhanced sentinel surveillance programme. *J. Hosp. Infect.* **95**, 365–375 (2017).
63. 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).
64. van der Steen, M., Leenstra, T., Kluytmans, J. A. J. W. & van der Bij, A. K. Trends in Expanded-Spectrum Cephalosporin-Resistant *Escherichia coli* and *Klebsiella pneumoniae* among Dutch Clinical Isolates, from 2008 to 2012. *PLoS One* **10**, e0138088 (2015).
65. Schlackow, I. *et al.* Increasing incidence of *Escherichia coli* bacteraemia is driven by an increase in antibiotic-resistant isolates: electronic database study in Oxfordshire 1999-2011. *J Antimicrob Chemother* **67**, 1514–1524 (2012).
66. de Kraker, M. E. *et al.* The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clin. Microbiol. Infect.* **19**, 860–868 (2013).
67. Vihta, K.-D. *et al.* Trends over time in *Escherichia coli* bloodstream infections, urinary tract infections, and antibiotic susceptibilities in Oxfordshire, UK, 1998-2016: a study of electronic health records. *Lancet Infect. Dis.* **18**, 1138–1149 (2018).
68. van der Mee-Marquet, N. L. *et al.* Marked increase in incidence for bloodstream infections due to *Escherichia coli*, a side effect of previous antibiotic therapy in the elderly. *Front. Microbiol.* **6**, 646 (2015).
69. 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).
70. Huttner, A. *et al.* Safety, immunogenicity, and preliminary clinical efficacy of a vaccine against extraintestinal pathogenic *Escherichia coli* in women with a history of recurrent urinary tract infection: a randomised, single-blind, placebo-controlled phase 1b trial. *Lancet Infect. Dis.* **17**, 528–537 (2017).
71. Overdevest, I. T. M. A. *et al.* Prevalence of phylogroups and O25/ST131 in susceptible and extended-spectrum β-lactamase-producing *Escherichia coli* isolates, the Netherlands. *Clin. Microbiol. Infect.* **21**, 570.e1-e4 (2015).
72. Van Der Bij, A. K. *et al.* Clinical and molecular characteristics of extended-spectrum-β-lactamase-producing *Escherichia coli* causing bacteremia in the Rotterdam Area, Netherlands. *Antimicrob. Agents Chemother.* **55**, 3576–3578 (2011).
73. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2016. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). (2016).
74. Carattoli, A. Plasmids and the spread of resistance. *Int. J. Med. Microbiol.* **303**, 298–304 (2013).
75. Mathers, A. J., Peirano, G. & Pitout, J. D. D. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clin. Microbiol. Rev.* **28**, 565–591 (2015).
76. van den Bunt, G. *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. *Eurosurveillance.* **24**, (2019).

2

Cost-effectiveness of selective digestive decontamination (SDD) versus selective oropharyngeal decontamination (SOD) in intensive care units with low levels of antimicrobial resistance: an individual patient data meta-analysis

Denise van Hout, Nienke Plantinga, Patricia Bruijning-Verhagen, Evelien Oostdijk, Anne Marie de Smet, Ardine de Wit, Marc Bonten and Henri van Werkhoven

BMJ Open. 2019

ABSTRACT

Objective: To determine the cost-effectiveness of selective digestive decontamination (SDD) as compared to selective oropharyngeal decontamination (SOD) in intensive care units (ICUs) with low levels of antimicrobial resistance.

Design: Post-hoc analysis of a previously performed individual patient data meta-analysis of two cluster-randomized cross-over trials.

Setting: 24 ICUs in the Netherlands.

Participants: 12,952 ICU patients that were treated with ≥1 dose of SDD (n= 6,720) or SOD (n=6,232).

Interventions: SDD versus SOD.

Primary and secondary outcome measures: The incremental cost-effectiveness ratio (ICER, i.e. costs to prevent one in-hospital death) was calculated by comparing differences in direct healthcare costs and in-hospital mortality of patients treated with SDD versus SOD. A willingness-to-pay curve was plotted to reflect the probability of cost-effectiveness of SDD for a range of different values of maximum costs per prevented in-hospital death.

Results: The ICER resulting from the fixed-effect meta-analysis, adjusted for clustering and differences in baseline characteristics, showed that SDD significantly reduced in-hospital mortality (adjusted absolute risk reduction 0.0195, 95% CI 0.0050 to 0.0338) with no difference in costs (adjusted cost difference €62 in favor of SDD, 95% CI –€1079 to €935). Thus, SDD yielded significantly lower in-hospital mortality and comparable costs as compared to SOD. At a willingness-to-pay value of €33,633 per one prevented in-hospital death, SDD had a probability of 90.0% to be cost-effective as compared to SOD.

Conclusion: In Dutch ICUs, SDD has a very high probability of cost-effectiveness as compared to SOD. These data support the implementation of SDD in settings with low levels of antimicrobial resistance.

INTRODUCTION

Patients that are admitted to an intensive care unit (ICU) are prone to acquire nosocomial infections, which increase morbidity and mortality. [1-5] Besides detrimental effects on health status, ICU-acquired infections are also responsible for increased expenditure in an already costly healthcare setting, further supporting the importance of optimal prevention. [2,6-8] Selective oropharyngeal decontamination (SOD) and selective decontamination of the digestive tract (SDD) are two infection prevention strategies which aim to eradicate colonization with aerobic Gram-negative bacteria, *S. aureus* and yeasts, while leaving the anaerobic flora intact. SOD comprises oropharyngeal application of bactericidal non-absorbable antibiotics, while in SDD this is supplemented with an intestinal suspension containing the same antibiotics (both applied until ICU discharge) and intravenous application of a third-generation cephalosporin during the first 4 days of ICU admission. Both selective decontamination regimens reduced ICU-acquired bacteremia and mortality rates in ICUs with low prevalence of antimicrobial resistance. [9-14] Both strategies are cost-effective as compared to no selective decontamination and are recommended as part of standard care in Dutch ICUs. [15,16]

Evidence that SDD is more effective than SOD in preventing ICU-acquired bacteraemia and mortality is accumulating. [17-19] However, the SDD regimen includes more antibiotics and more microbiological surveillance and hence is per patient day more expensive than SOD. Therefore, we aimed to evaluate the cost-effectiveness of SDD versus SOD in ICUs with low prevalence of antimicrobial resistance, from a healthcare perspective.

METHODS

Study selection

We performed a two-stage cost-effectiveness individual patient data meta-analysis (IPD-MA). Selection of studies was performed in a previous IPD-MA that aimed to assess whether the effect of selective decontamination differed between medical and surgical ICU patients. [19] Studies were included in the current cost-effectiveness analysis (CEA) if they performed a head-to-head comparison of the clinical effectiveness of SDD and SOD and if they were performed in ICU settings with low levels of antimicrobial resistance. Studies that only included either one of these strategies and compared that with usual care were excluded. This resulted in inclusion of patient-level data from two cluster-randomized cross-over (CRXO) trials in ICU patients that were included in the previous IPD-MA. [13,18] To assess the publication of any new trials that were

published after the previous IPD-MA, the same systematic PubMed search was performed, which included synonyms for domain and determinant (performed 11 December 2018, see original manuscript for search string). [19] One new trial was identified that made a head-to-head comparison of SDD and SOD. [20] This study was excluded for the current CEA because it did not meet criteria with regard to our domain, namely ICUs with low levels of antimicrobial resistance.

Description of included studies

Details of the two studies can be found elsewhere. [13,18] In short, in the first trial, patients were included in 13 Dutch ICUs from May 2004 until July 2006. [13] Patients were eligible if they were admitted to the ICU with an expected duration of mechanical ventilation of more than 48 hours or an anticipated ICU length of stay (ICU-LOS) of more than 72 hours. Each ICU was assigned to a randomized order of 6 month periods in which standard care, SOD or SDD was applied. In the second CRXO-trial, patients were recruited in 16 Dutch ICUs from August 2009 to January 2011 and were eligible for inclusion if they had an expected ICU-LOS of at least 48 hours. [18] In this study, SOD and SDD were implemented in 12-month periods in a randomized order. In both trials, the SOD regimen consisted of 4-times daily application of an oropharyngeal paste consisting of polymyxin E or colistin, tobramycin and amphotericin B (2% concentration). In addition to the oropharyngeal paste, the SDD regimen contained 4-times daily application of 10 mL non-absorbable suspension of 100 mg polymyxin E or colistin, 80 mg tobramycin and 500 mg amphotericin B through a nasogastric tube, and intravenous (IV) application of a third generation cephalosporin (cefotaxime 1000 mg 4 times daily or ceftriaxone 2000 mg once daily) during the first four days of ICU-admission. Furthermore, twice weekly microbiological surveillance for colonization with Gram-negative bacteria of the respiratory tract (SOD and SDD) and rectum (SDD) was performed. In the first study, individual informed consent was obtained for data collection, whereas in the second study, the requirement for individual informed consent was waived by the institutional review boards. [13,18] As with the previous IPD-MA, we included only the first ICU-admission of a patient within each hospital admission (further referred to as patients), from patients that received at least one dose of SOD or SDD. [19]

Patient and public involvement statement

Patients were not involved in the design and conduct of the current cost-effectiveness analysis.

Cost-effectiveness analysis

For the design and reporting of the CEA, the CHEERS reporting guidelines for health-economic evaluations were followed. [21] The CEA was performed from a healthcare perspective considering only direct costs that reflect healthcare expenditure and the time horizon of the CEA was defined as the time from study inclusion on the ICU until hospital discharge or in-hospital death.

SDD was considered the intervention and SOD the control treatment.

Measures of costs and effectiveness

Total healthcare costs were determined by multiplying healthcare resources used with corresponding unit costs (Table 1). The following healthcare resources were included: number of days in the ICU, number of days on the hospital ward after the index ICU-admission, study medication and microbiological investigations during ICU stay. For the latter we considered both surveillance and clinical samples from the respiratory tract, intestinal tract and blood. Costs for ICU-LOS, microbiology and study medication were counted from study inclusion until ICU discharge. Dutch guidelines for health economic evaluation were used to determine costs for days in the ICU and on the ward and included costs for storage, overhead and equipment. [22] For microbiological cultures, national reimbursement rates as advised by the Dutch Healthcare Authority were used which included overhead costs [23], whereas costs of study medication were retrieved from a Dutch database that includes average national reimbursement rates, without overhead costs. [24] These average national reimbursement rates were preferred over exact cost-prices per hospital, because of the heterogeneity and fluctuation in individual pricing agreements between different hospitals and pharmacies. Previous research has shown that nystatin is cheaper and has similar antifungal effectiveness as compared to amphotericin B; nystatin is now common practice in a large part of Dutch ICUs as the antifungal part of topical decontamination. [25] Total costs for the topical antimicrobials were, therefore, based on costs for colistin, tobramycin and nystatin. Accordingly, the daily price of the topical study medication was €2.56 for SOD and €16.74 for SDD. Daily costs for the third-generation cephalosporin were based on the costs for four doses of 1 gram IV cefotaxime per day (during the first four days in ICU). The reference year for all costs was 2017. If needed, costs were corrected for inflation based on the Dutch price index. [26] We used the absolute risk reduction of in-hospital death as a measure of effectiveness. There was no discounting for costs or effects, since all costs and effects were measured in the first year after ICU admission.

Outcomes measures

Outcome of the CEA was the incremental cost effectiveness ratio (ICER), defined as the ratio of the difference in mean costs and number of in-hospital deaths prevented per patient treated with SDD versus SOD. Consequently, the ICER is expressed as incremental costs per prevented in-hospital death.

Statistical analysis

A two-stage meta-analysis using individual patient data was performed to allow for optimal confounding adjustment within each study. We used separate generalized regression models per

Table 1. Costs per unit*

Hospital admission	***Costs per unit***
ICU admission day	€2061.64
Ward admission day	€487.02
Study medication	***Costs per day***
Oropharyngeal paste with non-absorbable AB†	€2.56
Suspension with non-absorbable AB‡	€14.18
Third-generation cephalosporin§	€20.92
Oropharyngeal paste with non-absorbable AB – including amphotericin B¶	*€6.96*
Suspension with non-absorbable AB – including amphotericin B#	*€65.60*
Microbiological costs	***Costs per unit***
Blood culture	€28.93 + €5.70 order rate
Respiratory and rectum cultures	€32.17 + €5.70 order rate
Species determination bacteria and yeasts	€8.81
Antibiotic susceptibility testing (per isolate)	€55.04

AB, antibiotics; ICU, intensive care unit; IV, intravenous. * All costs were indexed for the reference year 2017.
† Colistin/nystatin/tobramycin mouth paste (20MG/100000E/20MG per mL), 0.5mL 4 times daily.
‡ Colistin/nystatin/tobramycin suspension (10MG/200000E/8MG per mL), 10mL 4 times daily (only part of the SDD regimen).
§ IV cefotaxime, 1 gr 4 times daily (during first four days in ICU).
¶ Colistin/amphotericinB/tobramycin mouth paste (20MG/20MG/20MG per mL), 0.5mL 4 times daily (sensitivity analysis 3).
# Colistin/amphotericinB/tobramycin suspension (8.75MG/54.7MG/11.75MG per mL), 10mL 4 times daily (sensitivity analysis 3, only part of the SDD regimen).

study to estimate costs and effects and took clustering on a hospital level into account by using a fixed effect per study center. Linear regression was used to estimate the difference in costs between SDD and SOD. Similarly, logistic regression was performed to estimate an adjusted number of in-hospital deaths prevented with SDD versus SOD, with the absolute risk difference calculated by comparing the mean predicted probabilities per treatment arm. For comparison of these results with the previous IPD-MA, the pooled adjusted OR for in-hospital mortality was calculated as well. [19] Because CRXO trials are prone to selective inclusion, all analyses were corrected for possible confounders, which were selected based on previous knowledge: center, age, sex, APACHE II (De Smet study) or APACHE IV (Oostdijk study) score, admission type (medical or surgical), and mechanical ventilation at ICU admission (De Smet study, not available in Oostdijk study). The definition of surgical admission type differed per study. In the De Smet study, this was defined as "reason for ICU-admission is postoperative/surgical according to the treating ICU-physician" and for the Oostdijk study: "those who received any type of surgery in the week prior to ICU admission". [13,18] A random effect for cluster period did not improve model

fit based on Akaike's Information Criterion in any of the four models, and was therefore omitted. All analyses were performed on complete cases. Confidence intervals (CI) of non-parametric data and the ICER were calculated with the use of bootstrapping (10,000 repeats). A fixed-effect meta-analysis was used to obtain a pooled estimate of the ICER across the two trials, applying inverse variance weighting separately for costs and effects. The decision to use fixed-effects models was predefined and was based on the strong similarity of the two studies with regard to study design, ICU setting, in- and exclusion criteria and intervention.

The individual as well as the pooled results of the cost-effectiveness meta-analysis were plotted in a cost-effectiveness plane. Statistical heterogeneity was assessed by calculating the I^2 statistic. A willingness-to-pay plot was plotted to reflect the probability of cost-effectiveness of SDD versus SOD for a range of different values of the maximum incremental costs per averted in-hospital death. The curve represents the proportion of bootstrap samples that fall below the maximum acceptable incremental costs per averted in-hospital death (i.e. the willingness-to-pay to prevent one in-hospital death). Subsequently, we calculated the minimum required number of quality adjusted life-years (QALYs) gained per prevented in-hospital death, given the obtained incremental costs per prevented death for SDD compared to SOD, to reach cost-effectiveness in the context of the Dutch formal threshold of €80,000 per QALY for life-threatening illnesses. This was calculated by dividing the willingness-to-pay values corresponding to 90.0% and 95.0% probability of cost-effectiveness of SDD by €80,000. [28]

Sensitivity analyses were performed to estimate the robustness of the cost-effectiveness of SDD in case of fluctuation in market-prices of the medication. We measured the effect of increasing costs of the SDD and SOD medication regimen (including the IV component of SDD) by a factor 2 (Scenario 1) and 5 (Scenario 2). These factors were arbitrarily chosen. The third scenario included costs for amphotericin B instead of nystatin as the antifungal component of SDD and SOD (see Table 1).

All analyses were performed with Statistical Package for Social Sciences V.25.0 (SPSS, Chicago, Illinois, USA) and R version 3.4.1. Syntax for the cost-effectiveness meta-analysis is available at https://github.com/henrivanwerkhoven/meta2way.

RESULTS

Study population

A total of 3,949 and 11,997 patients were included in the SDD and SOD groups in the original trials. [13,18] For the current analysis, 197 patients were excluded from the De Smet *et al.* [13] study: 11 did not give permission to use clinical data, 1 was a duplicate, 176 were re-admissions within the same hospital admission and 9 patients had missing data for at least one variable in the

Table 2. Baseline characteristics, microbiological sampling and clinical outcomes

	De Smet *et al.* 2009 [13]		Oostdijk *et al.* 2017 [18]	
	SOD n = 1803	**SDD n = 1949**	**SOD n = 4429**	**SDD n = 4771**
Baseline characteristics				
Mean age, years (±SD)	61.5 (16.4)	62.4 (16.0)	62.8 (15.6)	63 (15.6)
Male (%)	1144 (63.4)	1203 (61.7)	2710 (61.2)	2880 (60.4)
Admission type: surgical (%)	841 (46.6)	898 (46.1)	1593 (36.0)	1805 (37.8)
Mean APACHE II score (±SD)	19.5 (8.2)	19.6 (7.8)	NA	NA
Mean APACHE IV score (±SD)	NA	NA	82.2 (33.4)	81.7 (33.8)
MV at ICU admission (%)	1698 (94.2)	1814 (93.1)	NA	NA
Microbiological sampling				
Median number of cultures (IQR)				
Blood	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-2)
Respiratory	5 (2-9)	5 (3-9)	3 (1-6)	2 (1-5)
Rectum	0	2 (1-4)	0 (0-1)	1 (0-3)
Clinical outcomes				
Median LOS – ICU, days (IQR)	9 (6-15)	9 (5-15)	7 (4-12)	6 (4-11)
Median LOS – hospital ward, days (IQR)*	12 (5-26)	13 (6-25)	11 (4-22)	11 (5-21)
In-hospital death (%)	552 (30.6)	623 (32.0)	1410 (31.8)	1384 (29.0)

IQR, interquartile range; LOS, length of stay; MV, mechanical ventilation; NA, not available; SC, standard care; SD, standard deviation; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination
*Patients that were not discharged from the ICU alive were excluded from this calculation.

regression analysis. 2797 patients were excluded from the Oostdijk *et al.* [18] study: 18 were duplicates, 2206 were not treated with SDD or SOD, 567 were re-admissions within the same hospital admission and 6 patients had missing data for at least one variable in the regression analysis. This resulted in a total study population of 12,952 patients. Of these, 6,720 and 6,232 patients were treated with SDD and SOD, respectively.

Baseline characteristics were similar between the two studies, except that patients were more often classified as surgical admission in the first trial (Table 2). There were small differences within studies between treatment arms, similar to the reported differences in the original studies (Table 2). [13,18]

Costs and effects

Patients in the first trial had a longer length of stay (LOS) on the ICU and hospital ward as

Table 3. Mean costs per patient

	De Smet *et al.* 2009 [13]		**Oostdijk *et al.* 2017 [18]**	
	SOD n =1803	**SDD n = 1949**	**SOD n = 4429**	**SDD n = 4771**
LOS – ICU (95% CI)*	€24,278 (€23,111 to €25,544)	€24,851 (€23,576 to €26,343)	€21,539 (€20,842 to €22,291)	€20,409 (€19,737 to €21,129)
LOS – hospital ward (95% CI)	€7,303 (€6,860 to €7,803)	€7,472 (€7,019 to €7,958)	€6,231 (€5,960 to €6,513)	€6,581 (€6,287 to €6,907)
Microbiology cultures (95% CI)*	€544 (€516 to €577)	€698 (€663 to €736)	€479 (€460 to €500)	€473 (€455 to €491)
Study medication (95% CI)*	€30 (€29 to €32)	€279 (€269 to €291)	€27 (€26 to €28)	€242 (€236 to €248)
Total (95% CI)	**€32,154 (€30,832 to €33,618)**	**€33,299 (€31,839 to €34,929)**	**€28,276 (€27,464 to €29,099)**	**€27,705 (€26,888 to €28,537)**

*Costs were calculated for days on the ICU after study inclusion.
ICU, intensive care unit; LOS, length of stay; SC, standard care; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination

compared to patients in the second trial (Table 2). Within the first trial, LOS on the ICU was similar in the SDD and SOD group, and LOS on the hospital ward for SDD and SOD patients that survived the ICU was 13 days (IQR 6 – 25) versus 12 days (IQR 2 – 26), respectively. In the second trial, SDD patients had shorter ICU LOS compared to SOD patients (6 days (IQR 4 – 11) versus 7 days (IQR 4 – 12)). Average LOS on the hospital ward for ICU survivors was comparable between the treatment arms.

Crude average total healthcare costs per patient (i.e. unadjusted for the CRXO design) were higher during the first trial compared to the second trial (Table 3). Average healthcare costs from inclusion until hospital discharge for an SDD patient were €33,299 (95% CI €31,877 to €34,981) in the first trial and €27,705 (95% CI €26,921 to €28,574) in the second trial. Total healthcare costs from inclusion until hospital discharge for an SOD patient were on average €32,154 (95% CI €30,883 to 33,638) in the first trial and €28,276 (95% CI €27,446 to €29,140) in the second trial. Total healthcare costs were mainly determined by costs for ICU-LOS (75%) and hospital ward-LOS (23%). In the first trial, crude in-hospital mortality was higher among SDD patients compared to SOD patients, 32.0% and 30.6%, respectively (Table 2). In the second trial, crude in-hospital mortality was lower in the SDD group than in the SOD group, 29.0% versus 31.8%, respectively.

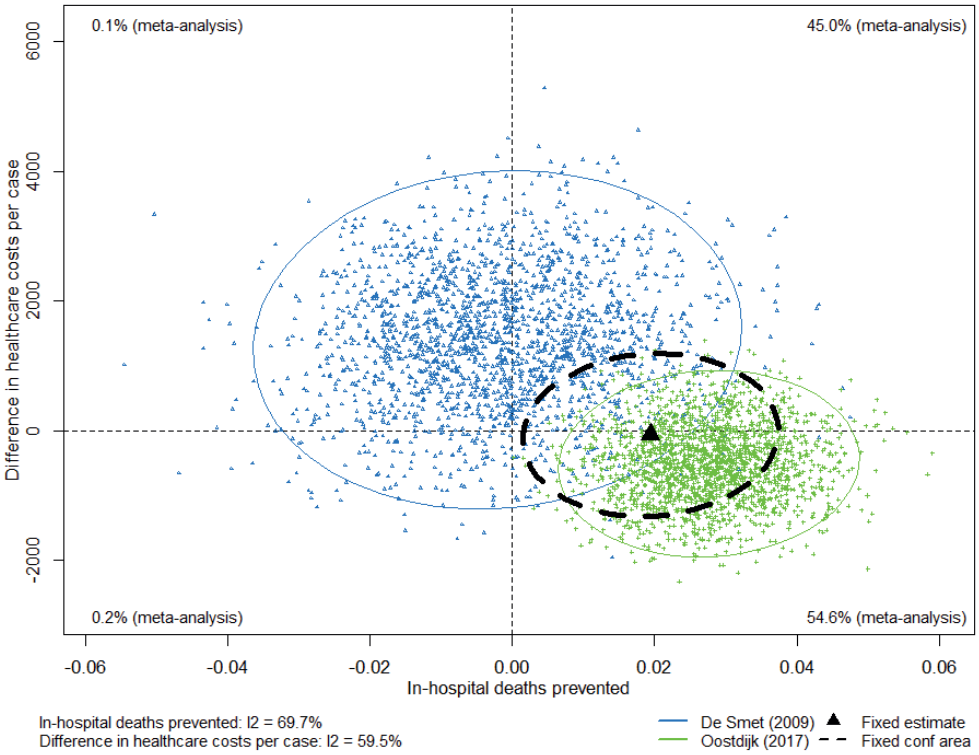


Figure 1 Cost-effectiveness plane of SDD versus SOD. The blue and green points represent the bootstrapped ICERs of the De Smet [13] and Oostdijk [18] trial, respectively. The coloured ellipses around these points represent the 95% confidence ellipses of the corresponding study. **The black ellipse represents the 95% confidence ellipse for the fixed effect meta-analysis (i.e. the pooled meta-analysis data).** The bootstrapped ICER points of the meta-analysis have been omitted from the figure to improve visuality of the plot. The proportions in each quadrant represent the proportion of bootstrap samples (i.e. ICER points) of the meta-analysis in that quadrant. ICER points in the lower right quadrant are in favour of SDD in terms of costs and effects, ICER points in the upper right quadrant are in favour of SDD in terms of beneficial effects but not in terms of incremental costs. ICER points in the upper left quadrant are in favour of SOD in terms of effects and costs, points in the lower left quadrant are in favour of SOD in terms of effects but not in terms of costs.

The adjusted paired bootstrapped ICERs of both trials as well as the results of the fixed-effect two-stage meta-analysis are depicted in a cost-effectiveness plane in Figure 1. I^2 was 59.5% (95% CI 0% to 99%) and 69.7% (95% CI 0% to 99%) for costs and effects, respectively. In the meta-analysis, SDD significantly reduced in-hospital mortality (adjusted absolute risk reduction 0.0195, 95% CI 0.0050 to 0.0338) with no difference in costs (adjusted cost difference €62 in favour of SDD, 95% CI –€1079 to €935). The adjusted pooled OR for in-hospital mortality was 0.90 (95% CI 0.82 to 0.97) for SDD versus SOD, which was identical to the previous IPD-MA. [27] In the cost-effectiveness plane these results are depicted in the different quadrants (Figure 1).

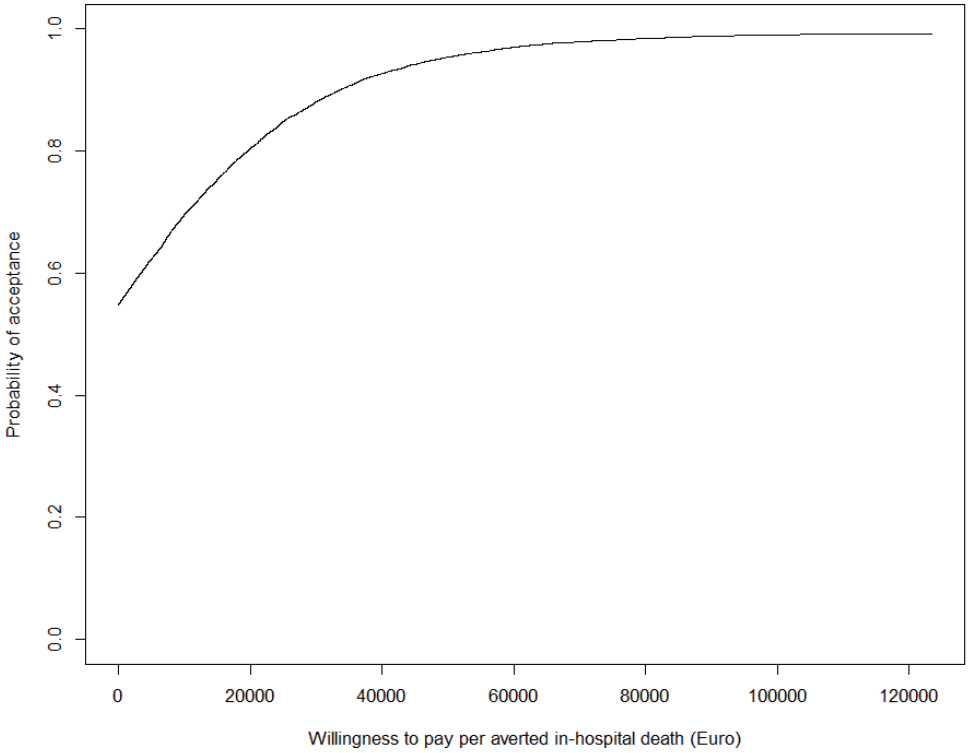


Figure 2 Willingness-to-pay plot. The curve represents the probability that SDD is below different thresholds of maximum willingness-to-pay values per one averted in-hospital death.

SDD was more effective (i.e. lower in-hospital mortality) and was less costly in 54.6% of the bootstrap samples (i.e. the lower right quadrant), compared to SOD. In 45.0% of the bootstrap samples, SDD was more effective, but was associated with higher costs (i.e. the upper right quadrant). There was 90.0% and 95.0% probability that SDD was cost-effective at a willingness to pay of €33,663 and €48,548 per prevented in-hospital death, respectively (Figure 2). Accordingly, at least 0.42 and 0.61 QALYs would need to be gained per prevented in-hospital death in order to reach cost-effectiveness of SDD at the Dutch threshold of €80,000 per QALY, respectively.

Sensitivity analyses

Increasing SDD and SOD medication costs by a factor 2 and 5 resulted in a reduction from 54.6% bootstrap samples being in the lower right quadrant (main analysis) to 37.8% and 5.7% of the bootstrap samples in the lower right quadrant, respectively (see Scenario 1 and 2 in the supplementary material). The willingness-to-pay to prevent one in-hospital death thresholds

corresponding to the 90.0% and 95.0% probabilities of cost-effectiveness of SDD were €47,360 and €65,607 for a doubling of medication costs of the SDD and SOD regimen, and €100,148 and €134,849 for an increase in SDD and SOD medication by a factor 5, respectively. Choosing amphotericin B instead of nystatin as the antifungal component of the topical medication, against average national reimbursement rates, resulted in 18.4% of the bootstrap samples in the lower right quadrant (i.e. SDD beneficial over SOD in terms of both costs and effects). In this scenario, the willingness-to-pay thresholds to prevent one in-hospital death were €68,924 and €94,591 for 90.0% and 95.0% probability of cost-effectiveness of SDD, respectively (see Scenario 3 in the supplementary material). The minimum numbers of QALYs gained per prevented in-hospital death in order for SDD to be cost-effective at the Dutch formal threshold of maximum €80,000 per QALY for the different scenarios can be found in the supplementary material.

DISCUSSION

In this individual patient data meta-analysis, SDD significantly reduced in-hospital mortality (adjusted absolute risk reduction 0.0195, 95% CI 0.0050 to 0.0338) with no difference in costs (adjusted cost difference €62 in favour of SDD, 95% CI –€1079 to €935) as compared to SOD. SDD had a 90.0% probability to be cost-effective compared to SOD at a willingness to pay of €33,663 to prevent one in-hospital death.

SDD and SOD are preventive regimens in a setting of critical care medicine. In the Netherlands, the willingness-to-pay threshold for one QALY gained is €80,000 in case of life threatening illnesses. [28] According to our results, in order for SDD to be cost-effective with 90.0% and 95.0% probability, one would need to gain at least 0.42 and 0.61 QALYs respectively, for each prevented in-hospital death. The Dutch National Intensive Care Evaluation (NICE) registry [29], in which 90% of all Dutch ICUs participate, was consulted to obtain life-expectancy data for ICU survivors. During the period 2006–2017, 111,608 patients admitted to the ICU for a minimum of 72 hours had left the hospital alive; of these 65% were still alive at 4 years after ICU discharge (Dutch NICE Registry, unpublished data, 2018). This patient group was similar to our study population with respect to age (63.3 ±15 years), proportion males (59.6%) and ICU-LOS (median 7.4 days, IQR 4.1 – 10.8), but had a lower mean APACHE IV score (70.9 ± 27.5). A large Dutch single-center study [30] that assessed long-term health-related QoL (HRQoL) of ICU patients, found a HRQoL index 1 year after ICU admission of 0.71 ± 0.26 for patients that were admitted to the ICU for 72 hours or more (Soliman, personal communication, 2018). So if we assume that those rescued by SDD have a similar life expectancy and HRQoL as the patients mentioned above, SDD has a very high probability of being cost-effective.

To the best of our knowledge, there is only one previous CEA on SDD and SOD which already showed cost-effectiveness of both SDD and SOD as compared to standard care. [15] That study was based on patient-level data of the De Smet *et al.* study [13] only, thus included 29% of the patients in the current CEA. Yet, in that study, SOD was cost-effective compared to SDD, which is in contrast with the results of the current individual patient data meta-analysis. There were important differences in our analysis methods as compared to the previous CEA. In the current CEA, additional costs for MV on the ICU were not included, because data were unavailable for the largest trial. Also, a different endpoint was chosen, namely incremental costs per prevented in-hospital death instead of incremental costs per life year gained, and the current analysis was corrected for clustering and differences in baseline characteristics between groups. Finally, in the current CEA, ICU re-admissions within one hospital admission were excluded, so patients could not be counted twice with relation to the occurrence of in-hospital mortality. The different result as compared to the previous CEA can also partly be explained by inclusion of the Oostdijk *et al.* study [18], in which SDD significantly improved in-hospital survival as compared to SOD (as opposed to the De Smet *et al.* study [13], where there was no significant difference in clinical effectiveness between SDD and SOD). Also, in the Oostdijk *et al.* study [18], the average ICU-LOS was shorter for patients treated with SDD in comparison to SOD, which was an important driver of the total healthcare costs per patient. As with any weighted meta-analysis, this larger study (N = 9,200) was assigned more weight in our meta-analysis as compared to the smaller first study (N = 3,752). As to date, it remains unclear why the first trial [13] did not show effectiveness of SDD over SOD in preventing in-hospital mortality. Inclusion criteria as well as the interventions were similar in both trials and both trials were performed in the same setting (Dutch ICUs with low levels of antimicrobial resistance). Although small differences in participating hospitals and patients between studies (and over time) cannot be ruled out, it is unlikely that such differences have modified the effectiveness of SDD and SOD to this extent. Therefore, we believe that chance is the best explanation for the statistical heterogeneity between the two trials.

In sensitivity analyses, doubling of medication costs for SDD and SOD had moderate impact on the cost-effectiveness, but a five-fold increase in medication costs would influence the cost-effectiveness estimates of SDD substantially. It is important to note that these scenarios were arbitrarily chosen to test the robustness of the cost-effectiveness estimate of SDD against fluctuation in market prices, and that such a large increase in medication costs is not likely. Using amphotericin B instead of nystatin as the topical antifungal component would also reduce the cost-effectiveness of SDD, as nystatin is the cheaper option at present. Still, in all three scenarios, the minimum number of QALYs gained per prevented in-hospital death, in order for SDD to be cost-effective at the Dutch maximum willingness-to-pay value of €80,000 per QALY, is reached with high probability if we compare our results to current available Dutch data on long-term survival and HR-QoL of ICU survivors.

One of the reasons that SDD is not yet widely implemented in the Netherlands is the fear that prolonged selective antibiotic pressure increases antibiotic resistance rates. However, for ICUs

with low prevalence of antibiotic resistance, there is no evidence that the use of SDD increases antibiotic resistance among Gram-negative bacteria, neither at ICU level nor at individual patient level, up to ten days after ICU discharge. [31-34] Naturally, surveillance of respiratory and rectal carriage with Gram-negative bacteria, including assessment of colistin and tobramycin resistance, remains an essential part of the SDD regimen.

Strengths of the current analysis are the inclusion of individual patient data from 24 Dutch hospitals that participated in CRXO trials on SDD and SOD, and the adjustment for baseline differences and clustering in the statistical analyses, which is crucial when analysing data from studies without individual randomization. Furthermore, patient characteristics were similar between the two studies, reflecting similar inclusion criteria and practices. This study also has some limitations. First, due to absence of post-hospital discharge data, health-economic evaluations could not be performed from a societal perspective, which is generally preferred by healthcare policy makers. However, we may assume that differences in costs after hospital discharge between SDD and SOD will be negligible. Secondly, we were not able to include costs for additional diagnostics, therapeutic antibiotics and other patient-level expenses that may have been influenced by the SDD and SOD strategy because these data were not available in one of the trials. Total absolute healthcare costs that were calculated in this study may therefore underestimate actual healthcare costs per patient. In the previous CEA that did include costs for therapeutic antibiotics, LOS still accounted for 98% of total costs. [15] Moreover, the analysis on antibiotic use in the study of De Smet *et al.* [13] showed that overall antibiotic use was lower during treatment with SDD as compared to SOD (1.10 defined daily dosage vs. 1.21 defined daily dosage per day in the ICU for SDD vs. SOD respectively) (De Smet, crude unpublished data, 2018). Also, in a post-hoc analysis, the proportion of patients on systemic antibiotics after day five of ICU admission (when IV cefotaxime per SDD protocol had stopped) was lower during SDD compared to SOD. [19] Therefore, it seems highly unlikely that including costs for therapeutic antibiotics would reduce the cost-effectiveness of SDD. Finally, it should be noted that both trials were performed in the Netherlands, where antimicrobial resistance levels in ICUs are low and selective decontamination has demonstrated clinical effectiveness. Therefore, the results of the current CEA may not be generalizable to countries with moderate to high antimicrobial resistance levels. In a recent CRXO trial in 13 European ICUs with moderate to high antibiotic resistance prevalence, SDD and SOD were not associated with statistically significant reductions in ICU-acquired bacteraemias caused by multidrug-resistant Gram-negative bacteria or mortality, as compared to standard care. [20] In that study, baseline period prevalence of rectal colonization with a third-generation cephalosporin-resistant Enterobacterales and vancomycin-resistant enterococci (VRE) was 15.8% and 2.2%, respectively. The proportion of ICU-acquired bacteraemia episodes caused by any highly-resistant micro-organism (i.e. multidrug-resistant Gram-negative bacteria, MRSA, VRE) and third-generation cephalosporin-resistant Enterobacterales was 25.5% and 15.1%, respectively. Results of the current study,

therefore, apply to all patients with an expected length of stay of >48 hours admitted to ICUs with low prevalence of antibiotic resistance. This critically-ill population is at increased risk of acquisition of ICU-acquired infections and subsequent in-hospital death. Results of the current study may assist healthcare policy makers and ICU-physicians from settings with similar levels of antimicrobial resistance as the Netherlands in the allocation of their resources for infection prevention.

In conclusion, SDD has a very high probability of being cost-effective as compared to SOD in Dutch ICU patients. These data support the implementation of SDD in ICU settings with low levels of antimicrobial resistance.

List of abbreviations
AB, antibiotics; CEA, cost-effectiveness analysis; CHEERS, consolidated health economic evaluation reporting standards; CI, confidence interval; CRXO, cluster-randomized cross-over; HR-QoL, health-related quality of life; ICER, incremental cost-effectiveness ratio; ICU, intensive care unit; IPD-MA, individual patient data meta-analysis; IV, intravenous; IQR, interquartile range; LOS, length of stay; MRSA, methicillin-resistant *S. aureus*; MV, mechanical ventilation; NICE, Dutch national intensive care evaluation; OR, odds ratio; QALY, quality-adjusted life year; SDD selective digestive decontamination; SOD, selective oropharyngeal decontamination; VRE, vancomycin-resistant Enterococcus

Acknowledgements: We sincerely thank Ivo W. Soliman for his extra subgroup analysis on the long-term health-related quality of life of ICU patients for the current study. Also, we would like to thank the NICE registry, in particularly Fabian Termorshuizen, for kindly providing us with Dutch long-term survival data of ICU patients. Finally, many thanks to Dylan de Lange for his help in retrieving these extra data estimates.

Funding: This study is a post-hoc analysis of studies that were funded by the Netherlands Organization for Scientific Research and has not received a specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflicts of interest: None to declare.

REFERENCES

1. Laupland KB, Zygun DA, Davies HD, ***et al.*** Population-based assessment of intensive care unit-acquired bloodstream infections in adults: Incidence, risk factors, and associated mortality rate. ***Crit Care Med*** 2002;30:2462–7. doi:10.1097/01.CCM.0000034731.48473.
2. Rello J, Ollendorf DA, Oster G. Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. ***Chest*** 2002;122:2115–21. doi:10.1378/chest.122.6.2115.
3. Garrouste-Orgeas M, Timsit J, Tafflet M, ***et al.*** Excess risk of death from intensive care unit-acquired nosocomial bloodstream infections: a reappraisal. ***Clin Infect Dis*** 2006;42:1118–26. doi:10.1086/500318.
4. Melsen WG, Rovers MM, Groenwold RHH, ***et al.*** Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. ***Lancet Infect Dis*** 2013;13:665–71. doi:10.1016/S1473-3099(13)70081-1.
5. Adrie C, Garrouste-Orgeas M, Ibn Essaied W, ***et al.*** Attributable mortality of ICU-acquired bloodstream infections: Impact of the source, causative micro-organism, resistance profile and antimicrobial therapy. ***J Infect*** 2017;74:131–41. doi:10.1016/j.jinf.2016.11.001.
6. Warren DK, Shukla SJ, Olsen MA, ***et al.*** Outcome and attributable cost of ventilator-associated pneumonia among intensive care unit patients in a suburban medical center. ***Crit Care Med*** 2003;31:1312–7. doi:10.1097/01.CCM.0000063087.93157.06.
7. Laupland KB, Lee H, Gregson DB, ***et al.*** Cost of intensive care unit-acquired bloodstream infections. ***J Hosp Infect*** 2006;63:124–32. doi:10.1016/j.jhin.2005.12.016.
8. Kollef MH, Hamilton CW, Ernst FR. Economic impact of ventilator-associated pneumonia in a large matched cohort. ***Infect Control Hosp Epidemiol*** 2012;33:250–6. doi:10.1086/664049.
9. Krueger WA, Lenhart F-P, Neeser G, ***et al.*** Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. ***Am J Respir Crit Care Med*** 2002;166:1029–37. doi:10.1164/rccm.2105141.
10. de Jonge E, Schultz MJ, Spanjaard L, ***et al.*** Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. ***Lancet*** 2003;362:1011–6. doi:10.1016/S0140-6736(03)14409-1.
11. Silvestri L, Saene HKF Van, Milanese M. Selective decontamination of the digestive tract reduces bacterial bloodstream infection and mortality in critically ill patients. Systematic review of randomized, controlled trials. ***J Hosp Infect*** 2007;65:187–203. doi:10.1016/j.jhin.2006.10.014.
12. Liberati A, D'Amico R, Pifferi S, ***et al.*** Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care. ***Cochrane database Syst Rev*** 2009:CD000022. doi:10.1002/14651858.CD000022.pub3.
13. de Smet AMGA, Kluytmans JAJW, Cooper BS, ***et al.*** Decontamination of the digestive tract and oropharynx in ICU patients. ***N Engl J Med*** 2009;360:20–31. doi:10.1056/NEJMoa0800394.
14. Price R, Maclennan G, Glen J. Selective digestive or oropharyngeal decontamination and topical oropharyngeal chlorhexidine for prevention of death in general intensive care: systematic review and network meta-analysis. ***BMJ*** 2014;348:g2197. doi:10.1136/bmj.g2197.
15. Oostdijk EAN, de Wit GA, Bakker M, ***et al.*** Selective decontamination of the digestive tract and selective oropharyngeal decontamination in intensive care unit patients: a cost-effectiveness analysis. ***BMJ Open*** 2013;3:e002529. doi:10.1136/bmjopen-2012-002529.
16. Stichting Werkgroep Antibioticabeleid (SWAB). SWAB Richtlijn: selectieve decontaminatie bij patiënten op de intensive care. 2018:1–29.
17. de Smet AMGA, Kluytmans JAJW, Blok HEM, ***et al.*** Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. ***Lancet Infect Dis*** 2011;11:372–80. doi:10.1016/S1473-3099(11)70035-4.

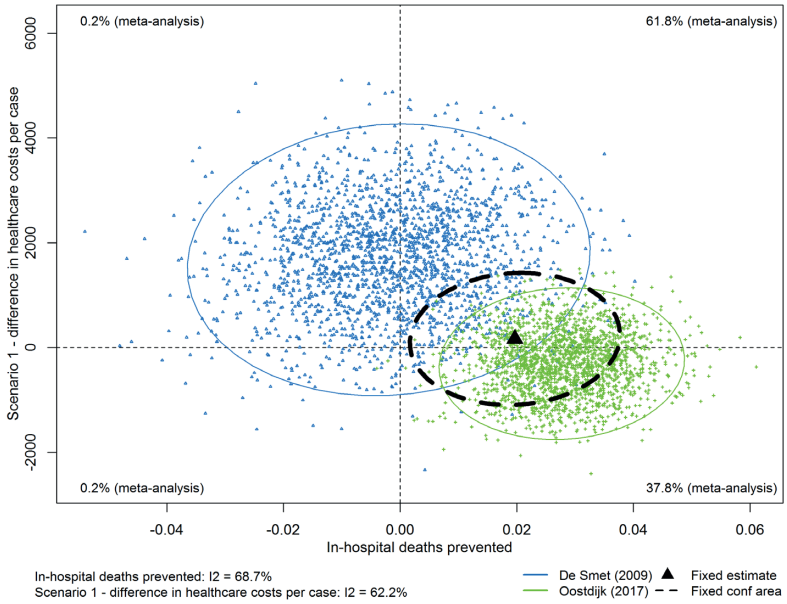
18. Oostdijk EAN, Kesecioglu J, Schultz MJ, ***et al.*** Notice of Retraction and Replacement: Oostdijk et al. Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. JAMA. 2014;312(14):1429-1437. ***JAMA*** 2017;317:1583–4. doi:10.1001/jama.2017.1282.
19. Plantinga NL, de Smet AMGA, Oostdijk EAN, ***et al.*** Selective digestive and oropharyngeal decontamination in medical and surgical ICU patients: individual patient data meta-analysis. ***Clin Microbiol Infect*** 2017;24:505–13. doi:10.1016/j.cmi.2017.08.019.
20. Wittekamp BH, Plantinga NL, Cooper BS, ***et al.*** Decontamination Strategies and Bloodstream Infections with Antibiotic-Resistant Microorganisms in Ventilated Patients: A Randomized Clinical Trial. ***JAMA*** 2018;320:2087–98. doi:10.1001/jama.2018.13765.
21. Husereau D, Drummond M, Petrou S, ***et al.*** Consolidated Health Economic Evaluation Reporting Standards (CHEERS) statement. ***BMJ*** 2013;346:f1049. doi:10.1136/bmj.f1049.
22. Hakkaart-van Roijen. Kostenhandleiding: Methodologie van kostenonderzoek en referentieprijzen voor economische evaluaties in de gezondheidszorg. 2016.
23. Nederlandse Zorg Authoriteit, NZA. https://puc.overheid.nl/nza/doc/PUC_13010_22/1. Date accessed 17 Jan 2018.
24. Available: www.medicijnkosten.nl. Date accessed 26 January 2018.
25. Wittekamp BH, Ong DSY, Cremer OL, ***et al.*** Nystatin versus amphotericin B to prevent and eradicate Candida colonization during selective digestive tract decontamination in critically ill patients. ***Intensive Care Med.*** 2015;41:2235–6. doi:10.1007/s00134-015-4081-x.
26. Centraal Bureau voor de Statistiek. StatLine: Consumentenprijzen; prijsindex 2014-2017.
27. Plantinga NL, Bonten MJM. Selective digestive and oropharyngeal decontamination in medical and surgical ICU patients: authors' reply. ***Clin. Microbiol. Infect.*** 2018;24:552–3. doi:10.1016/j.cmi.2017.09.019.
28. van Rijen AJG. Zinnige en duurzame zorg: advies uitgebracht door de Raad voor de Volksgezondheid en Zorg aan de minister van Volksgezondheid, Welzijn en Sport. Zoetermeer: 2006.
29. Dutch National Intensive Care Evaluation (NICE) registry. http://www.stichting-nice.nl (accessed 9 Nov 2018).
30. Soliman IW, de Lange DW, Peelen LM, ***et al.*** Single-center large-cohort study into quality of life in Dutch intensive care unit subgroups, 1 year after admission, using EuroQoL EQ-6D-3L. J Crit Care 2015;30:181–6. doi:10.1016/j.jcrc.2014.09.009.
31. Daneman N, Sarwar S, Fowler RA, ***et al.*** Effect of selective decontamination on antimicrobial resistance in intensive care units: a systematic review and meta-analysis. ***Lancet Infect Dis*** 2013;13:328–41. doi:10.1016/S1473-3099(12)70322-5.
32. Plantinga NL, Bonten MJM. Selective decontamination and antibiotic resistance in ICUs. ***Crit Care*** 2015;19:259. doi:10.1186/s13054-015-0967-9.
33. Buelow E, Bello Gonzalez TDJ, Fuentes S, ***et al.*** Comparative gut microbiota and resistome profiling of intensive care patients receiving selective digestive tract decontamination and healthy subjects. ***Microbiome*** 2017;5:88. doi:10.1186/s40168-017-0309-z.
34. de Jonge E, de Wilde RBP, Juffermans NP, ***et al.*** Carriage of antibiotic-resistant Gram-negative bacteria after discontinuation of selective decontamination of the digestive tract (SDD) or selective oropharyngeal decontamination (SOD). ***Crit care*** 2018;22:243. doi:10.1186/s13054-018-2170-2.

SUPPLEMENTARY MATERIAL

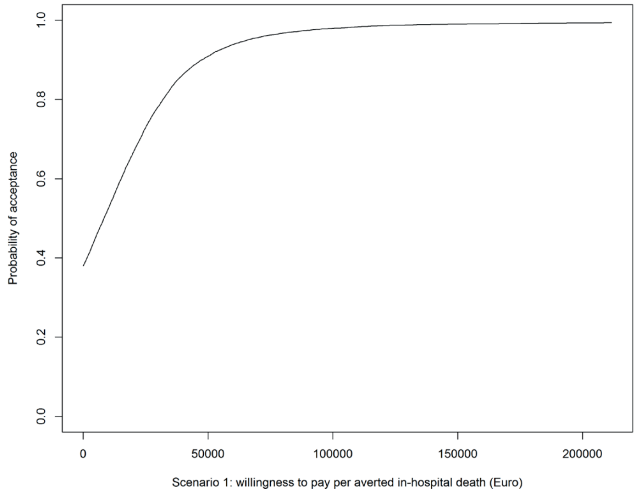
Supplementary Table 1. Cost and effect estimates from two-stage meta-analysis

	Median estimate	95% CI – Lower bound	95% CI – Upper bound
Main analysis			
Adjusted absolute in-hospital mortality risk reduction	0.0195	0.0050	0.0338
Adjusted cost difference (€/patient)	-€62	-€1079	€935
Sensitivity analyses			
*Scenario 1 (medication costs*2)*			
Adjusted absolute in-hospital mortality risk reduction	0.0198	0.0050	0.0340
Adjusted cost difference (€/patient)	€158	-€856	€1168
*Scenario 2 (medication costs*5)*			
Adjusted absolute in-hospital mortality risk reduction	0.0196	0.0052	0.0338
Adjusted cost difference (€/patient)	€824	-€206	€1873
Scenario 3 (amphotericin B instead of nystatin)			
Adjusted absolute in-hospital mortality risk reduction	0.0197	0.0053	0.0340
Adjusted cost difference (€/patient)	€470	-€564	€1481

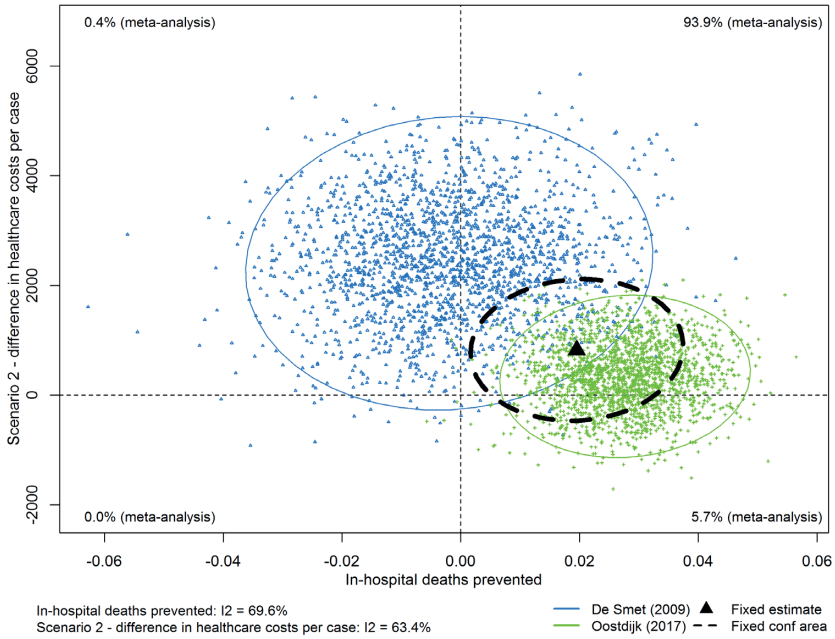
The estimates and 95% confidence intervals represent the median estimate and 95% confidence bounds (i.e. the black ellipse) from the fixed-effects meta-analysis cost-effectiveness planes.



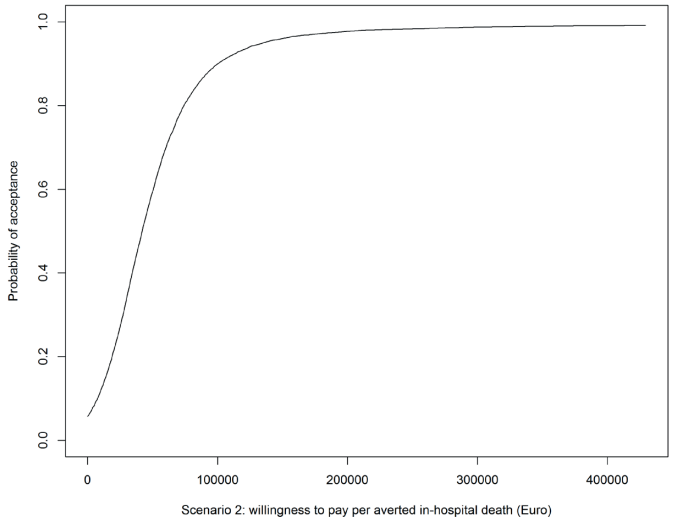
Supplementary Figure 2. Cost-effectiveness plane sensitivity analysis - Scenario 1
The red and green points represent the bootstrapped ICERs of the De Smet and Oostdijk trial, respectively, **in case of an increase in medication by a factor 2**. The coloured ellipses represent the 95% CI of the corresponding study. The black ellipse represents the 95% CI for the fixed effect meta-analysis. The bootstrapped ICERs of the meta-analysis have been omitted from the figure to improve visuality of the plot.



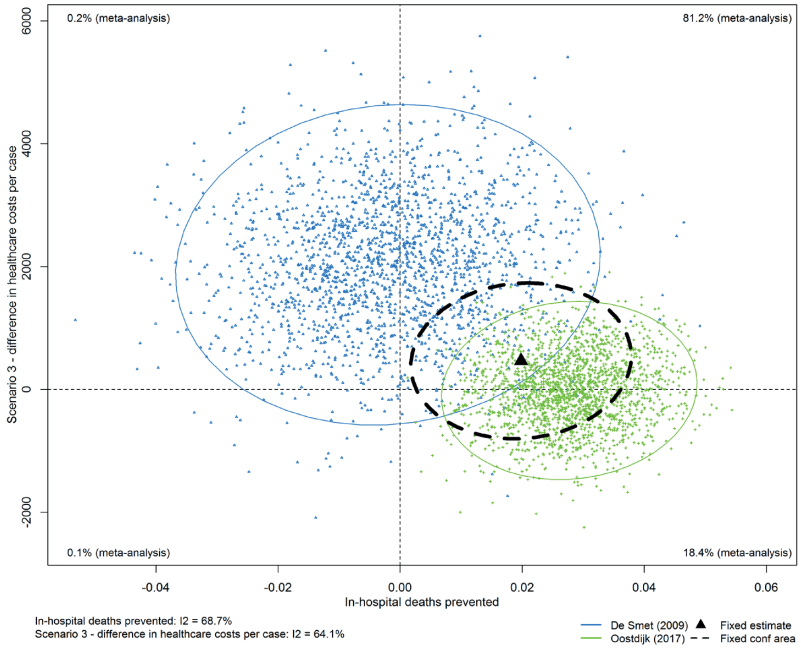
Supplementary Figure 3. Willingness-to-pay curve sensitivity analysis - Scenario 1
The curve represents the probability that SDD is below different thresholds of maximum acceptability prices to pay per one averted in-hospital death, **in case of an increase in medication by a factor 2**. The willingness-to-pay thresholds corresponding to 90.0% and 95.0% probabilities are €47,370 and €65,607. Corresponding minimum numbers of QALYs gained per averted in-hospital death in order for SDD to be cost-effective at the Dutch formal threshold of maximum €80,000 per QALY, are 0.59 and 0.82, respectively.



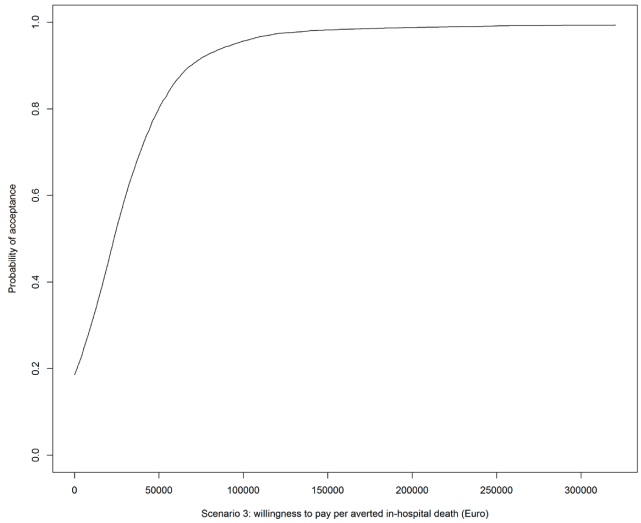
Supplementary Figure 4. Cost-effectiveness plane sensitivity analysis - Scenario 2
The red and green points represent the bootstrapped ICERs of the De Smet and Oostdijk trial, respectively, **in case of an increase in medication by a factor 5**. The coloured ellipses represent the 95% CI of the corresponding study. The black ellipse represents the 95% CI for the fixed effect meta-analysis. The bootstrapped ICERs of the meta-analysis have been omitted from the figure to improve visuality of the plot.



Supplementary Figure 5. Willingness-to-pay curve sensitivity analysis - Scenario 2
The curve represents the probability that SDD is below different thresholds of maximum acceptability prices to pay per one averted in-hospital death, **in case of an increase in medication by a factor 5**. The willingness-to-pay thresholds corresponding to 90.0% and 95.0% probabilities are €100,148 and €134,849. Corresponding minimum numbers of QALYs gained per averted in-hospital death in order for SDD to be cost-effective at the Dutch formal threshold of maximum €80,000 per QALY, are 1.25 and 1.69, respectively.



Supplementary Figure 6. Cost-effectiveness plane sensitivity analysis - Scenario 3
The red and green points represent the bootstrapped ICERs of the De Smet and Oostdijk trial, respectively, **in case of choosing amphotericin B instead of nystatin as the antifungal component of SOD and SDD**. The coloured ellipses represent the 95% CI of the corresponding study. The black ellipse represents the 95% CI for the fixed effect meta-analysis. The bootstrapped ICERs of the meta-analysis have been omitted from the figure to improve visuality of the plot.



Supplementary Figure 7. Willingness-to-pay curve sensitivity analysis - Scenario 3
The curve represents the probability that SDD is below different thresholds of maximum acceptability prices to pay per one averted in-hospital death, **in case of choosing amphotericin B instead of nystatin as the antifungal component of SOD and SDD.** The willingness-to-pay thresholds corresponding to 90.0% and 95.0% probabilities are €68,924 and €94,591. Corresponding minimum numbers of QALYs gained per averted in-hospital death in order for SDD to be cost-effective at the Dutch formal threshold of maximum €80,000 per QALY, are 0.86 and 1.18, respectively.

3

The added value of the selective SuperPolymyxin™ medium in detecting rectal carriage of Gram-negative bacteria with acquired colistin resistance in intensive care unit patients receiving selective digestive decontamination

Denise van Hout, Axel Janssen, Rob Rentenaar, Judith Vlooswijk, Edwin Boel and Marc Bonten

Eur J Clin Microbiol Infect Dis. 2019

ABSTRACT

Objectives: To determine the value of using SuperPolymyxin™ selective medium (ELITech Group, Puteaux, France) in addition to conventional non-selective inoculation methods in the detection of acquired colistin resistance in a Dutch intensive care unit (ICU) that routinely uses selective decontamination of the digestive tract (SDD).

Methods: We performed a cross-sectional study with prospective data collection in a tertiary-care ICU. All consecutive surveillance rectal swabs of ICU-patients receiving SDD were included and cultured in an observer-blinded approach using: (1) a conventional culture method using non-selective media and (2) SuperPolymyxin™ selective medium. MIC values for colistin of non-intrinsically colistin-resistant Gram-negative isolates were determined with broth microdilution (BMD) using Sensititre™ and colistin resistance was confirmed using BMD according to EUCAST guidelines.

Results: 1105 rectal swabs of 428 unique ICU-patients were inoculated using both culture methods, yielding 346 and 84 Gram-negative isolates for BMD testing with the conventional method and SuperPolymyxin™ medium, of which 308 and 80 underwent BMD, respectively. The number of identified rectal carriers of isolates with acquired colistin resistance was 3 (0.7%) for the conventional method, 4 (0.9%) for SuperPolymyxin™, and 5 (1.2%) for both methods combined. The number of isolates with acquired colistin resistance was 4 (1.0%) for the conventional method, 8 (2.1%) for SuperPolymyxin™ and 9 (2.3%) for both methods combined.

Conclusion: In a surveillance setting of low prevalence of acquired colistin resistance in patients that receive SDD in a Dutch tertiary-care ICU, SuperPolymyxin™ had a higher diagnostic yield than conventional inoculation methods, but the combination of both had the highest diagnostic yield.

INTRODUCTION

Selective digestive decontamination (SDD) is a preventive antibiotic regimen that contains, among others, colistin as one of the topical components. SDD has been shown to reduce intensive care unit (ICU)-acquired infections and mortality in settings with low levels of antimicrobial resistance and is therefore standard of care in the Netherlands for ICU-patients. [1,2] Colistin is increasingly regarded as a last-resort antibiotic against infections with multidrug-resistant Gram-negative bacteria (GNB). [3] Dissemination of colistin resistance among GNB already resistant to other classes of antibiotics could potentially limit treatment options for patients infected with multidrug-resistant GNB, emphasizing the importance of optimizing surveillance and laboratory detection of colistin resistance.

Testing for phenotypic colistin susceptibility however, is problematic. [4-8] A 2016 joint CLSI-EUCAST Working Group recommended that only broth microdilution (BMD) methods be used for testing of colistin susceptibility. [9] However, BMD requires manual preparation leading to potential errors, is labour-intensive and is difficult to implement in many routine clinical microbiology laboratories. Previous studies have suggested that the use of the commercially available SuperPolymyxin™ selective medium (ELITech Group, Puteaux, France) may improve the detection of colistin-resistant GNB in surveillance samples. [10,11]

The aim of the current study was to determine the added value of using SuperPolymyxin™ in addition to the conventional screening method with non-selective media in the detection of rectal carriage with acquired colistin-resistant GNB in a Dutch tertiary-care ICU that routinely uses SDD.

MATERIALS AND METHODS

Study design

A cross-sectional study with prospective data collection was performed from 9-7-2018 until 24-1-2019 in a 40-bed ICU of a tertiary care hospital in the Netherlands (University Medical Center Utrecht, Utrecht). All consecutive rectal swabs of ICU-patients taken during routine SDD surveillance were included and were taken at ICU-admission and twice weekly thereafter until ICU-discharge. Swabs were excluded in case of missing inoculation in either method. Ethical approval of patients was not deemed applicable because this was a laboratory quality improvement study and only anonymized medical microbiology data were used.

Use of conventional non-selective media and the SuperPolymyxin™ medium

Rectal swabs were first inoculated on non-selective media (conventional method): tryptic soy, 5% sheep blood agar (BA, BD254087, Becton Dickinson, Erembodegem, Belgium), secondly on non-selective MacConkey agar (McC, BD257286) and thirdly on Malt extract agar (MEA, in-house manufactured). Fourthly, the swab was inoculated on the selective SuperPolymyxin™ medium. Plates were incubated at 37°C for 48 hours either in 5% CO2 (BA), or in ambient air (McC, MEA, SuperPolymyxin™). All plates were visually examined after 24 and 48 hours of incubation. Technicians that visually inspected growth on the conventional method were blinded for results of the SuperPolymyxin™ medium, and vice versa. All different colony morphologies that were suspected of being GNB were subjected to species identification by MALDI-TOF MS (Bruker, Bremen, Germany) and all Gram-negative isolates were stored at -80°C.

Colistin broth microdilution

MIC determination was performed on all Gram-negative isolates of species that are not intrinsically resistant to colistin using Sensititre™ FRCOL plates (Thermo Fisher Scientific, Wesel, Germany) according to the manufacturer's instructions. *E. coli* ATCC25922 and *E. coli* NCTC13846 were included as control strains daily. Colistin MICs were interpreted according to EUCAST 2019 guidelines. [12] Colistin resistance of isolates that were tested colistin-resistant with Sensititre™ was confirmed using a broth microdilution method in line with EUCAST guidelines. [9,13] *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* were excluded from analyses because of missing EUCAST and CLSI susceptibility breakpoints for colistin.

Mcr-gene detection

Genomic sequences of the isolates that tested colistin-resistant with BMD were subjected to screening for *mcr*-genes using ResFinder 3.2. [14]

Statistical analyses

Contingency tables were made for the conventional method compared to SuperPolymyxin™ in the detection of ICU-patients with a rectal swab positive for ≥1 isolate that exhibited acquired colistin resistance at any time point during ICU-stay and rectal swabs positive for ≥1 isolate that exhibited acquired colistin resistance. Acquired colistin resistance was defined as colistin resistance determined with BMD in species that are usually susceptible to colistin. Isolates from a single rectal swab with different colony morphologies on the SuperPolymyxin™ plate but belonging to identical species and with similar colistin MICs were counted only once. The number of isolates that grew on SuperPolymyxin™ but were colistin-susceptible with BMD using Sensititre™ (i.e. false-resistant result) was reported, as well as the number of colistin-resistant

isolates that was found in the conventional method but did not grow on SuperPolymyxin™ medium (i.e. false-susceptible result).

The value of SuperPolymyxin™ as a screening method for routine colistin susceptibility testing was examined by comparing the use of SuperPolymyxin™ with implementation of routine colistin BMD using Sensititre™. Three scenarios were compared: 1) implementation of routine colistin Sensititre™ BMD for all Gram-negative isolates detected in the conventional (non-selective) method, 2) addition of SuperPolymyxin™ to the current laboratory pipeline and performing colistin Sensititre™ BMD on all isolates detected in either the conventional method or SuperPolymyxin™ and 3) addition of SuperPolymyxin™ to the current pipeline and only performing colistin Sensititre™ BMD on isolates detected through SuperPolymyxin™ (i.e. using SuperPolymyxin™ as a screening medium). We calculated maximum costs per SuperPolymyxin™ plate for the use of SuperPolymyxin™ as a screening medium to be under the costs of performing colistin BMD using Sensititre™ on all isolates detected with the conventional method.

This was a pragmatic study without a formal sample size calculation; the aim was to include 1000 rectal swabs. All analyses were performed with Statistical Package for Social Sciences V.25.0 (SPSS, Chicago, Illinois, USA).

RESULTS

ICU-patients, rectal swabs and Gram-negative isolates for colistin broth microdilution

A total of 1105 rectal swabs of 428 unique ICU patients were included (Fig. 1). The conventional method and SuperPolymyxin™ medium yielded 308 and 77 Gram-negative isolates that were tested with colistin BMD using Sensititre™ and were included in further analyses, respectively (Fig. 1).

Diagnostic yield

The number of carriers and positive rectal swabs was highest when combining results of both methods (Table 1 and 2). Colistin susceptibility pattern was reclassified from resistant into susceptible in 2 isolates after BMD with cation-adjusted Mueller Hinton broth (1 *E. coli* and 1 *P. aeruginosa*). The number of isolates with acquired colistin resistance was 4 (4/385 = 1.0%) with the conventional method, 8 (8/385 = 2.1%) with SuperPolymyxin™ medium and 9 (9/385 = 2.3%) with both methods combined (Table 1 and 3). In total, 373 colistin-susceptible isolates were identified, of which 69 (18.5%, 95% CI 14.9%–22.8%) grew on the SuperPolymyxin™ medium. Of the 9 unique isolates with acquired colistin resistance, 1 (11.1%, 95% CI 2.0%–43.5%) did not exhibit growth on SuperPolymyxin™ (Table 3 and Supplementary material).

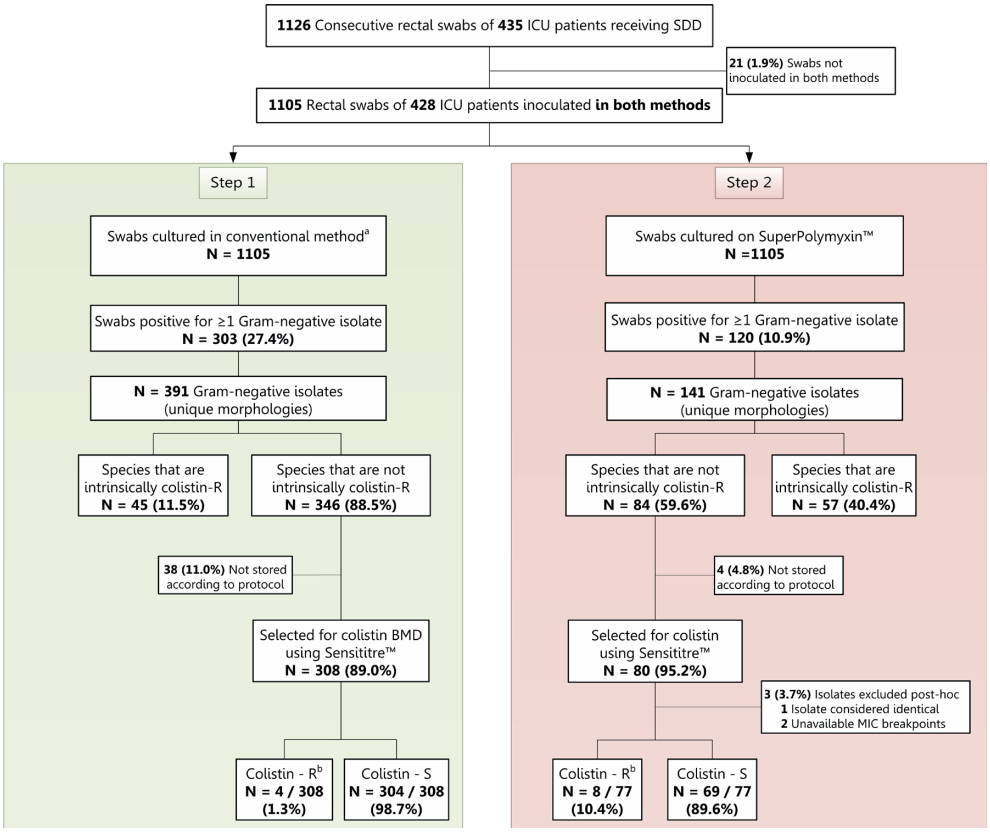


Figure 1. Study flowchart. BMD, broth microdilution; ICU, intensive care unit; McC, MacConkey agar; MIC, minimum inhibitory concentration; MEA, Malt extract agar; R, resistant; S, susceptible; SDD, selective digestive decontamination.
a. The conventional method consisted of inoculation on non-selective Blood (BA) and MacConkey (McC) agar and Malt extract agar. Only BA and McC were used for isolation of Gram-negative isolates.
b. Acquired colistin resistance was confirmed using a broth microdilution method in line with EUCAST guidelines [9,13] (see Methods section).

Characteristics of isolates with acquired colistin resistance

Colistin MICs of isolates with acquired colistin resistance ranged from 4µg/mL to >128µg/mL (Table 3). One *E. coli* isolate tested positive for *mcr*-1; this isolate was identified with SuperPolymyxin™ and had an colistin MIC of 8µg/mL. No other *mcr*-genes were identified.

Added value of SuperPolymyxin™ medium as screening method

A strategy of BMD testing of all isolates that grew on non-selective media and/or the SuperPolymyxin™ medium (Scenario 2, Fig. 2) would have the highest diagnostic yield and would require 430 Sensititre™ BMD tests.

Table 1. Diagnostic yield per inoculation method

	Conventional method[a]	SuperPolymyxin™
ICU Patients	N = 428 (%)	N = 428 (%)
Rectal carriers of GNB with acq. colistin resistance	3 (0.7)	4 (0.9)
Rectal swabs	N = 1105 (%)	N = 1105 (%)
Rectal swabs with ≥1 GNB with acq. colistin resistance	4 (0.4)	8 (0.7)
Colistin-resistant isolates		
Species intrinsically colistin-resistant	N = 45 (%)	N = 57 (%)
Proteus mirabilis	23 (51.1)	19 (33.3)
Morganella morganii	7 (15.6)	16 (28.1)
Serratia marcescens	8 (17.8)	11 (19.3)
Providencia rettgeri	4 (8.9)	3 (5.2)
Hafnia alvei	1 (2.2)	4 (7.0)
Ochrobactrum intermedium	1 (2.2)	2 (3.5)
Proteus vulgaris	1 (2.2)	1 (1.8)
Providencia species	-	1 (1.8)
Species non-intrinsically colistin-resistant	N = 4 (%)	N = 8 (%)
Escherichia coli	2 (50.0)	6 (75.0)
Klebsiella aerogenes	1 (25.0)	2 (25.0)
Enterobacter asburiae	1 (25.0)	-

acq, acquired; GNB, Gram-negative bacteria; ICU, intensive care unit

[a] The conventional method consisted of inoculation on non-selective Blood (BA) and MacConkey (McC) agar and Malt extract agar. Only BA and McC were used for isolation of Gram-negative isolates.

A strategy in which only isolates identified with SuperPolymyxin™ would undergo Sensititre™ BMD would require 84 BMD tests, a reduction of 75.7% (1-84/346) (Scenario 3 versus 1), at the cost of 1 missed isolate with acquired colistin resistance. Not using SuperPolymyxin™ medium (Scenario 1) would require 346 Sensititre™ BMD tests (19.5% less than when also using SuperPolymyxin™ medium) and would have let to 5 missed isolates with acquired colistin resistance (including 1 *mcr*-1 positive *E. coli*). Considering the direct costs of Sensititre™ BMD (in our laboratory being €21.45 per test), addition of SuperPolymyxin™ to the conventional inoculation methods as a screening medium (Scenario 3) would be cheaper than performing Sensititre™ BMD on all isolates that grew in the conventional method (Scenario 1) if the costs of adding SuperPolymyxin™ would not exceed €5.09 per Sensititre™ BMD test (including laboratory technician time) (see Supplementary material).

Table 2. Comparison of the conventional method[a] and SuperPolymyxin™ medium in the detection of rectal carriers of Gram-negative isolates that exhibited acquired colistin resistance and rectal swabs positive for Gram-negative isolates that exhibited acquired colistin resistance

			SuperPolymyxin™	
Conventional method[a]	*A. ICU patients*	Carrier	Non-carrier	
	Carrier	**2**	**1**	3
	Non-carrier	**2**	**423**	425
		4	424	
	B. Rectal swabs	Positive	Negative	
	Positive	**3**	**1**	4
	Negative	**5**	**1096**	1101
		8	1097	

[a] The conventional method consisted of inoculation on non-selective Blood (BA) and MacConkey (McC) agar and Malt extract agar. Only BA and McC were used for isolation of Gram-negative isolates. ICU, intensive care unit
A. Number of detected rectal carriers and non-carriers of ≥1 non-intrinsically colistin-resistant isolate with acquired colistin resistance. B. Number of swabs detected that were positive or negative for ≥1 non-intrinsically colistin-resistant isolate with acquired colistin resistance.

Table 3. Culture results from the conventional method and SuperPolymyxin™ medium for all rectal swabs with growth of acquired colistin-resistant Gram-negative isolates

PT	**Swab**	**Date**	**Growth on conventional method[a]**			**Growth on SuperPolymyxin™**		
			Species	Colistin MIC[b] (µg/mL)	Colistin profile	Species	Colistin MIC[b] (µg/mL)	Colistin profile
27	282	30-08-2018	*E. coli*	0.5	S	-	-	-
			-	-	-	*E. coli*[c]	8	R
			K. pneumoniae	1	S	*K. pneumoniae*	1	S
31	643	01-11-2018	*E. coli*	16	R	*E. coli*	16	R
	661	05-11-2018	*E. coli*	NA	NA	*E. coli*	16	R
	681	08-11-2018	*E. coli*	NA	NA	*E. coli*	16	R
	722	14-11-2018	*E. coli*	16	R	*E. coli*	16	R
37	146	06-08-2018	*K. aerogenes*	0.5	S	-	-	-
			-	-	-	*K. aerogenes*	32	R
	177	09-08-2019	*K. aerogenes*	32	R	*K. aerogenes*	32	R
			-	-	-	*M. morganii*	-	R
307	331	07-09-2018	*E. asburiae*	>128	R	-	-	-
			E. coli	0.5	S	-	-	-
311	636	01-11-2018	*E. coli*	NA	NA	*E. coli*	4	R

MIC, minimum inhibitory concentration; NA, not available (i.e. not stored); PT, patient; S, sensitive; R, resistant. Total growth of all rectal swabs with growth of a Gram-negative isolate with acquired colistin resistance is presented (including intrinsically colistin-resistant and/or colistin-sensitive Gram-negative isolates, if these grew on the rectal swabs). Grey background indicates discordant growth of isolates with acquired colistin resistance between the two methods.

[a] The conventional method consisted of inoculation on non-selective Blood (BA) and MacConkey (McC) agar and Malt extract agar. Only BA and McC were used for isolation of Gram-negative isolates.

[b] Colistin MICs by using the commercial Sensititre™ broth microdilution method. Presence of acquired colistin resistance was confirmed using a broth microdilution method in line with EUCAST guidelines (see Methods section).

[c] Tested positive for *mcr*-1.

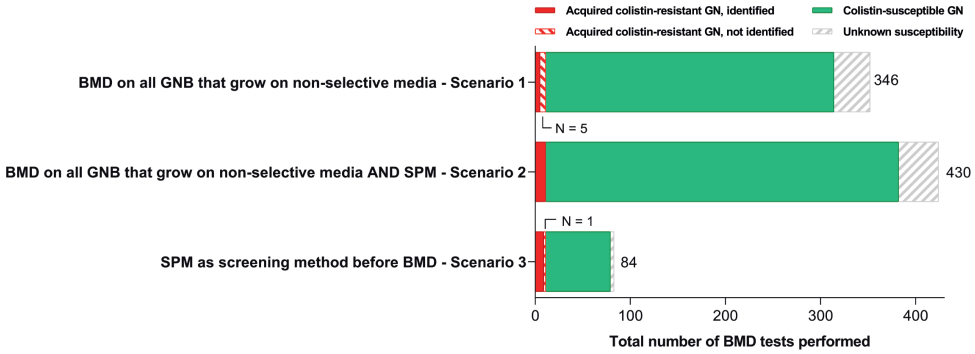


Figure 2. Different scenarios for implementing colistin BMD using Sensititre™ in the current laboratory pipeline. BMD, broth microdilution; GN, Gram-negative isolates; SPM, SuperPolymyxin™ medium

DISCUSSION

In this prospective study, embedded in routine surveillance of ICU-patients that receive SDD, the combined use of conventional inoculation methods and selective SuperPolymyxin™ medium had the highest diagnostic yields in detecting rectal carriers of isolates that exhibited acquired colistin resistance, rectal swabs positive for isolates that exhibited acquired colistin resistance and the total number of detected colistin-resistant Gram-negative isolates.

Previous studies that assessed the diagnostic performance and applicability of the commercial SuperPolymyxin™ medium in routine screening reported varying results. [10,15,16] In one study, rectal swabs were spiked with 94 well-characterized Enterobacterales (of which 53 with acquired colistin resistance) and sensitivity and specificity of the SuperPolymyxin medium™ were 86.8% (95% CI 74.0%–94.0%) and 97.5% (95% CI 85.6%–99.9%), respectively. [16] In another study 100% (33/33) sensitivity and 90.3% (56/62) specificity of SuperPolymyxin™ were reported. [10] In the current study, we did not aim to determine sensitivity and specificity of the test. However, we did find one false-negative result of the SuperPolymyxin™ medium (11.1%, 95% CI 2.0%–43.5%). A possible explanation could be that SuperPolymyxin™ was inoculated as the fourth medium, potentially leading to reduced bacterial loads on rectal swabs upon inoculation of SuperPolymyxin™. Our inoculation method might bias our results towards false-negative results of SuperPolymyxin™ in case of low density inocula. However, it is currently standard procedure to inoculate plates directly from rectal swabs, with a standard order from non-selective media to selective media. Thus, this is how SuperPolymyxin™ medium would be used in our routine practice, which was the main research aim of the current study.

Strengths of the current study were the prospective data collection and study design, which was embedded in our current routine laboratory pipeline of SDD surveillance. To decrease observer bias, all observers were blinded for the results of the alternative method. One of the study limitations was the number of isolates that were not stored according to protocol, as one of the technicians was not aware of the study instruction to store all Gram-negative isolates that grew on either method. Another limitation was that the total amount of identified isolates with acquired colistin resistance was relatively low. It is known that the performance of SuperPolymyxin™ is different for different Gram-negative species, so it is important to note that some important species were not encountered during our study period (i.e. *Salmonella* sp., *Acinetobacter baumannii*). [10] This could have influenced the determination of error rates of the SuperPolymyxin™ medium (in unknown direction). Use of an enrichment broth might have increased diagnostic yield, however this was not included as part of the current study. Lastly, this study was performed in a single-center ICU that routinely uses SDD and results therefore may not be generalizable to all other clinical settings.

We tested a two-step approach, in which screening through SuperPolymyxin™ was followed by colistin BMD testing using Sensititre™. Naturally, it is important to consider both additional value and costs before implementing new diagnostic tools. The added value depends on the aim of colistin susceptibility testing (i.e. research, surveillance, or direct patient care), the clinical impact of identifying colistin resistance and the impact of potential missed cases. In our setting with low prevalence of colistin resistance, total diagnostic yield will always be low. Still, given the resource-dense nature of colistin BMD testing, results of the current study support the use of colistin-selective media as a screening method in case of daily large numbers of screening samples, such as in our SDD surveillance setting. Future research could determine the value of SuperPolymyxin™ in other settings, for example in laboratories in which colistin BMD testing is already part of routine practice or in countries with higher prevalence of colistin resistance.

In conclusion, in a routine surveillance setting of ICU-patients that receive SDD, the combined use of non-selective media and selective SuperPolymyxin™ medium had the highest diagnostic yield in detecting Gram-negative isolates with acquired colistin resistance. However, overall prevalence of acquired colistin resistance was low.

List of abbreviations

BA, blood agar; BMD, broth microdilution; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee for Antimicrobial Susceptibility Testing; GNB, Gram-negative bacteria; ICU, intensive care unit; McC, MacConkey; MEA, malt extract agar; MIC, minimal inhibitory concentration; NA, not available; R, resistant; S, susceptible; SDD selective digestive decontamination; SPM, SuperPolymyxin™ medium

Acknowledgements: We would sincerely like to thank Sjoukje van Gorkum and Arjen Cornelisse for their part in the laboratory execution of this study. Preliminary results of this paper were presented at the 29th European Congress of Clinical Microbiology & Infectious Diseases, Amsterdam, the Netherlands, 13-16 April 2019 (P1401).

Funding and conflicts of interest: The SuperPolymyxin™ medium was supplied free of charge by the manufacturer. All authors guarantee that the supplying company did not have any part in study design, data collection, data-analyses, interpretation of the data, preparation of the manuscript and the decision to submit the manuscript for publication. All authors report no other conflicts of interests.

REFERENCES

1. Plantinga NL, de Smet AMGA, Oostdijk EAN, de Jonge E, Camus C, Krueger WA, ***et al.*** Selective digestive and oropharyngeal decontamination in medical and surgical ICU patients: individual patient data meta-analysis. ***Clin Microbiol Infect*** 2018;24:505–13. doi: 10.1016/j.cmi.2017.08.019.
2. Stichting Werkgroep Antibioticabeleid (SWAB). SWAB Richtlijn: selectieve decontaminatie bij patiënten op de intensive care. 2018;1–29. doi: https://nvic.nl/swab-richtlijn-sdd.
3. Mendelson M, Brink A, Gouws J, Mbelle N, Naidoo V, Pople T, ***et al.*** The One Health stewardship of colistin as an antibiotic of last resort for human health in South Africa. ***Lancet Infect Dis*** 2018;18:e288-94. doi: 10.1016/S1473-3099(18)30119-1.
4. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. ***Clin. Microbiol. Rev.*** 2017;30:557–96. doi: 10.1128/CMR.00064-16.
5. Karvanen M, Malmberg C, Lagerback P, Friberg LE, Cars O. Colistin Is Extensively Lost during Standard In Vitro Experimental Conditions. ***Antimicrob. Agents Chemother.*** 2017;61:e00857-17. doi: 10.1128/AAC.00857-17.
6. Jayol A, Nordmann P, Lehours P, Poirel L, Dubois V. Comparison of methods for detection of plasmid-mediated and chromosomally encoded colistin resistance in Enterobacteriaceae. ***Clin. Microbiol. Infect.*** 2018;24:175–9. doi: 10.1016/j.cmi.2017.06.002.
7. Vourli S, Dafopoulou K, Vrioni G, Tsakris A, Pournaras S. Evaluation of two automated systems for colistin susceptibility testing of carbapenem-resistant *Acinetobacter baumannii* clinical isolates. ***J. Antimicrob. Chemother.*** 2017;72:2528–30. doi: 10.1093/jac/dkx186.
8. Tan TY, Ng SY. Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin. Clin. Microbiol. Infect. 2007;13:541–4. doi: 10.1111/j.1469-0691.2007.01708.x.
9. The European Committee on Antimicrobial Susceptibility Testing. Joint EUCAST and CLSI recommendation - Recommendations for colistin (polymyxin E) MIC testing. 2016. doi: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf.
10. Jayol A, Poirel L, Andre C, Dubois V, Nordmann P. Detection of colistin-resistant Gram-negative rods by using the SuperPolymyxin medium. ***Diagn. Microbiol. Infect. Dis.*** 2018;92:95–101. doi:10.1016/j.diagmicrobio.2018.05.008.
11. Nordmann P, Jayol A, Poirel L. A Universal Culture Medium for Screening Polymyxin-Resistant Gram-Negative Isolates. ***J. Clin. Microbiol.*** 2016;54:1395–9. doi: 10.1128/JCM.00446-16.
12. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. 2019. doi: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf.
13. Andrews JM. Determination of minimum inhibitory concentrations. ***J. Antimicrob. Chemother.*** 2001;48 Suppl 1:5–16. doi: 10.1093/jac/48.suppl_1.5.
14. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, ***et al.*** Identification of acquired antimicrobial resistance genes. ***J. Antimicrob. Chemother.*** 2012;67:2640–4. doi: 10.1093/jac/dks261.
15. Przybysz SM, Correa-Martinez C, Kock R, Becker K, Schaumburg F. SuperPolymyxin Medium for the Screening of Colistin-Resistant Gram-Negative Bacteria in Stool Samples. ***Front. Microbiol.*** 2018;9:2809. doi: 10.3389/fmicb.2018.02809.
16. Girlich D, Naas T, Dortet L. Comparison of the Superpolymyxin and ChromID Colistin R Screening Media for the Detection of Colistin-Resistant Enterobacteriaceae from Spiked Rectal Swabs. ***Antimicrob. Agents Chemother.*** 2018;63:pii: e01618-18. doi: 10.1128/AAC.01618-18.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Performance of the SuperPolymyxin™ medium in the detection of Gram-negative isolates with acquired colistin resistance

		Colistin resistance as determined with BMD[a]		
		+	-	
Growth on SuperPolymyxin™	+	**8**	**69**	77
	-	**1**	**304**	305
		9[b]	373	382

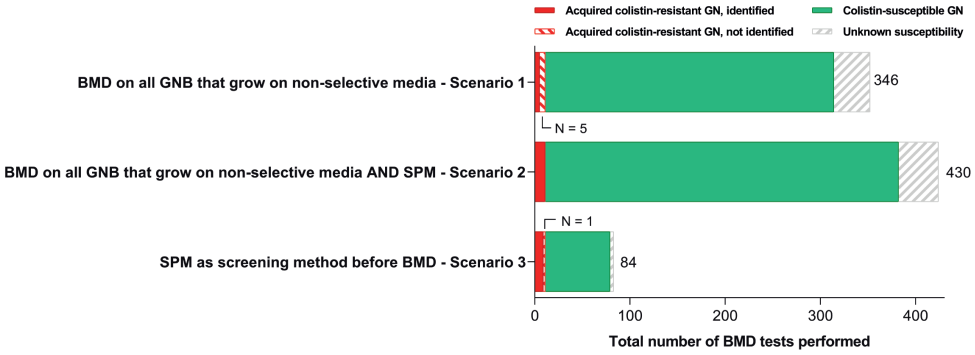
BMD, broth microdilution

[a] BMD was only performed on Gram-negative isolates that are not intrinsically resistant to colistin and was performed by first using Sensititre™ on all isolates, followed by BMD using Mueller Hinton cation-adjusted broth for the isolates that were tested colistin resistant with Sensititre™.

[b] Unique Gram-negative isolates with acquired colistin resistance (i.e. the 3 colistin-resistant isolates that were detected in both methods were only counted once in this table)

Supplementary Table S2. Cost analysis different scenarios of implementing colistin broth microdilution

Scenario 1: Implementation of routine colistin broth microdilution (BMD) on all Gram-negative isolates detected in the conventional inoculation method.
Scenario 2: Addition of SuperPolymyxin™ medium (SPM) to the current laboratory pipeline and performing colistin BMD on all isolates detected in either the conventional method or SuperPolymyxin™ medium.
Scenario 3: Addition of the SuperPolymyxin™ medium to the current laboratory pipeline and only performing colistin BMD on isolates detected through SuperPolymyxin™ (i.e. using SuperPolymyxin™ medium as a screening medium).



Notes:
1. Costs of conventional inoculation methods are considered the same in each scenario; because SuperPolymyxin would be added to the current pipeline and would never "replace' all conventional methods.
2. The costs per colistin BMD test (21.45 Euro) are based on internal UMCU calculations and are available from the corresponding author. These costs include material, lab technician time, overhead, etc.
3. The current cost analysis is based on the number of isolates that were found during the study period: 9-7-2018 until 24-1-2019 (approx 6.5 months).

	Scenario 1			**Scenario 2**			**Scenario 3**		
	N tests performed	*Costs per test*	*Total costs*	*N tests performed*	*Costs per test*	*Total costs*	*N tests performed*	*Costs per test*	*Total costs*
Costs SPM (hypothetical)	0	0	0	1105	€5.09	€5619.90	1105	€5.09	€5619.90
Costs BMD	346	€21.45	€7421,70	430	€21.45	€9223.50	84	21.45	€1801.80
Total costs			**€7421.70**			**€14843.40**			€**7421.70**

Interpretation:
1. If the SPM medium would be 5.09 Euro per plate, including lab technician time, Scenario 1 would be equal in costs as compared to Scenario 3.
2. If the SPM medium would be <5.09 Euro per plate (including material, lab technician time, etc.), then Scenario 3 would be cheaper as compared to Scenario 1.
3. Scenario 2 is the most expensive scenario

Universal risk assessment upon hospital admission for screening of carriage with multidrug-resistant microorganisms (MDRO) in a Dutch tertiary care centre (2016 – 2019)

Denise van Hout, Patricia Bruijning-Verhagen, Hetty Blok, Annet Troelstra and Marc Bonten

Submitted

ABSTRACT

Background

In Dutch hospitals a 6-point questionnaire is mandatory for risk-assessment to identify carriers of multidrug-resistant organisms (MDRO) at the time of hospitalization. Presence of one or more risk factors is followed by microbiological culturing and pre-emptive isolation. We evaluated the test characteristics of this screening tool in identifying new MDRO carriers.

Methods

A cross-sectional study using routinely collected healthcare data was performed in a Dutch tertiary hospital between 1 January 2015 and 1 August 2019 including all admissions with an MDRO risk assessment performed on the day of admission. MDRO risk-assessment included: (1) known MDRO carriage, (2) previous hospitalization in another Dutch hospital during a known outbreak, (3) previous hospitalization in a foreign hospital, (4) living in an asylum centre, (5) professional exposure to livestock farming and (6) household membership of a meticillin-resistant *Staphylococcus aureus* (MRSA) carrier. Sensitivity of the risk assessment was estimated by comparing observed prevalence of newly detected MDRO carriage to expected prevalence of carriage in the Dutch population upon hospital admission.

Results

144,051 hospital admissions of 84,485 unique patients were included. In total, 4,480 (3.1%) admissions had a positive MDRO risk-assessment (i.e. ≥1 risk factors present). In 1,516 (34%) admissions microbiological screening was performed, of which 341 (23%) yielded MDRO. 81 patients were categorized as new MDRO carriers, as identified through MDRO risk-assessment, reflecting 0.06% (95% CI: 0.04%–0.07%) of all admissions and 1.8% (95% CI: 1.4%–2.2%) of those with positive risk assessment. MDRO included ESBL-producing and/or multidrug-resistant Enterobacterales (n=52, 64%), MRSA (n=26, 32%), carbapenem-resistant Enterobacterales (CRE) (n=2, 3%) and VRE (n=1, 1%). The numbers of "MDRO risk-assessments needed to perform" and individual "MDRO risk-assessment questions needed to ask" to detect one new MDRO carrier upon admission were 1,778 and 10,420, respectively. Estimated sensitivities of the risk-assessment for detecting MDRO carriage were <1%, for ESBL-E and VRE, <2% for CRE and 18% for MRSA.

Conclusions

The number of risk-assessments needed to perform to detect one new MDRO carrier upon hospital admission was high, and the vast majority of carriers most likely remained undetected. The current MDRO risk assessment upon admission strategy needs thorough reconsideration.

BACKGROUND

Dissemination of multidrug-resistant micro-organisms (MDRO) in healthcare settings may lead to more infections caused by MDRO, which may reduce effectiveness of empirical antibiotic therapy. [1-4] The hospital setting facilitates patient-to-patient transmission of MDRO because of the high antibiotic selective pressure, frequent contact between healthcare workers and patients and vulnerability of patients to acquire carriage with MDRO. Optimizing control strategies is, therefore, important to prevent dissemination and associated risks of infections caused by MDRO. Hospital-based surveillance is recommended for timely detection of MDRO carriage and installation of transmission-based contact precautions. In the Netherlands, hospitals have adopted a risk-based screening for asymptomatic MDRO carriage upon admission. This originated in the mid-1980s to control the emergence of meticillin-resistant *S. aureus* (MRSA), as one of the elements of the Dutch 'search and destroy' strategy. [5-10] Over the years, this risk-based screening was extended to also control other MDRO, such as multidrug-resistant Gram-negative bacteria (MDR-GNB). [11,12] MDRO risk assessment is, for each patient, based on a 6-point questionnaire that needs to be checked upon admission. These questions include risk factors for carriage of MRSA, MDR-GNB, and vancomycin-resistant enterococci (VRE). In patients at risk of MDRO carriage, according to this screening, pre-emptive contact precautions should be installed and screening cultures should be obtained. Adherence to this strategy is monitored by the Dutch Healthcare Inspectorate. Yet, this approach requires time for questioning patients, pre-emptive isolation measures that may affect care of other patients and resources for microbiological testing. The benefits of the strategy have not yet been quantified.

The aim of the current study was to evaluate the current risk assessment for screening of MDRO carriage upon hospital admission in a Dutch tertiary care hospital. We, therefore, determined the number of newly identified MDRO carriers and the number of questions needed to ask to identify one new MDRO carrier. We also compared the detected prevalence of MDRO carriage with the expected prevalence of MDRO carriage in the Dutch population upon hospital admission.

METHODS

Study design

This observational study was performed in the University Medical Center Utrecht (UMCU) in the Netherlands. The UMCU is a tertiary care medical centre with 1,042 beds for adults and children, all medical specialties represented and around 180,000 inpatient days per year. A cross-sectional study using routinely collected healthcare data was performed of all hospital admissions

between 1 January 2015 and 1 August 2019. For this study we extracted data from all hospital admissions with completion of the MDRO risk assessment in the electronic medical record (EMR) on the same day as hospitalization. A hospital admission was defined as any admission to any ward, including admissions for single-day treatments, and for all ages. Characteristics available per admission were age, sex and length of stay (LOS). Results of this study were reported following the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) criteria. [13]

MDRO risk assessment

The screening strategy consisted of two consecutive steps. Step one was an individual 6-item risk assessment for MDRO carriage. The six questions referred to (1) known MDRO carriage, (2) previous hospitalization in another Dutch hospital during the past 2 months with an ongoing outbreak during hospitalization, (3) previous hospitalization in a foreign hospital in the past 2 months, (4) living in an asylum shelter, (5) professional exposure to livestock farming (i.e. living pigs, veal calves or broilers), and (6) living with a known MRSA carrier (the entire questionnaire is provided in Table S1). The MDRO-assessment was obligatory and embedded in the EMR, to be completed within 24 hours for each patient admitted, visiting the emergency department or out-patient clinic for pre-operative screening. Answers of the assessment remained valid for 62 days after completion and answers were automatically completed if a new assessment was started within this time-window. In case of more than one MDRO-assessment obtained on the day of admission, only the first one was used for the current study. A positive MDRO risk assessment was defined as at least one question answered with 'yes'. A positive assessment automatically generated an isolation label in the EMR with a responsive order for pre-emptive contact precautions for that patient. The second step entailed obtaining screening cultures from these patients, unless someone was a known carrier and/or there were culture results with MDRO that had been obtained in the past 2 months. Screening cultures were routinely assessed for growth of MDR-GNB and MRSA; other MDRO were assessed upon indication (e.g. previous carriage, outbreak in previous hospital). If screening cultures yielded MDRO, contact precautions were continued and if not, the EMR isolation label was removed and contact precautions were discontinued. All steps were coordinated semi-automatically by the Infection Prevention (IP) specialists, who manually reviewed positive MDRO-assessments within 24 hours and who modified infection control measures, where needed. IP specialist were also automatically notified in case of any (screening or clinical) culture yielding MDRO and manually assigned isolation labels in the EMR if contact precautions were needed.

Microbiology

We defined screening cultures as nasal, throat, rectal or perineal swabs obtained at the day of admission or the day thereafter in patients with a positive MDRO-assessment (Table S2). MDRO

included MRSA, VRE, extended-spectrum beta-lactamase (ESBL)-producing and/or multidrug-resistant Enterobacterales (ESBL/MDR-E), carbapenem-resistant Enterobacterales (CRE), multidrug-resistant *Acinetobacter* spp (MDR-A), carbapenem-resistant *Acinetobacter* spp (CRA), multi-drug resistant *P. aeruginosa*, cotrimoxazol-resistant *S. maltophilia* and penicillin-resistant *S. pneumoniae* (PSP) (See Table S3 for definitions). The categories ESBL/MDR-E and CRE were mutually exclusive (i.e. strains categorized as ESBL/MDR-E were not carbapenem-resistant, because these were categorized separately). Definitions of MDRO were based on the Dutch Working Party Infection Prevention (WIP) guidelines and were adapted to local definitions of the UMCU if applicable. [12]

Statistical analyses

We determined 'the number of MDRO risk assessments to perform' and 'the number of MDRO-assessment questions needed to ask' to detect one new MDRO carrier upon hospital admission by dividing the total number of admissions and the corresponding MDRO-assessment questions by the total number of newly identified MDRO carriers, respectively. The positive predictive value (PPV) was determined for each of the individual questions of the MDRO-assessment. The PPV was calculated as the number of admissions in which the question was answered positively and screening identified new MDRO carriage, divided by the total number of times the question was answered positively. Naturally, patients admitted might already have an isolation label in the EMR (usually based upon prior culture results), yet, in routine care, such patients are also part of the risk assessment. We, therefore, determined in admissions with a positive MDRO-assessment and with MDRO in screening cultures the presence of prior isolation labels in the EMR. The observed prevalence of detected MDRO carriage through risk assessment was compared to expected MDRO carriage of the Dutch population, based on recent studies (if available; of the last 10 years), to estimate the sensitivity of the risk assessment and the proportion of MDRO carriers that still remained undetected upon admission.

False-positive risk assessment leads to unnecessary (pre-emptive) isolation days until screening cultures turn out to be negative for MDRO. In the absence of our risk assessment strategy, true positives would remain undetected until clinical cultures yield MDRO or until patient discharge. We, therefore, determined the average length of stay until the first clinical culture yielding MDRO for admissions with newly identified MDRO carriage identified through risk assessment. In absence of MDRO in clinical cultures the total duration of hospital stay was used. These days were used as a proxy of the maximum duration of pre-emptive contact precautions gained by the screening strategy. The total number of unjustified isolation days was calculated as the total number of isolation days until negative screening results were available, for which we assumed 0.5 days for MRSA (based upon PCR testing of nasal swabs) and 1.5 days for other MDRO (based upon conventional cultures).

Data were reported with means ± standard deviation (SD), medians with first and third quartile

(Q1-Q3) or percentages, where appropriate. 95% confidence intervals (CI) of proportions were calculated using the Exact method. [14] All statistical analyses were performed with Statistical Package for Social Sciences V.25.0.2 (SPSS, Chicago, Illinois, USA) and R Version 3.4.1.

Ethical statement

This study was performed in line with the Declaration of Helsinki, as revised in 2013. [15] Because this study does not fall under the scope of the Medical Research Involving Human Subjects Act (in Dutch: WMO), the Medical Research Ethics Committee of the UMCU waived the need for official approval by the UMCU Ethics Committee (IRB correspondence number 18-574C) and individual informed consent was not obtained. All data were analysed and stored pseudonymised.

RESULTS

Patient population

In all, 171,974 MDRO assessments of non-cancelled admissions were obtained. As two or more assessments were obtained in 27,923 (16.2%) admissions, exclusion of duplicate assessments led to 144,051 hospital admissions of 84,485 unique patients for analysis (Fig. 1). MDRO risk assessment was performed on the day of admission in 90.3% of hospital admissions. The median age of admissions was 49 years (Q1-Q3 19-67) and 48% were female. Median length of stay (LOS) was 1 day (Q1-Q3 0-4) and 64.6% of all admissions included an overnight stay.

Identification of new MDRO carriers

In total, 4,480 (3.1%) admissions had a positive MDRO-assessment and pre-emptive contact precautions installed, which was mainly based on the presence of known carriage with MDRO (n=3,206, 71.6%) (Table 1). In 1,516 (33.8%) of these admissions screening cultures were obtained, of which 341 (22.5%) yielded MDRO (Fig. 1). Predominant reasons for not obtaining screening cultures were known MDRO carriage status (77.8%) or re-categorization to low risk by IP specialists (13.7%). Of the remaining 253 (8.5%) episodes, discharge was on the same day as admission in 109 (3.7%) and reasons for not obtaining screening cultures were unknown in 144 (4.9%) admissions.

In 260 (76.2%) admissions with MDRO growing in screening cultures an isolation label was already present in the EMR at the time of hospitalization (of which 29 (11.1%) due to a previous risk assessment). In all, 81 admissions (of 81 unique patients) were categorized as newly identified MDRO carriers due to the MDRO risk assessment screening strategy (Fig. 1). This reflects 0.06% (95% CI: 0.04%-0.07%) of all admissions and 1.8% (95% CI: 1.4%-2.2%) of all admissions with

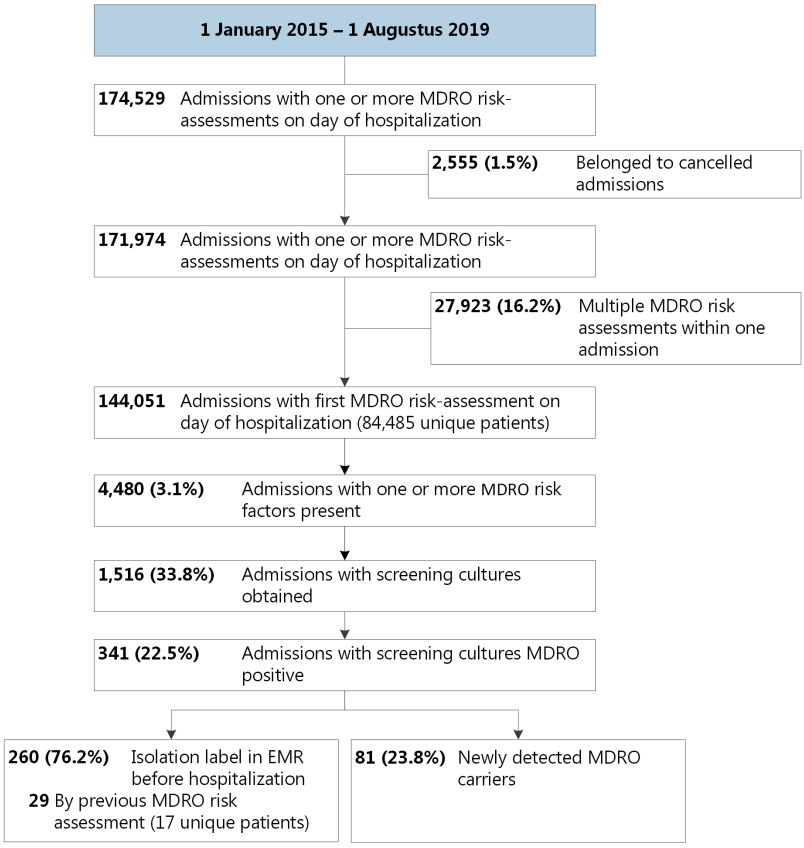


Fig 1. Study flowchart. EMR, electronic medical record; MDRO, multidrug-resistant organism

a positive risk assessment. Of these, 52 (64.2%) carried MDR-E/ESBL-E and 26 (32.1%) carried MRSA. MDR-E isolates (n=33) were resistant to fluoroquinolones and aminoglycosides, but susceptible to carbapenems. The MDRO risk assessment strategy identified CRE carriage (rectal carriage with OXA-48-like *Enterobacter cloacae* and OXA-48 *Klebsiella pneumoniae*, respectively) in two patients with recent hospitalization abroad, and one VRE carrier with known carriage due to screening in another hospital. The number of newly identified MDRO carriers through risk-based screening was stable over time (See Table S4) and the identified MDRO per risk factor is provided in Table S5.

MDRO risk assessment

Positive predictive values of the individual questions for identifying new MDRO carriage ranged from 1.0% (95% CI: 0.7%-1.3%) for "Are you a known carrier of an MDRO?" to 7.0% (95% CI:

Table 1. Admissions with positive MDRO risk assessment, positive MDRO screening cultures and positive predictive value for new identified MDRO carriage per question.

	Answered positively, n (%[a])	Screening cultures obtained, n (%)	New identified MDRO carriage, n (%[b])	PPV (%) (95% CI)	NNA[c]
1. Are you a known carrier of an MDRO (e.g. MRSA, VRE, MDR-GNR)?	3,206 (2.8)	901 (28.1)	31 (3.4)	1.0 (0.7-1.3)	4,647
2. During the past 2 months, were you hospitalized in another Dutch hospital during a known MDRO outbreak?	200 (0.1)	46 (23.0)	3 (6.5)	1.5 (0.5-4.4)	47,798
3. During the past 2 months, were you hospitalized in a foreign hospital?	673 (0.5)	372 (55.3)	34 (9.1)	5.0 (3.9-6.5)	4,228
4. In the past 2 months, did you live in an asylum shelter?	187 (0.2)	106 (56.7)	13 (12.3)	7.0 (4.3-11.1)	8,875
5. Do you work with living pigs, veal calves or broilers?	340 (0.2)	141 (41.5)	8 (5.7)	2.4 (1.2-4.5)	17,919
6. Are you a household member of an MRSA carrier?	116 (0.1)	54 (46.6)	2 (3.7)	1.7 (0.4-6.5)	71,563
One or more of six questions answered positively	4,480 (3.1)	1,516 (33.8)	81 (5.3)	1.8 (1.5-2.2)	1,778

CI, confidence interval; MDRO, multi-drug resistant organism; MDR-GNR, multi-drug resistant Gram-negative rod; MRSA, methicillin-resistant *S. aureus*; NNA, number needed to ask; PPV, positive predictive value; VRE, vancomycin-resistant Enterococcus.

[a] Proportion of the total number of times the particular question was asked. Missings were excluded from the denominator.

[b] Individual column counts count up to >81 because risk assessment could have a positive reply to multiple questions.

[c] "Number of questions needed to ask"; calculated as the total number of times the question was asked divided by the number of newly identified MDRO carriers with a positive reply to this question.

4.3%-11.1%) for "Did you live in an asylum shelter during the past 2 months?" (Table 1). Yet, the number needed to ask of the individual questions to detect one new MDRO carrier ranged from 4,647 for "Are you a known carrier of an MDRO?" to 71,563 for "Are you a household member of an MRSA carrier?". The numbers of 'MDRO risk assessments needed to perform' and individual 'MDRO-questions needed to ask' to detect one new MDRO carrier upon hospital admission were 1,778 (144,041/81) and 10,420 (844,031/81), respectively.

When comparing the observed prevalence of newly identified carriers based on the screening strategy to the perceived prevalence of MDRO carriage upon hospital admission based on recent epidemiological studies in the Netherlands, estimated sensitivities of the risk assessment for detecting MDRO carriage were <1% for ESBL-E and VRE, <2% for CRE and 18.2% for MRSA carriage (Table 2).

Table 2. Sensitivity of risk assessment strategy for detecting MDRO carriage at the time of hospital admission.

	Prevalence of newly identified carriage upon admission by risk-based screening (95% CI) – current study (%)	**Reported prevalence of carriage upon admission in other Dutch studies (%)**	**Estimated proportion detected by risk-based screening (%)**
ESBL-positive Enterobacterales	0.03 (0.02-0.04)	6.4 to 7.0 [16]	0.4 to 0.5
MRSA	0.02 (0.01-0.03)	0.11 [17] to 0.13 [18]	15.4 to 18.2
Carbapenem-R Enterobacterales	0.001 (0.0002-0.005)	<0.06 [19] to 0.25 [20-22]	0.4 to 1.7
VRE	0.0007 (0.00002-0.004)	1.3 [22] to 1.5[a] [23-24]	0.05

ESBL, extended-spectrum beta-lactamase; MDRO, multidrug-resistant organism; MRSA, meticillin-resistant *S. aureus*; R, resistant; VRE, vancomycin-resistant Enterococcus

[a] NB. Estimates for VRE carriage derive from point-prevalence surveys in patients during admission and a population-based study on community intestinal carriage.

MDRO in clinical cultures during hospital stay

In 1,279 (0.9%) hospital admissions clinical cultures yielded MDRO during hospital stay, and 765 (59.8%) of these admissions had negative risk assessments at the time of admission (See Table S6 and S7). In 12 (14.8%) of the 81 admissions with newly identified MDRO carriage, the same type of MDRO was also identified in clinical cultures during hospital stay. For these 12 patients, the median LOS until MDRO detection in clinical cultures was 4 days (Q1-Q3 2–6), and the total number of days was 53. Most clinical cultures were from urine (n=5, 29.4%) (Table S8). The total LOS of the 69 MDRO carriers that would not have been detected without risk-based screening was 513, making 566 days of unprotected ward stay that was prevented by the screening strategy. The total number of unjustified isolation days due false-positive risk assessment was 1,436 days.

DISCUSSION

In this analysis of 144,051 hospital admissions a strategy of risk-based screening for MDRO carriage upon hospital admission identified previously unknown MDRO carriage in 0.06% (95% CI: 0.04%-0.07%) of all admissions and in 1.8% (95% CI: 1.4%-2.2%) of all patients considered to be at high risk of MDRO carriage. The numbers of 'MDRO risk assessments needed to perform' and individual 'MDRO risk assessment questions needed to ask' to detect one new MDRO carrier upon hospital admission were 1,778 and 10,420, respectively. Still, the vast majority of MDRO carriers most likely remained undetected.

The calculated numbers needed to ask are actually even underestimated as 16% of admissions had more than one MDRO risk assessment completed on the same day, and these copy-assessments were excluded from our analysis. If included, the numbers of "MDRO risk assessments actually performed" and "MDRO risk assessment questions actually asked" to detect one new MDRO carrier upon admission would have been 2,123 (171,974/81) and 12,440 (1,007,640/81), respectively. If we, conservatively, estimate one minute of labour time per MDRO risk assessment and one minute for administration, at least 160 36-hour working weeks were spent on performing assessments during these four and a half years. This reflects at least two working weeks spent per newly identified MDRO carrier (160 weeks divided by 81 new carriers).

Newly identified carriers were most often colonized with ESBL-producing and/or Enterobacterales strains resistant to both an aminoglycoside and ciprofloxacin (70%), MDRO of which the value of screening upon admission for the prevention of transmission and hospital-acquired infections is not well-established. [25-27] In our study, the prevalence of newly detected ESBL carriage upon admission was 0.03% (95% CI: 0.02-0.04), which is considerably lower than the prevalence of faecal ESBL carriage in the Dutch community; which was 5% in randomly selected subjects [19] and 6.4% to 7.0% upon admission to our hospital. [16] As a result, in our hospital, the proportion of ESBL carriers that still remained undetected upon admission despite risk-based screening was probably more than 99%. For CRE and VRE the proportion of undetected carriers was equally high, being >98% and >99%, respectively.

The second most common MDRO in new carriers was MRSA (26%), which was identified in 0.02% (95% CI: 0.01%-0.03%) of all admissions. Screening and pre-emptive isolation of high-risk patients for MRSA has been an important part of the Dutch 'search and destroy' policy for the prevention of MRSA transmission. [6-8,17,28-31] In our study, positive predictive values to detect - among others - MRSA carriage ranged from 2.4% (95% CI: 1.2%-4.5%) (working with living pigs, veal calves or broilers) to 5.0% (95% CI: 3.9%-6.5%) (previous hospitalization in a foreign hospital). Still, presence of these risk factors was rare and even lowest for the question about being a household member of an MRSA carrier (0.1%), which needed to be asked 71,563 times in order to identify one new MRSA carrier upon hospital admission. In a recent analysis of routine universal pre-operative screening for nasal *S. aureus* carriage during a 7-year period in another Dutch hospital the prevalence of MRSA carriage was 0.13%, comparable to the reported prevalence of 0.11% upon admission in a study performed eight years earlier. [17,18] Assuming a similar prevalence in patients admitted to our hospital would imply that the current screening strategy identified only 15% of all MRSA carriers upon admission, suggesting that 85% still remained undetected. This is in line with other studies that report that currently most MRSA carriers do not have the classical risk factors (i.e. as inquired with our risk assessment) for MRSA carriage. [7,32-34]

The assessment question on known MDRO carriage had the highest yield, as it was answered positively in 2.8%. Indeed, 76% (n=260) of all patients with an MDRO positive screening culture

were already labelled in our EMR as a known MDRO carrier, of which 11% (n=29) had this label due to previous risk-based screening. This implies that if the risk assessment would have been replaced by the use of existing MDRO labelling in the EMR 68% (231/341) of MDRO carriers – that were now identified by risk-based screening – would still be captured.

Typically, the unexpected identification of an MDRO carrier during admission (i.e. through a positive clinical culture) is associated with extra workload, for screening of exposed roommates or healthcare workers of the index patient. This is not needed if the carrier was already identified upon admission (and thus contact precautions had already been installed). In our study only 15% (n=12) of detected carriers had a clinical culture positive for MDRO during admission, for which contact tracing would have been implemented if screening upon admission had not been applied. We estimated that abandoning risk-assessment based screening would have led to 566 patient days without protective measures for MDRO carriers in the 4.5 years of the observation period. As the vast majority of MDRO carriers remained undetected, these 566 days add little to the total number of patient days without protective measures for – unknown – MDRO carriers. The number of prevented episodes of cross-transmission due to the identification of new MDRO carriers upon admission is difficult to determine. Yet, the total number of hospital-acquired bacteraemia episodes caused by MDRO in our hospital during the 54 months of the study period was 44; 37 were caused by ESBL/MDR-Enterobacterales, four by multidrug-resistant *P. aeruginosa*, two by MRSA and one by VRE. Of these 44 patients eleven were known MDRO carriers at the time of admission and two were newly identified as MDRO carrier through risk assessment and screening. The remaining 31 patients (including those with hospital-acquired bacteraemia caused by MRSA and VRE) had no risk factors for MDRO carriage upon admission.

A strength of the current analysis was the combination of routine care data and medical microbiology information of 90% of all admissions during the predefined study period. There are also important limitations of this study that should be acknowledged. Firstly, retrograde manual changes to the MDRO risk assessment during hospital admission could not be retrieved. It is, therefore, not excluded that the MDRO risk assessment (e.g. the first question) was manually changed to 'positive' in case of MDRO positive cultures during admission. If so, the value of MDRO risk assessment would have been overestimated. Secondly, this was a real-life evaluation of clinical practice, without confirmation whether the individual questions of the MDRO risk assessment were answered correctly. Thirdly, this was a single-centre analysis in an academic medical centre in the Netherlands, with a well-developed system of identification, labelling and isolation of MDRO carriers as well as good adherence to standard precautions in routine care. Extrapolation of findings to other settings or countries should always occur in light of local epidemiology and established routine infection and prevention practices within a hospital.

The current low levels of AMR in hospitals in the Netherlands are partly explained by a restrictive use of antibiotics combined with the well-established 'search and destroy' policy over the last decades. Still, critical appraisal as well as continuous improvement is a fundamental part of infection prevention and control, considering that local epidemiology and target populations

may change over time. Results of this study imply that the majority of MDRO carriers in the community remains undetected upon admission despite current risk-based screening. Combined with the low prevalence of risk factors and the types of MDRO that are most often identified, the question arises whether the number of newly identified MDRO carriers truly justifies the invested workload across all hospital wards in risk assessment upon admission. We propose a system in which risk-based screening is abandoned and instead, contact precautions are installed upon hospitalization of patients that are known (previous) carriers of MDRO. This captures the majority of MDRO carriers that would else wise be identified through risk assessment.

CONCLUSIONS

In conclusion, in an academic Dutch hospital with a well-established MDRO surveillance system, individual risk assessment and screening for MDRO carriage upon hospital admission resulted in a low yield of new identified MDRO carriers in comparison to overall invested workload, while the majority of carriers remained undetected. Our findings justify a thorough reconsideration of the current individual risk assessment for MDRO carriage upon admission.

List of abbreviations
AMR, antimicrobial resistance; CI, confidence interval; CRA, carbapenem-resistant *Acinetobacter* spp.; CRE, carbapenem-resistant Enterobacterales; EMR, electronic medical record; ESBL, extended-spectrum beta-lactamase; ESBL/MDR-E, ESBL-producing and/or multidrug-resistant Enterobacterales; IRB, institutional review board; LOS, length of stay; MDR-E, multidrug-resistant Enterobacterales; MDR-GNB, multidrug-resistant Gram-negative bacteria; MDR-A, multidrug-resistant *Acinetobacter* spp.; MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *S. aureus*; NNA, number needed to ask; PPV, positive predictive value; PSP, penicillin-resistant *S. pneumoniae*; SD, standard definition; STROBE, strengthening the reporting of observational studies in epidemiology; VRE, vancomycin-resistant *E. faecium*; WIP, werkgroep infectiepreventie

Acknowledgements: We sincerely thank Roel Elbers for his important part in the data-extraction and data-management part of the current study. We are grateful for the assistance of Maaike van Mourik in the transfer of the risk assessment data to the UMCU Research Data Platform that was needed to start the study.

Funding: No funding to report.

Potential conflicts of interest: No conflicts of interest to disclose.

REFERENCES

1. Mehl A, Asvold BO, Kummel A, Lydersen S, Paulsen J, Haugan I, ***et al.*** Trends in antimicrobial resistance and empiric antibiotic therapy of bloodstream infections at a general hospital in Mid-Norway: a prospective observational study. ***BMC Infect Dis.*** 2017;17(1):116.
2. de Kraker MEA, Jarlier V, Monen JCM, Heuer OE, van de Sande N, Grundmann H. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. ***Clin Microbiol Infect.*** 2013;19(9):860–8.
3. Van Der Steen M, Leenstra T, Kluytmans JAJW, Van Der Bij AK. Trends in expanded-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* among Dutch clinical isolates, from 2008 to 2012. ***PLoS One.*** 2015;10(9):e0138088.
4. Vihta K-D, Stoesser N, Llewelyn MJ, Quan TP, Davies T, Fawcett NJ, ***et al.*** Trends over time in Escherichia coli bloodstream infections, urinary tract infections, and antibiotic susceptibilities in Oxfordshire, UK, 1998-2016: a study of electronic health records. ***Lancet Infect Dis.*** 2018;18(10):1138–49.
5. Werkgroep Infectiepreventie. WIP Richtlijn Meticilline-resistente *Staphylococcus aureus* (MRSA). 2012. Available from: https://www.rivm.nl/sites/default/files/2018-11/121205%20MRSA%20v1a%20def.pdf.
6. Vos MC, Ott A, Verbrugh HA. Successful search-and-destroy policy for methicillin-resistant *Staphylococcus aureus* in The Netherlands. ***Journal of Clinical Microbiology.*** 2005;43 2034; author reply 2034-5.
7. Vos MC, Behrendt MD, Melles DC, Mollema FPN, de Groot W, Parlevliet G, ***et al.*** 5 years of experience implementing a methicillin-resistant *Staphylococcus aureus* search and destroy policy at the largest university medical center in the Netherlands. ***Infect Control Hosp Epidemiol.*** 2009;30(10):977–84.
8. van Rijen MML, Bosch T, Heck MEOC, Kluytmans JAJW. Meticillin-resistant *Staphylococcus aureus* epidemiology and transmission in a Dutch hospital. ***J Hosp Infect.*** 2009;72(4):299–306.
9. Vandenbroucke-Grauls CM. Methicillin-resistant *Staphylococcus aureus* control in hospitals: the Dutch experience. ***Infect Control Hosp Epidemiol.*** 1996;17(8):512–3.
10. Werkgroep Infectiepreventie. Beleid bij meticilline-resistente *Staphylococcus aureus*. Leiden; 1988.
11. Werkgroep Infectiepreventie. WIP Richtlijn Maatregelen tegen overdracht van bijzonder resistente micro-organismen (BRMO). 2005.
12. Werkgroep Infectiepreventie. WIP Richtlijn Bijzonder Resistente Micro-organismen (BRMO). 2013. Available from: https://www.rivm.nl/sites/default/files/2018-11/130424 BRMO.pdf
13. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. ***PLoS Med***. 2007;4(10):e296.
14. Tobi H, van den Berg PB, de Jong-van den Berg LTW. Small proportions: what to report for confidence intervals? ***Pharmacoepidemiol Drug Saf.*** 2005;14(4):239–47.
15. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. ***JAMA***. 2013;310(20):2191–4.
16. Kluytmans-van den Bergh MFQ, van Mens SP, Haverkate MR, Bootsma MCJ, Kluytmans JAJW, Bonten MJM, ***et al.*** Quantifying hospital-acquired carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* among patients in Dutch hospitals. ***Infect Control Hosp Epidemiol*** 2018;39(1):32–9.
17. Bode LGM, Wertheim HFL, Kluytmans JAJW, Bogaers-Hofman D, Vandenbroucke-Grauls CMJE, Roosendaal R, ***et al.*** Sustained low prevalence of meticillin-resistant *Staphylococcus aureus* upon admission to hospital in The Netherlands. ***J Hosp Infect.*** 2011;79(3):198–201.
18. Weterings V, Veenemans J, van Rijen M, Kluytmans J. Prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* in patients at hospital admission in The Netherlands, 2010-2017: an observational study. ***Clin Microbiol Infect.*** 2019;25(11):1428.e1-1428.e5.
19. van den Bunt G, van Pelt W, Hidalgo L, Scharringa J, de Greeff SC, Schurch AC, ***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. ***Eurosurveillance***. 2019;24(41).
20. Dautzenberg MJ, Ossewaarde JM, de Kraker ME, van der Zee A, van Burgh S, de Greeff SC, ***et al***. Successful control of a hospital-wide outbreak of OXA-48 producing *Enterobacteriaceae* in the Netherlands, 2009 to 2011. ***Eurosurveillance***. 2014;19(9):20723.
21. Reuland EA, Overdevest ITMA, Al Naiemi N, Kalpoe JS, Rijnsburger MC, Raadsen SA, ***et al.*** High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints. ***Clin Microbiol Infect.*** 2013;19(6):542–9.
22. Zhou X, Garcia-Cobos S, Ruijs GJHM, Kampinga GA, Arends JP, Borst DM, ***et al.*** Epidemiology of extended-spectrum beta-lactamase-producing *E. coli* and vancomycin-resistant Enterococci in the northern Dutch-German cross-border region. ***Front Microbiol.*** 2017;8:1914.
23. van den Braak N, Ott A, van Belkum A, Kluytmans JA, Koeleman JG, Spanjaard L, ***et al.*** Prevalence and determinants of fecal colonization with vancomycin-resistant Enterococcus in hospitalized patients in The Netherlands. ***Infect Control Hosp Epidemiol.*** 2000;21(8):520–4.
24. van den Bunt G, Top J, Hordijk J, de Greeff SC, Mughini-Gras L, Corander J, ***et al.*** Intestinal carriage of ampicillin- and vancomycin-resistant *Enterococcus faecium* in humans, dogs and cats in the Netherlands. ***J Antimicrob Chemother.*** 2017:607–14. Available from: http://academic.oup.com/jac/advance-article/doi/10.1093/jac/dkx455/4774625
25. Otter JA, Mutters NT, Tacconelli E, Gikas A, Holmes AH. Controversies in guidelines for the control of multidrug-resistant Gram-negative bacteria in EU countries. ***Clin Microbiol Infect.*** 2015;21(12):1057–66.
26. Tacconelli E, Cataldo MA, Dancer SJ, De Angelis G, Falcone M, Frank U, ***et al.*** ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. ***Clin Microbiol Infect***. 2014;20 Suppl 1:1–55.
27. Gardam MA, Burrows LL, Kus J V, Brunton J, Low DE, Conly JM, ***et al.*** Is surveillance for multidrug-resistant enterobacteriaceae an effective infection control strategy in the absence of an outbreak? ***J Infect Dis.*** 2002;186(12):1754–60.
28. Bootsma MCJ, Diekmann O, Bonten MJM. Controlling methicillin-resistant *Staphylococcus aureus*: quantifying the effects of interventions and rapid diagnostic testing. ***Proc Natl Acad Sci USA.*** 2006;103(14):5620–5.
29. Wertheim HFL, Vos MC, Boelens HAM, Voss A, Vandenbroucke-Grauls CMJE, Meester MHM, ***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(4):321–5.
30. Souverein D, Houtman P, Euser SM, Herpers BL, Kluytmans J, Den Boer JW. Costs and Benefits Associated with the MRSA Search and Destroy Policy in a Hospital in the Region Kennemerland, The Netherlands. ***PLoS One.*** 2016;11(2):e0148175.
31. Clancy M, Graepler A, Wilson M, Douglas I, Johnson J, Price CS. Active screening in high-risk units is an effective and cost-avoidant method to reduce the rate of methicillin-resistant *Staphylococcus aureus* infection in the hospital. ***Infect Control Hosp Epidemiol.*** 2006;27(10):1009–17.
32. Lekkerkerk WSN, Sande-Bruinsma N van de, van der Sande MAB, Tjon-A-Tsien A, Groenheide A, Haenen A, ***et al.*** Emergence of MRSA of unknown origin in the Netherlands. ***Clin Microbiol Infect.*** 2012;18(7):656–61. Available from: https://www.sciencedirect.com/science/article/pii/S1198743X14645602?via%3Dihub
33. de Greeff SC, Mouton JW. Nethmap 2019. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. 2019.
34. Donker T, Bosch T, Ypma RJF, Haenen APJ, van Ballegooijen WM, Heck MEOC, ***et al.*** Monitoring the spread of meticillin-resistant *Staphylococcus aureus* in The Netherlands from a reference laboratory perspective. ***J Hosp Infect.*** 2016;93(4):366–74

SUPPLEMENTARY MATERIAL

Table S1-A. MDRO questionnaire – ADULTS

1. Were you ever carrier of an antibiotic-resistant bacterium[a] (for example MRSA, VRE, MDR-GNB, *Acinetobacter*, PRSP)?	○ No ○ Yes*
*If yes: Which one?	○ MRSA ○ VRE ○ MDR-GNB ○ PRSP ○ Other, …..
2. Were you admitted to another Dutch hospital during the past 2 months?	○ No ○ Yes*
*If yes: - What hospital? - Was there an outbreak?[b]	○ …… ○ No ○ Yes
3. Were you admitted to – or treated at – a foreign hospital during the past 2 months?	○ No ○ Yes*
*If yes: Was this during military deployment to a war zone?	○ No ○ Yes
4. Were you living in an asylum seekers' centre during the past 2 months?[c]	○ No ○ Yes
5. Do you live on – or work at – a company with living pigs, veal calves or broilers?	○ No ○ Yes
6. Do you live with – or take care of – someone that is carrier of MRSA?	○ No ○ Yes
MDRO risk present (AUTOMATICALLY FILLED-IN)	○ **No** ○ **Yes**
Contact precautions (AUTOMATICALLY FILLED-IN)	○ **Contact isolation** ○ **Contact isolation – PLUS**[d] ○ **Droplet isolation** ○ **Airborne isolation** ○ **Strict isolation**

MDR-GNB, multidrug-resistant Gram-negative bacterium; MRSA, methicillin-resistant *S. aureus*; PRSP, penicillin-resistant *S. pneumoniae*; VRE, vancomycin resistant Enterococcus

a. In Dutch: 'ziekenhuisbacterie'

b. Only if this subquestion is answered positively, it will give a positive MDRO-assessment result for question 2.

c. This question was added to the MDRO-assessment on 21-10-2015.

d. Contact isolation plus disinfection of the patient room after discharge (not indicated in routine contact isolation)

Table S1-B. MDRO questionnaire – CHILDREN

1. Is your child carrier of an antibiotic-resistant bacterium[a] (for example MRSA, VRE, MDR-GNB, Acinetobacter, PRSP)?	○ No ○ Yes*
*If yes: Which one?	○ MRSA ○ VRE ○ MDR-GNB ○ PRSP ○ Other, …..
2. Was your child admitted to another Dutch hospital during the past 2 months?	○ No ○ Yes*
*If yes: - What hospital? - Was there an outbreak?[b]	○ …… ○ No ○ Yes
3. Was your child admitted to a foreign hospital during the past 2 months?	○ No ○ Yes*
4. Was your child living in an asylum seekers' centre during the past 2 months?[c]	○ No ○ Yes
5. Does your child live on a farm with living pigs, veal calves or broilers?	○ No ○ Yes
6. Does your child live with someone that is carrier of MRSA?	○ No ○ Yes
MDRO risk present (AUTOMATICALLY FILLED-IN)	○ **No** ○ **Yes**
Contact precautions (AUTOMATICALLY FILLED-IN)	○ **Contact isolation** ○ **Contact isolation – PLUS**[d] ○ **Droplet isolation** ○ **Airborne isolation** ○ **Strict isolation**

MDR-GNB, multidrug-resistant Gram-negative bacterium; MRSA, methicillin-resistant *S. aureus*; PRSP, penicillin-resistant *S. pneumoniae*; VRE, vancomycin resistant Enterococcus

a. In Dutch: 'ziekenhuisbacterie'

b. Only if this subquestion is answered positively, it will give a positive MDRO-assessment result for question 2.

c. This question was added to the MDRO-assessment on 21-10-2015.

d. Contact isolation plus disinfection of the patient room after discharge (not indicated in routine contact isolation)

Table S2. Classification and definition of culture types for the current study

	1. Screening cultures	2. Clinical cultures[a]
Definition	- rectum, or - perineum, or - faeces (only in children), or - anal, or - nose, or - throat swab AND - taken on day 0 or 1 of admission, and - in an admission with a positive MDRO risk assessment	- abdominal fluid, - ascites, - bile, - biopsy, - blood, - bronchoalveolar lavage (BAL), - bronchial washing, - catheter tip, - cervix, - drain (fluid) - faeces (only adults), - liquor, - mouth, - pleural effusion, - prosthesis material, - pus, - sinus fluid - skin, - sputum, - urine midstream, - urine catheter, - vagina, - wound, - other

a. This list is based on the different types of MDRO-positive cultures that were present in the current study.
NB. This means that, for example, rectal cultures taken during hospital stay are NOT classified as a clinical culture.

Table S3. Definitions of multi-drug resistant organisms (MDRO) for the current study

		MDRO IF	Abbreviation	
1.	Enterobacterales	Resistant* to: Quinolones (i.e. ciprofloxacin, norfloxacin or levofloxacin) AND Aminoglycosides (i.e. gentamicin, tobramycin of amikacin)	MDR-E	MDR-E and/ or ESBL-E: ESBL/MDR-E
2.	Enterobacterales	ESBL positive	ESBL-E	
3.	Enterobacterales	Resistant* to: Carbapenems	CRE	
4.	*Staphylococcus aureus*	Resistant* to: Penicillin, flucloxacillin or oxacillin	MRSA	
5.	*Acinetobacter* spp	Resistant* to: Carbapenems	CRA	
6.	*Acinetobacter* spp	Resistant* to: Quinolones (i.e. ciprofloxacin of levofloxacin) AND Aminoglycosides (i.e. gentamicin, tobramycin of amikacin)	MDR-A	
7.	*Pseudomonas aeruginosa*	Resistant* to ≥3 of: - Ceftazidime - Quinolones - Aminoglycosides - Carbapenems - Piperacillin or piperacillin/tazobactam	-	
8.	*Stenotrophomonas maltophilia*	Resistant* to: Cotrimoxazol	-	
9.	*Enterococcus faecium* or *Enterococcus faecalis*	Resistant* to: Amoxicillin AND Vancomycin	VRE	
10.	*Streptococcus pneumoniae*	Resistant* to: Penicillin OR Vancomycin	PSP	

*Resistant = intermediate or resistant according to EUCAST MIC guidelines.

Table S4. Number of included hospital admissions and yield of risk-based screening per study year

	Admissions, N	**New identified MDRO carrier, N (row %)**
2015	33,311	17 (0.05)
2016	33,436	20 (0.06)
2017	34,365	21 (0.06)
2018	25,193	9 (0.04)
2019	17,746	14 (0.08)
Total	**144,051**	**81 (0.06)**

MDRO, multi-drug resistant organism

Table S5. Prevalence of new identified MDRO carriage per risk assessment question.

	Positive reply and screening cultures obtained, n (%)	**ESBL/ MDR-E n (%[a])**	**MRSA n (%[a])**	**CRE n (%[a])**	**VRE n (%[a])**
1. Are you a known carrier of an MDRO (e.g. MRSA, VRE, MDR-GNR)?	901 (28.1)	23 (2.6)	7 (0.8)	-	1 (0.1)
2. During the past 2 months, were you hospitalized in another Dutch hospital during a known MDRO outbreak?	46 (23.0)	3 (6.5)	-	-	-
3. During the past 2 months, were you hospitalized in a foreign hospital?	372 (55.3)	25 (6.7)	7 (1.9)	2 (0.5)	-
4. In the past 2 months, did you live in an asylum shelter?	106 (56.7)	4 (3.8)	9 (8.5)	-	-
5. Do you work with living pigs, veal calves or broilers?	141 (41.5)	-	8 (5.7)	-	-
6. Are you a household member of an MRSA carrier?	54 (46.6)	-	2 (3.7)	-	-
Total	**1,516 (33.8)**	**52 (3.4)[b]**	**26 (1.7)[b]**	**2 (0.1)**	**1 (0.07)**

a. Row percentages (i.e. proportion carriers in patients at high-risk).
b. Individual counts count up to more than total because of multiple carriers with positive reply to more than one risk assessment question.

Table S6. Types of multi-drug resistant organisms (MDRO) identified in (any) clinical culture during hospital stay, stratified for negative and positive risk assessment upon hospital admission.

	Admissions with ≥1 clinical culture positive for MDRO		
	All n = 1,279 (%[a])	*Positive risk assessment[b] n = 514 (%[a])*	*Negative risk assessment n = 765 (%[a])*
ESBL/MDR Enterobacterales	906 (70.8)	343 (66.7)	563 (73.6)
MDR *P. aeruginosa*	290 (22.7)	132 (25.7)	158 (20.7)
MRSA	54 (4.2)	36 (7)	18 (2.4)
Cotrimoxazol-R *S. maltophilia*	40 (3.1)	13 (2.5)	27 (3.5)
Carbapenem-R Enterobacterales	7 (0.5)	2 (0.4)	5 (0.7)
PSP	6 (0.5)	-	6 (0.8)
MDR *Acinetobacter* spp	5 (0.4)	1 (0.2)	4 (0.5)
VRE	3 (0.2)	1 (0.2)	2 (0.3)
Carbapenem-R *Acinetobacter* spp	1 (0.1)	1 (0.2)	-

ESBL, extended-spectrum beta-lactamase; ESBL/MDR-E, ESBL-producing or MDR Enterobacterales; MDR, multi-drug resistant; MDRO, multi-drug resistant organism; MRSA, methicillin-resistant *S. aureus*; PSP, penicillin-resistant *S. pneumoniae*; R, resistant; VRE, vancomycin-resistant Enterococci.

a. Proportions count up to >100% because of admissions with multiple clinical cultures positive for MDRO.

b. NB. Positive risk assessment upon admission in this table only indicates ≥1 risk factors present in the risk assessment upon admission and does not take in to account results of screening cultures (i.e. whether they were obtained or whether they were positive).

Table S7. Types of multi-drug resistant organisms (MDRO) identified in hospital-acquired bloodstream infection (BSI) episodes caused by MDRO, stratified for negative and positive risk assessment upon hospital admission.

	Admissions with hospital-acquired BSI caused by MDRO		
	All n = 44	*Positive risk assessment upon admission*[a] *n = 9 (known carrier in EMR)*	*Negative risk assessment upon admission n = 35 (known carrier in EMR)*
ESBL/MDR Enterobacterales	37	8 (6)	29 (3[b])
MDR *P. aeruginosa*	4	1 (1)	3 (1[b])
MRSA	2	-	2 (0)
Cotrimoxazol-R *S. maltophilia*	-	-	-
Carbapenem-R Enterobacterales	-	-	-
PSP	-	-	-
MDR *Acinetobacter* spp	-	-	-
VRE	1	-	1 (0)
Carbapenem-R *Acinetobacter* spp	-	-	-

BSI, bloodstream infection; ESBL, extended-spectrum beta-lactamase; ESBL/MDR-E, ESBL-producing or MDR Enterobacterales; MDR, multi-drug resistant; MDRO, multi-drug resistant organism; MRSA, methicillin-resistant *S. aureus*; PSP, penicillin-resistant *S. pneumoniae*; R, resistant; VRE, vancomycin-resistant Enterococci. Hospital-acquired BSI was defined as a positive blood culture for MDRO taken >2 days after hospital admission.

a. NB. Positive risk assessment in this table indicates ≥1 risk factors present in the risk assessment upon admission and does not take in to account results of screening cultures (i.e. whether they were obtained or positive).

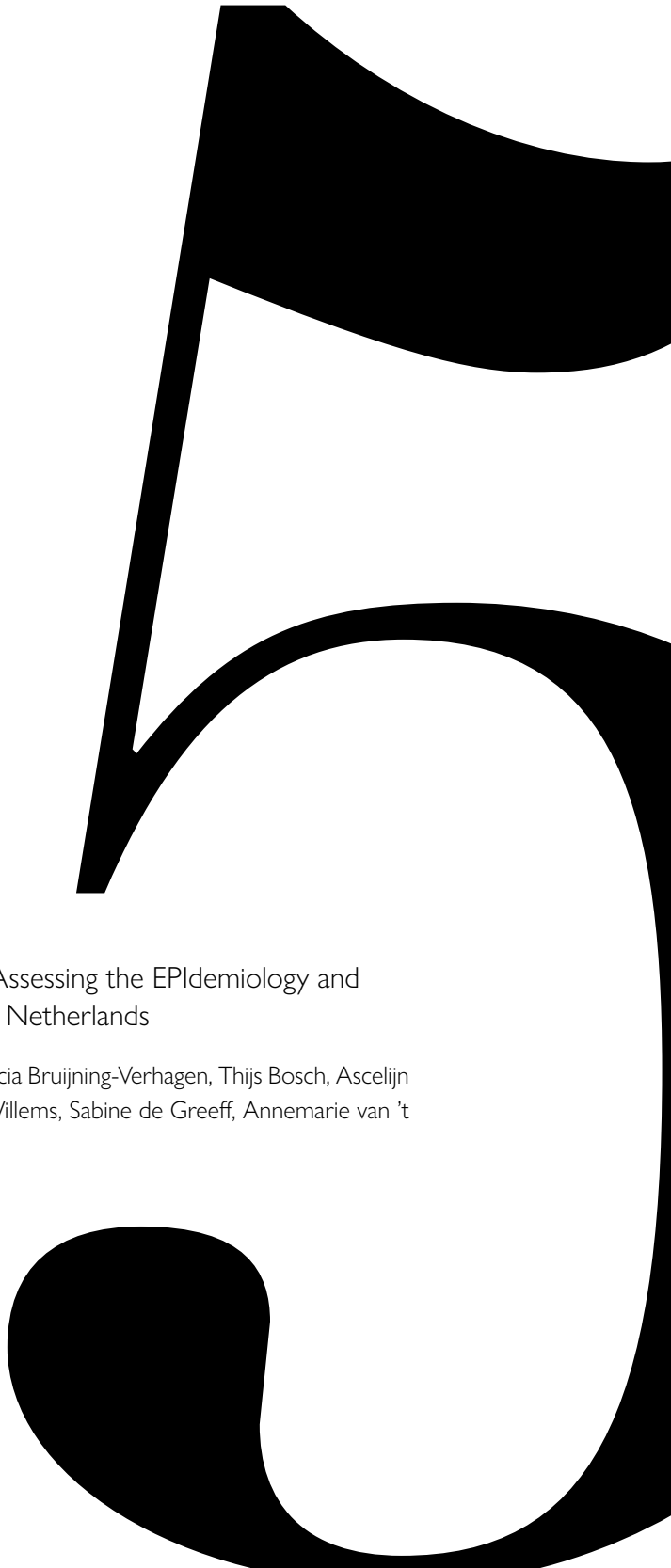
b. This indicates a false-negative MDRO risk assessment upon admission.

Table S8. Clinical culture types positive for a multi-drug resistant organism (MDRO).

	All admissions with any clinical culture positive for MDRO (N = 1,279)	**Identified as MDRO carrier through screening that also had clinical culture positive for MDRO during subsequent admission (N = 12)**
Body site	**Cultures N = 2,220 (%)**	**Cultures N = 17 (%)**
Sputum	749 (33.7)	1 (5.9)
Urine	744 (33.5)	5 (29.4)
Wound	162 (7.3)	4 (23.5)
Blood	146 (6.6)	2 (11.8)
Tissue/biopsy	54 (2.4	2 (11.8)
Bronchial washing	50 (2.3)	-
Bronchoalveolar lavage (BAL)	49 (2.2)	-
Pus	43 (1.9)	1 (5.9)
Drain fluid	33 (1.5)	
Mouth wash	20 (0.9)	-
Bile	14 (0.6)	-
Skin	13 (0.6)	-
Abdominal fluid	10 (0.5)	-
Catheter tip	9 (0.4)	-
Vagina	7 (0.3)	-
Ascites fluid	6 (0.3)	-
Other[a]	111 (5.0)	2 (11.8)

MDRO, multi-drug resistant organism

a. All other culture types that occurred <6 times.



Design of the EPIGENEC Study: Assessing the EPIdemiology and GENetics of *Escherichia coli* in the Netherlands

Denise van Hout, Tess Verschuuren, Patricia Bruijning-Verhagen, Thijs Bosch, Ascelijn Reuland, Ad Fluit , Anita Schürch, Rob Willems, Sabine de Greeff, Annemarie van 't Veen, Jan Kluytmans and Marc Bonten

Available at preprints.org. 2019

ABSTRACT

Background: Infections caused by *E. coli* cause considerable disease burden and range from frequently occurring and relatively innocent urinary tract infection (UTI) to severe bloodstream infection (BSI). The incidence of infections caused by ESBL-producing *E. coli* (ESBL-PEc) is increasing, justifying surveillance and development of preventive strategies in several domains. Faecal carriage is universal and believed to be the most important reservoir for *E. coli* from which infections can originate. It is currently unknown to what extent Dutch *E. coli* carriage strains in the community reflect isolates causing disease. In this study, we will perform comparative genomics to infer the population structures of human-derived ESBL-PEc from community- and hospital-acquired infections and from community-based faecal carriage samples in the Netherlands. Furthermore, we will describe the molecular epidemiology of *E. coli* isolates causing invasive disease (BSI).

Methods: This study uses four different microbiological data sources: 1) ESBL-PEc from patients with community-acquired UTI tested in primary care between May and November 2017, 2) ESBL-PEc from urine cultures obtained from patients hospitalized between January 2014 and December 2016, 3) *E. coli* from blood cultures obtained from patients hospitalized between January 2014 and December 2016, and 4) ESBL-PEc from faecal samples collected in a national population-prevalence study performed between January 2014 and January 2017. Clinical epidemiological data was collected from all patients and all isolates were subjected to whole genome sequencing.

Discussion: The EPIGENEC study (EPIdemiology and GENetics of *E. coli*) will describe the molecular epidemiology of *E. coli* BSI and assess the genomic population structure of ESBL-PEc strains from community-acquired and nosocomial infections, and of ESBL-PEc reflecting community-based faecal carriage. Information from these studies may assist in optimizing surveillance strategies and determining targets and potential impact of future new preventive measures.

BACKGROUND

Escherichia coli (*E. coli*) is commonly found as a gut commensal in humans. Besides its commensal lifestyle *E. coli* is also an important pathogen in humans, as it can establish disease in tissues other than the gastrointestinal tract. These so-called extra-intestinal pathogenic *E. coli* (ExPEC) can cause a wide spectrum of diseases, from uncomplicated cystitis to bloodstream infections (BSI) with 30-day mortality up to 18%. [1–3] *E. coli* is a very heterogeneous species, only 20% of the genes in a typical *E. coli* genome is usually shared among all strains. [4] *E. coli* is known to easily acquire antimicrobial resistance. Molecular characterization studies have shown that *E. coli* strains predominantly become resistant through the exchange of mobile genetic elements carrying resistance genes, such as those encoding for extended-spectrum beta-lactamases (ESBL). [5] ESBL-producing *E. coli* (ESBL-PEc) are often co-resistant to other classes of antibiotics. [6] Infections caused by antibiotic-resistant *E. coli* strains occur with increasing frequency, which potentially increase the total overall *E. coli* disease burden. [3,7,8] Furthermore, in a recent modelling study, ESBL-PEc was found to be responsible for approximately a third of the estimated 33,000 antibiotic-resistance related deaths in Europe in 2015. [9] The increasing availability of whole genome sequencing (WGS) has allowed a more detailed insight into the genetics of *E. coli* virulence and resistance and provided further insight into the distribution of acquired virulence and resistance genes in pathogenic and commensal *E. coli* strains of different genetic backgrounds. [10–12]

Intestinal carriage is believed to be the most important human reservoir for ESBL-PEc from which infections can originate. [13] The estimated prevalence of ESBL-PEc faecal carriage in Dutch community-dwelling inhabitants ranges from 5.2% in the general population [14] to 10.1% in urbanized areas [15], and from 5.0% [16] to 6.1% [17] in hospitalized patients. Surveillance of the molecular epidemiology of antibiotic resistance in the community reservoir is important to identify trends in resistance development. Yet, such surveillance is labour-intensive and costly, and, therefore, not regularly performed. It is currently unknown to what extent the molecular epidemiology of these ESBL-PEc strains present in the Dutch community relates to the molecular epidemiology of ESBL-PEc strains causing community-acquired and nosocomial *E. coli* infections. In case of good correlations, urine *E. coli* isolates from primary care patients or from hospitalized patients could be used for surveillance of the molecular epidemiology of antibiotic-resistant *E. coli* in the community in the Netherlands.

Information on to what extent *E. coli* strains from different niches and patient populations in the Netherlands differ genomically, is scarce. Possibly, there is also a difference in pathogenic potential within invasive *E. coli* isolates, reflected for example by molecular differences at the genome level in strains that have caused community-acquired BSI as compared to strains that cause BSI in a population that is already vulnerable to infection. Such information is critical for

informing strategies around surveillance, prevention and treatment of this important pathogen. In particular for *E. coli* BSI, which is characterized by high morbidity and mortality, more insight in the clinical as well as molecular epidemiology in the Netherlands is needed to help identify targets and potential impact of future preventive strategies such as *E. coli* vaccines, of which one is currently being developed. [18]

Here, the rationale and study design of the EPIGENEC Study (EPIdemiology and GENetics of *E. coli*) is described, which aims to 1) Investigate the association between the molecular epidemiology of faecal ESBL-PEc isolates obtained from carriage in the community and from clinical cultures (UTI and BSI) in the Netherlands, and 2) Characterize *E. coli* causing BSI in the Netherlands by evaluating clinical characteristics and mapping the genomic population structure of *E. coli* causing BSI based on WGS.

METHODS

Primary objectives

1. To compare the genomic population structure of ESBL-PEc isolates from community-acquired and nosocomial infections with ESBL-PEc isolates found in community faecal samples based on WGS.

2. To describe the clinical and molecular epidemiology of *E. coli* isolates causing BSI, and in particular, to assess the association between different clinical and molecular characteristics.

Study design and population

This observational study consists of a prospective as well as a retrospective part. Four sources of data and samples will be obtained from clinical care and the community (see Figure 1).

1. Community-acquired UTI

Patients with a community-acquired UTI caused by ESBL-PEc were identified prospectively by a positive urine culture result at Saltro, a medical laboratory providing service to primary care practices, primarily in the Utrecht (city) region. Urine samples were either inoculated in enrichment broth (Isobouillon with tobramycin, vancomycin and nystatin) if specifically requested on ESBL or identified by elevated MIC for cephalosporins. Screening for ESBL-producing *Enterobacteriaceae* was performed by inoculation onto a selective screening agar, the Brilliance ESBL screening agar (Oxoid, Basingstoke, United Kingdom). All broths and plated were incubated overnight at 36°C.

Species identification and antibiotic susceptibility testing of colonies growing on the Brilliance ESBL plates were performed with respectively the MALDITOF-MS (Bruker, Bremen, Germany) and the Vitek 2 system (Vitek AST, bioMérieux, Marcy-l'Étoile, France). The MIC breakpoints used for interpreting the results were according to the criteria of the EUCAST. Phenotypic confirmation of ESBL was performed by combination disk diffusion test, as recommended by the Dutch national guideline for laboratory ESBL detection. [19] All ESBL-PEc isolates from positive urine cultures between May 2017 and November 2017 were stored at Saltro, at -80°C.

2. Nosocomial UTI

Patients with nosocomial UTI caused by ESBL-PEc were retrospectively identified from medical microbiological records in two participating hospitals: 1) University Medical Center Utrecht (UMCU), and 2) Amphia Hospital in Breda. The UMCU is a 1,042-bed tertiary hospital, providing care to the Utrecht (province) region and serves as a regional referral center. The Amphia Hospital is an 837-bed teaching hospital that provides service to a region of approximately ~400,000 residents. Sample inoculation and confirmation of phenotypic ESBL production was performed as described for the community UTI samples, except that CHROMagar ESBL plates were used (CHROMagar, Paris, France). In both hospitals, every first ESBL-PEc isolate per patient is routinely stored and frozen at -80°C by the medical microbiology department. For this study, we selected all ESBL-PEc isolates from nosocomial UTIs (sample taken >2 days after hospital admission) during the years 2014, 2015 and 2016.

3. BSI

In the same two hospitals, patients with *E. coli* BSI, both ESBL-producing and non-ESBL-producing, were retrospectively identified from medical microbiological records by growth of *E. coli* in blood cultures. In these hospitals, *E. coli* isolates from blood cultures are routinely stored at -80°C. For the years 2014, 2015 and 2016, a random sample of 40 isolates per year, comprising ~25% of all bacteraemic *E. coli* isolates in a year, was drawn from each hospital. In addition to the random sample, all ESBL-PEc isolates from 2014-2016 were selected for WGS. Consequently, this set of ESBL-PEc, together with the random sample of the bacteraemic *E. coli* strains, comprises the total blood isolate collection for the current study (see Figure 1). All ESBL-PEc isolates obtained from the clinical samples (community UTI, nosocomial UTI and BSI) and the random sample of *E. coli* BSI isolates were included for further molecular analyses at The Netherlands National Institute for Public Health and the Environment (RIVM). Because we expected follow-up cultures to often grow the same *E. coli* isolate as the first culture, and for efficiency reasons, we selected only the first available *E. coli* isolate for each patient (all ages), irrespective of time between cultures.

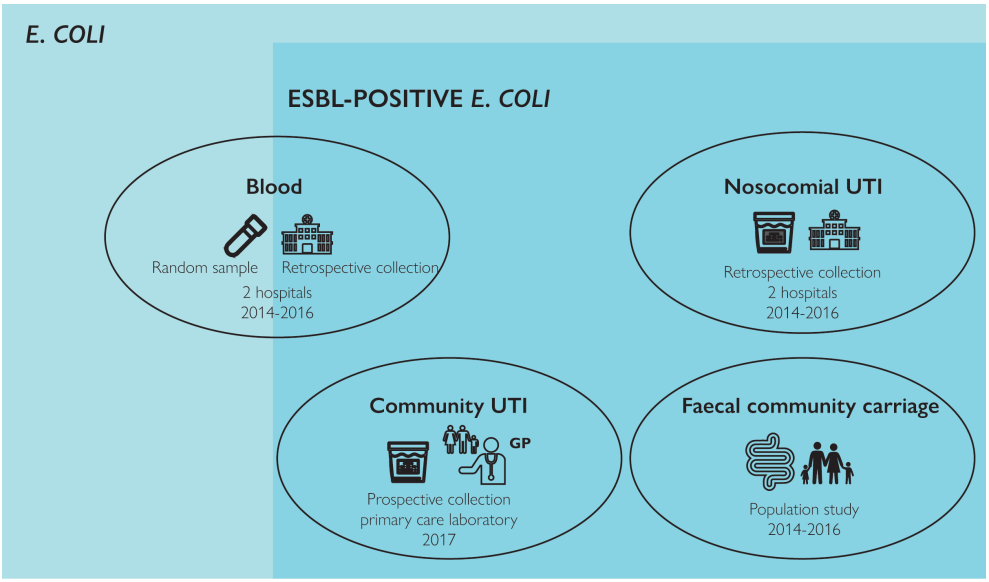


Figure 1. Data sources. This figure depicts the sources and time-span of the different *E. coli* collections that are used in this study. GP, general practitioner; UTI, urinary tract infection

4. Community-based intestinal carriage

The fourth dataset consists of ESBL-PEc isolates collected from faecal samples of a national population study for ESBL-producing *Enterobacteriaceae*, performed between November 2014 and November 2016. In this cross-sectional study, every month a random sample of ~2,000 residents of the Netherlands was drawn from Dutch municipalities (covering the entire population of the Netherlands). One person per household was invited to fill in a web-based questionnaire, and upon completion of the questionnaire, the participant was asked to provide a faecal sample. ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, and the *Enterobacter cloacae* complex were isolated using MacConkey agar with 1 mg/L cefotaxime or after enrichment 2 mL of LB with 1 mg/mL cefotaxime. Up to five colonies with different morphologies were selected. Species identification was performed using MALDITOF-MS (Bruker, Bremen, Germany). ESBL-encoding genes were identified by PCR and isolates negative in the PCR were tested for the presence of other ESBL encoding genes by the Check MDR CT-101 microarray (Check-points, Wageningen, the Netherlands). The genes were identified by conventional sequencing. PCR-based Replicon Typing (PBRT) was performed to identify the plasmid type that encoded the ESBL. [20] All ESBL-producing *Enterobacteriaceae* were stored at -80°C in the UMCU and were subjected to WGS (see Genotyping). Further details of the study design can be found elsewhere. [21] For the current study, only genetic data of the first sampled faecal ESBL-PEc isolate of a patient were collected. No age restrictions were used.

Epidemiological variables

The following information was collected from all patients: age, sex, postal code, type of infection (community UTI, nosocomial UTI, BSI), date of sample collection, and community or nosocomial (i.e. sample taken >2 days after hospital admission) onset of infection. In addition, for UTIs it was recorded whether the urine sample was a catheter sample. For patients with *E. coli* BSI, additional information regarding presence of a urinary catheter, hospital ward (ICU versus non-ICU), 30-day and 1-year mortality and the primary focus of BSI was obtained from electronic medical records. Possible primary foci were: urinary tract (i.e. pyelonephritis, prostatitis), gastro-intestinal (i.e. diverticulitis, bacterial translocation), hepatic-biliary (i.e. cholangitis), respiratory, gynaecological, other (i.e. meningitis, venous catheter), and unknown. The primary focus of BSI (portal of entry) was defined on the basis of clinical and/or radiologic features and the isolation of *E. coli* from the presumed source of infection. If *E. coli* was not isolated from the presumed primary focus (i.e. because of previous antimicrobial treatment or invasive procedure that was needed to isolate *E. coli* from primary source), the presumed primary focus was based on a firm clinical suspicion (given that all other possible sources of infection were excluded). In case of multiple possible primary foci, consensus was reached by discussion by DH and TV.

Genotyping

All *E. coli* isolates that were selected for the current study were inoculated on (non-selective) blood agar and species confirmation was performed by MALDITOF-MS prior to WGS, which was performed at the RIVM. All *E. coli* strains, except the strains from the external dataset, were subjected to WGS using the Illumina HiSeq 2500 (BaseClear, Leiden, the Netherlands). For this, cell pellets were made from 1500µl of overnight culture in Brain Heart Infusion (BHI) broth (Tritium Microbiologie BV, Eindhoven, the Netherlands). The pellets were washed in 500µl saline and subsequently re-suspended in 200µl DNA/RNA Shield (Zymo Research, Irvine CA, U.S.A.). From the pellets, nucleic acid extraction was performed by BaseClear (Leiden, the Netherlands) using an in-house protocol. Library preparation and sequencing of the bacterial genomes was performed using the Illumina Nextera XT kit and the HiSeq 2500 with a paired-end 100 cycles protocol. The ESBL-PEc from the fourth dataset, i.e. ESBL-PEc recovered from faeces of healthy humans, were sequenced using the MiSeq or NextSeq platforms (Illumina, San Diego, CA). Contigs of all four datasets were assembled with SPAdes genome assembler v.3.6.2. The resulting WGS data were used to determine the multi-locus sequence type (MLST), virulence gene and resistance gene content using the mlst2.0 (https://github.com/tseemann/mlst) to scan contig files against the *E. coli* PubMLST typing scheme (updated May 12th, 2018), and Abricate (https://github.com/tseemann/abricate) for mass screening of contigs for antimicrobial resistance and virulence genes. Abricate comes bundled with multiple resistance gene and virulence gene databases. For this study, the ResFinder and VFDB databases were used. Serotypes were assigned by using the web-tool SerotypeFinder 2.0 from the Center for Genomic Epidemiology at the

Danish Technical University, Lyngby, Denmark (http://www.genomicepidemiology.org). This tool uses presence of O- and H-antigen-processing genes to predict *E. coli* serotypes. [22]

Planned analyses

Primary objective 1

The population structure of ESBL-PEc from the clinical and faecal samples will be compared on three levels. Firstly, the core genome will be assessed with MLST, a core genome phylogeny based on SNP and allelic profile variation using SeqSphere, and the ESBL-PEc populations will be partitioned in sequence clusters. For this, different methods are available like hierarchical Bayesian Analysis of Population Structure (BAPS) or PopPUNK. [23,24] Secondly, the accessory genome will be assessed by comparing acquired resistance genes in the ESBL-PEc populations using Resfinder, and the plasmid composition will be predicted using the recently developed mlplasmid© algorithm. [25] Lastly, a pan-genome analysis will be performed using PANINI, to assess if the total gene content differs per different ESBL-PEc population. [26]

Primary objective 2

To assess the association between epidemiological characteristics and molecular characteristics of *E. coli* blood isolates, MLST, virulence and antimicrobial resistance gene content will be described according to the different epidemiological subgroups. A core-genome tree will be constructed with the same method as mentioned above. A virulence score will be made per isolate and will be defined as the total number of virulence genes present in that strain. These virulence scores will then be compared between isolates with different epidemiological characteristics and between ST131 and non-ST131 isolates, respectively. Serotype distribution of the bacteraemia population will be compared to current *E. coli* vaccine candidates. Furthermore, a genome-wide association approach will be used to see whether any epidemiological characteristics are associated with certain molecular traits.

Ethics

This study is conducted according to the principles of the Declaration of Helsinki (World Medical Association, 2013) and does not fall under the scope of the Medical Research Involving Human Subjects Act, the Medical Research Ethics Committee of the UMCU has therefore waived the need for official approval by the Ethics Committee (IRB number 18/056). The study uses pseudonymised data and informed consent is not obtained from study participants. Patients that participated in the open population study (ESBLAT study, IRB number 14/219-C) have provided informed consent for the use of clinical data and faecal samples in future studies such as the current study. In this study, in case of age <13 years, parents provided informed consent. In case of age 13-17 years, both the child and parents provided informed consent.

DISCUSSION

The EPIGENEC study aims to assess the genomic population structure of ESBL-PEc strains from community and nosocomial infections and ESBL-PEc strains representing community faecal carriage. It will also carefully describe the clinical epidemiology and genomic population structure of *E. coli* BSI, which is important in determining the targets and impact of possible new preventive measures.

Strengths

One of the key aspects of the current study is the combined use of epidemiological data and detailed whole genome sequence data of strains from several different domains in order to obtain a more complete picture of the current molecular epidemiology of (ESBL-producing) *E. coli* in the Netherlands. Furthermore, the use of WGS techniques allows us to map the population structure of *E. coli* and the association of the genomic make-up of strains with their isolation source with high resolution and discriminatory power. Also, all strains were uniformly assembled and analysed, reducing the risk of information bias.

Limitations

This study also has limitations. Guidelines for Dutch primary care physicians recommend to only send in urine cultures for microbiological testing for patients with complicated UTI (i.e. symptoms accompanied with fever, or in case of male patients with UTI symptoms), clinical treatment failure, recurrent UTIs, or a possibly resistant infection, which implies selection of patients with community UTI. However for our study, we do not consider this to cause selection bias, since we are particularly interested in the molecular epidemiology of ESBL-PEc from urine samples in the way they are currently being performed, so as according to clinical practice. Also, ideally we would be able to pick up time-trends in the change in molecular epidemiology of community faecal carriage of ESBL-PEc and assess whether these trends are reflected in the molecular epidemiology of clinical cultures, for example from community or nosocomial UTI. One could imagine using such results to assess the possible value of ESBL-PEc isolates from clinical cultures as a proxy of changes in the molecular epidemiology of community faecal carriage. However, considering the heterogeneity in the *E. coli* species and the limited amount of years of which we have faecal samples, this will prohibit us to draw hard conclusions. We still believe this comparison will provide us valuable information and will guide future research on the possible use of routine clinical samples in the assessment of the molecular epidemiology of ESBL-PEc.

List of abbreviations

BSI, bloodstream infection; ESBL, extended-spectrum beta-lactamase; ESBL-PEc, ESBL-producing *E. coli*; EUCAST, European Committee for Antimicrobial Susceptibility Testing; GP, general practitioner; ICU, intensive care unit; MIC, minimal inhibitory concentration; MLST, multi-locus sequence type; UTI, urinary tract infection; WGS, whole genome sequencing

Acknowledgements: We sincerely thank Kim van der Zwaluw, Judith Vlooswijk, Carlo Verhulst and Jelle Scharringa for their contribution in the preparation of this study.

Conflicts of interest: no conflicts of interest to disclose.

Funding: This study is internally funded by the Netherlands National Institute for Public Health and the Environment (RIVM) and the UMCU and has not received external funding or assistance from any commercial organization.

REFERENCES

1. Abernethy, J. K. *et al.* Thirty day all-cause mortality in patients with *Escherichia coli* bacteraemia in England. *Clin. Microbiol. Infect.* **21**, 251.e1-8 (2015).
2. 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).
3. Vihta, K. D. *et al.* Trends over time in *Escherichia coli* bloodstream infections, urinary tract infections, and antibiotic susceptibilities in Oxfordshire, UK, 1998-2016: a study of electronic health records. *Lancet. Infect. Dis.* **18**, 1138-1149 (2018).
4. Lukjancenko, O., Wassenaar, T. M. & Ussery, D. W. Comparison of 61 Sequenced *Escherichia coli* Genomes. *Microb. Ecol.* **60**, 708–720 (2010).
5. Mathers, A. J., Peirano, G. & Pitout, J. D. D. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clin. Microbiol. Rev.* **28**, 565–591 (2015).
6. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2016. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). (2016).
7. Schlackow, I. *et al.* Increasing incidence of *Escherichia coli* bacteraemia is driven by an increase in antibiotic-resistant isolates: electronic database study in Oxfordshire. *J Antimicrob. Chemother.* **67**, 1514–1524 (2012).
8. 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**, 860–868 (2013).
9. 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).
10. Köhler, C. D. & Dobrindt, U. What defines extraintestinal pathogenic *Escherichia coli*? *Int. J. Med. Microbiol.* **301**, 642–647 (2011).
11. Johnson, J. R. *et al.* Epidemiological Correlates of Virulence Genotype and Phylogenetic Background among *Escherichia coli* Blood Isolates from Adults with Diverse-Source Bacteremia. *J. Infect. Dis.* **185**, 1439–1447 (2002).
12. Johnson, J. R. *et al.* Accessory Traits and Phylogenetic Background Predict *Escherichia coli* Extraintestinal Virulence Better Than Does Ecological Source. *J. Infect. Dis.* **219**, 121-132 (2019).
13. Yamamoto, S. *et al.* Genetic evidence supporting the fecal-perineal-urethral hypothesis in cystitis caused by *Escherichia coli*. *J. Urol.* **157**, 1127–1129 (1997).
14. van Hoek, A. H. *et al.* Molecular characteristics of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* from humans in the community. *PLoS One* **10**, e0129085 (2015).
15. Reuland, E. A. *et al.* High prevalence of ESBL-producing *Enterobacteriaceae* carriage in Dutch community patients with gastrointestinal complaints. *Clin. Microbiol. Infect.* **19**, 542–549 (2013).
16. Willemsen, I. *et al.* Trends in Extended Spectrum Beta-Lactamase (ESBL) Producing *Enterobacteriaceae* and ESBL Genes in a Dutch Teaching Hospital, Measured in 5 Yearly Point Prevalence Surveys (2010-2014). *PLoS One* **10**, e0141765 (2015).
17. Zhou, X. *et al.* Epidemiology of Extended-Spectrum beta-Lactamase-Producing *E. coli* and Vancomycin-Resistant Enterococci in the Northern Dutch-German Cross-Border Region. *Front. Microbiol.* **8**, 1914 (2017).
18. Huttner, A. *et al.* Safety, immunogenicity, and preliminary clinical efficacy of a vaccine against extraintestinal pathogenic *Escherichia coli* in women with a history of recurrent urinary tract infection: a randomised, single-blind, placebo-controlled phase 1b trial. *Lancet. Infect. Dis.* **17**, 528–537 (2017).
19. Netherlands Society for Medical Microbiology. NVMM Guideline Laboratory detection of highly resistant microorganisms. 24–46 (2012).
20. Carattoli, A. *et al.* Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* **63**,

219–228 (2005).
21. van den Bunt, G. *et al.* Intestinal carriage of ampicillin- and vancomycin-resistant *Enterococcus faecium* in humans, dogs and cats in the Netherlands. *J. Antimicrob. Chemother.* **73**, 607–614 (2017).
22. Joensen, K. G. *et al.* Rapid and Easy In Silico Serotyping of *Escherichia coli* Isolates by Use of Whole-Genome Sequencing Data. *J. Clin. Microbiol.* **53**, 2410–2426 (2015).
23. Cheng, L. *et al.* Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. *Mol. Biol. Evol.* **30**, 1224–1228 (2013).
24. Lees, J. *et al.* Fast and flexible bacterial genomic epidemiology with PopPunk. *BioRxiv* doi: https://doi.org/10.1101/360917 (2018).
25. Arredondo-Alonso, S. *et al.* Mlplasmids: a User-Friendly Tool To Predict Plasmid- and Chromosome-Derived Sequences for Single Species. *Microb. Genomics* doi:10.1099/mgen.0.000224 (2018).
26. Abudahab, K. *et al.* PANINI: Pangenome Neighbour Identification for Bacterial Populations. *Microb. Genomics* doi:10.1099/mgen.0.000220 (2018).

Extended-spectrum beta-lactamase (ESBL)-producing and non-ESBL-producing *Escherichia coli* isolates causing bacteraemia in the Netherlands (2014 – 2016) differ in clonal distribution, antimicrobial resistance gene and virulence gene content

Denise van Hout, Tess Verschuuren, Patricia Bruijning-Verhagen, Thijs Bosch, Anita Schürch, Rob Willems, Marc Bonten and Jan Kluytmans

PLOS ONE. 2019

ABSTRACT

Background: Knowledge on the molecular epidemiology of *Escherichia coli* causing *E. coli* bacteraemia (ECB) in the Netherlands is mostly based on extended-spectrum beta-lactamase-producing *E. coli* (ESBL-Ec). We determined differences in clonality and resistance and virulence gene (VG) content between non-ESBL-producing *E. coli* (non-ESBL-Ec) and ESBL-Ec isolates from ECB episodes with different epidemiological characteristics.

Methods: A random selection of non-ESBL-Ec isolates as well as all available ESBL-Ec blood isolates was obtained from two Dutch hospitals between 2014 and 2016. Whole genome sequencing was performed to infer sequence types (STs), serotypes, acquired antibiotic resistance genes and VG scores, based on presence of 49 predefined putative pathogenic VG.

Results: ST73 was most prevalent among the 212 non-ESBL-Ec (N=26, 12.3%) and ST131 among the 69 ESBL-Ec (N=30, 43.5%). Prevalence of ST131 among non-ESBL-Ec was 10.4% (N=22, *P* value < .001 compared to ESBL-Ec). O25:H4 was the most common serotype in both non-ESBL-Ec and ESBL-Ec. Median acquired resistance gene counts were 1 (IQR 1 – 6) and 7 (IQR 4 – 9) for non-ESBL-Ec and ESBL-Ec, respectively (*P* value < .001). Among non-ESBL-Ec, acquired resistance gene count was highest among blood isolates from a primary gastro-intestinal focus (median 4, IQR 1 – 8). Median VG scores were 13 (IQR 9 – 20) and 12 (IQR 8 – 14) for non-ESBL-Ec and ESBL-Ec, respectively (*P* value = .002). VG scores among non-ESBL-Ec from a primary urinary focus (median 15, IQR 11 – 21) were higher compared to non-ESBL-Ec from a primary gastro-intestinal (median 10, IQR 5 – 13) or hepatic-biliary focus (median 11, IQR 5 – 18) (*P* values = .007 and .04, respectively). VG content varied between different *E. coli* STs.

Conclusions: Non-ESBL-Ec and ESBL-Ec blood isolates from two Dutch hospitals differed in clonal distribution, resistance gene and VG content. Also, resistance gene and VG content differed between non-ESBL-Ec from different primary foci of ECB.

INTRODUCTION

Escherichia coli is the leading causative pathogen in Gram-negative bacteremia and is associated with 30-day mortality up to 18%. [1-4] Antibiotic treatment options of *E. coli* bacteremia (ECB) are getting compromised by the pandemic presence of extended-spectrum beta-lactamases (ESBLs); conferring resistance to antibiotics commonly used for ECB treatment such as third-generation cephalosporins. Worryingly, the incidence of ECB is increasing and in some European countries, the incidence of ECB with antibiotic-resistant strains seems to increase faster than ECB caused by susceptible strains. [3-6] Even though the individual patient and financial burden is increased for resistant ECB episodes, ECB due to susceptible strains is far more common and therefore determines the major part of the ECB disease burden. The majority of ECBs is of community onset and is preceded by an infection in the urinary tract, but other sources, such as the hepatic-biliary tract, also comprise important primary foci. [3,7] These clinical characteristics of ECB episodes are important because they indicate different target populations for prevention. Thorough insight in the molecular epidemiology of both ESBL-negative and ESBL-positive ECB episodes with different clinical characteristics is key in identifying targets for the development of future preventive strategies, such as *E. coli* vaccines that are currently being developed. [8] Up to now, the molecular epidemiology of ECB in the Netherlands was mainly described in single-center studies [9] or among antimicrobial resistant isolates only. [10]

In this study, we aimed to analyze the current population structure of ECB in the Netherlands, with special attention to differences in antimicrobial resistance and virulence gene (VG) content and clonal and serotype distribution between isolates with different clinical epidemiological characteristics and between non-ESBL-producing *E. coli* (non-ESBL-Ec) and ESBL-producing *E. coli* (ESBL-Ec) blood isolates.

METHODS

Study design

Details of the study design is fully described elsewhere. [11] In short, unique patients with ECB were retrospectively identified in the University Medical Center Utrecht, a 1,042-bed tertiary care center and the Amphia Hospital in Breda, an 837-bed teaching hospital. In each hospital, a random sample of 40 isolates per year for the years 2014, 2015 and 2016 was selected, comprising ~24% of all first bacteraemic *E. coli* isolates in a year. In addition, all ESBL-Ec blood isolates from 2014 to 2016 were selected. Whole genome sequencing (WGS) was performed

by The Netherlands National Institute for Public Health and the Environment (RIVM) using the Illumina HiSeq 2500 (BaseClear, Leiden, the Netherlands). All generated raw reads were submitted to the European Nucleotide Archive (ENA) of the European Bioinformatics Institute (EBI) under the study accession number PRJEB35000. De novo assembly was performed using SPAdes genome assembler v.3.6.2 and quality of assembles was assessed using QUAST. [12] ESBL-production was defined as confirmed phenotypic ESBL-positivity, unless described otherwise. [11] Baseline characteristics were compared between non-ESBL-Ec and ESBL-Ec ECB episodes by the Fisher's Exact or Pearson $\chi 2$ test for categorical variables and by Mann-Whitney U test for continuous variables when applicable. A two-tailed *P* value <.05 was considered statistically significant.

This study does not fall under the scope of the Medical Research Involving Human Subjects Act. The Medical Research Ethics Committee of the UMCU has therefore waived the need for official approval by the Ethics Committee (IRB number 18/056). Individual informed consent was not obtained and all study data were analyzed and stored in a pseudonymized form. All statistical analyses were performed with Statistical Package for Social Sciences V.25.0 (SPSS, Chicago, Illinois, USA) and R Version 3.4.1.

Multi-locus sequence types (MLST)

Multi-locus sequence types (STs) were based on the allelic profile of seven housekeeping genes and were determined using mlst2.0 (https://github.com/tseemann/mlst), by scanning contig files against the *E. coli* PubMLST typing scheme (updated May 12th, 2018). Clonal (i.e. ST) distribution was presented stratified for non-ESBL-Ec and ESBL-Ec isolates and by epidemiological subgroups. Genotype (ST) diversity was analysed by Simpson's diversity index. [13]

Serotyping

Serotypes were assigned by using the web-tool SerotypeFinder 2.0 from the Center for Genomic Epidemiology at the Danish Technical University, Lyngby, Denmark (https://cge.cbs.dtu.dk/services/SerotypeFinder). [14] Simpson's index for serotype diversity was calculated for non-ESBL-Ec and ESBL-Ec isolates. Serotype distribution among non-ESBL-Ec and ESBL-Ec was compared to two current *E. coli* vaccine candidates [8,15], excluding those isolates in which no definitive serotype could be defined.

Antimicrobial resistance genes and virulence genes

Abricate (https://github.com/tseemann/abricate) v0.8.13 was used for (i) mass screening of contigs for (acquired) antimicrobial resistance genes using ResFinder 3.1.0 (download 24 January 2019), and (ii) to determine presence of VG by BLAST against the VFDB database (http://www.

mgc.ac.cn/VFs) (download 8 February 2019). [16,17] We searched for 49 putative VG that were previously described as extra-intestinal pathogenic *E. coli* (ExPEC)-associated VG. [18-22] If any of the predefined VG were not included in VFDB, BLAST against the ecoli_VF_collection database was performed (date 8 February 2019). [23] Coverage length and sequence identity thresholds were 80% and 95%. Resistance gene count was defined as the total number of unique identified acquired resistance genes per isolate. Resistance gene counts were compared between non-ESBL-Ec and ESBL-Ec with the non-parametric Wilcoxon rank sum test (for this comparison only, resistance gene count of ESBL-Ec was corrected for presence of the ESBL gene). The VG score was defined as the total number of pre specified VG within an isolate, adjusted for multiple detection of the *afa/dra* (Afa/Dr adhesins), *pap* (P fimbrial adhesins), *sfa/foc* (S and F1C fimbrial adhesins) and *kpsM* (group 2 and III capsule) operons, as described previously. [20] If a VG was detected multiple times within a single isolate (i.e. different quality measures), it was only counted once. The *kpsM*, *afa/dra* and *sfa/foc* operons were considered present if any of the corresponding genes or allelic variants were identified. Resistance gene counts and VG scores were further analysed for non-ESBL-Ec and ESBL-Ec separately and were compared between isolates with different epidemiological characteristics and different STs using Kruskal-Wallis one-way ANOVA. In case of an overall ANOVA *P* value <.05, post-hoc pairwise comparisons were made with the non-parametric Wilcoxon-rank sum test and the Holm-Bonferroni *P* value correction was applied to account for multiple testing.

RESULTS

Patient characteristics

The isolate collection consisted of 212 phenotypic non-ESBL-Ec and 69 ESBL-Ec blood isolates (Fig 1). Distribution of age, sex, onset of infection and primary foci were comparable between non-ESBL-Ec and ESBL-Ec bacteremia episodes (Table 1). As compared to non-ESBL-Ec, ECB episodes with ESBL-Ec were less often of community onset (63.8% versus 81.1%, *P* value = .003). Crude 30-day and 1-year mortality were higher in ECB episodes caused by ESBL-Ec (27.5% and 50.7%, respectively) compared to ECB episodes caused by non-ESBL-Ec (11.3% and 29.2%, respectively) (both *P* values = .001).

Clonal distribution

Among non-ESBL-Ec, ST73 was the most frequently observed ST (N = 26, 12.3%), followed by ST131 (N = 22, 10.4%). Isolates of ST73, 95, 127, 141, 80 and 1193 were solely identified among non-ESBL-Ec (Fig 2). ST131 was dominant among ESBL-Ec (N = 30, 43.5%) and prevalence was higher than among non-ESBL-Ec (*P* value < .001). Simpson's index for clonal diversity was 95.6%

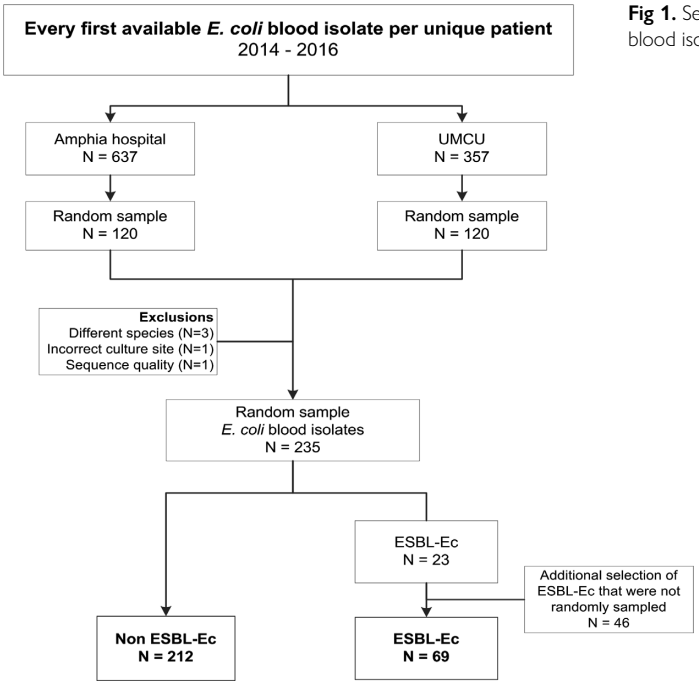


Fig 1. Selection of *E. coli* blood isolates.

Table 1. Baseline epidemiological characteristics of *E. coli* bacteraemia episodes.

	Non-ESBL-Ec[a] N = 212		ESBL-Ec[a] N = 69		*P* value[b]
Median age, years (IQR)	69	(59 – 77)	69	(56 – 76)	.80
Female sex (%)	102	(48.1)	32	(46.4)	.80
Community onset (%)	172	(81.1)	44	(63.8)	*.003*
Primary focus of ECB (%)					
Urinary tract	103	(48.6)	30	(43.5)	.79
Hepatic-biliary	46	(21.7)	14	(20.3)	
Gastro-intestinal	23	(10.8)	7	(10.1)	
Other	10	(4.7)	5	(7.2)	
Unknown	30	(14.2)	13	(18.8)	
Urinary catheter (%)	69	(32.5)	28	(40.6)	.22
Ward (%)					
Non-ICU	182	(85.8)	58	(84.1)	.71
ICU	30	(14.2)	11	(15.9)	
Mortality (%)					
30-day	24	(11.3)	19	(27.5)	*.001*
1-year	62	(29.2)	35	(50.7)	*.001*

ECB, *E. coli* bacteraemia; ESBL, extended-spectrum beta-lactamase; ESBL-Ec, ESBL-producing *E. coli*; ICU, intensive care unit; IQR, interquartile range; non-ESBL-Ec, non-ESBL-producing *E. coli*. [a]ESBL-positivity based on phenotype. [b]*P* value of comparison between non-ESBL-Ec versus ESBL-Ec, calculated with Pearson's χ2, Fisher's exact, or Mann-Whitney U test when applicable. *P* values in italic represent *P* values <.05.

(95% CI 94.4% – 96.8%) and 80.6% (95% CI 70.9% – 90.4%) for non-ESBL-Ec and ESBL-Ec, respectively. The occurrence of different STs did not differ between nosocomial and community onset ECB (Supplementary Figure S1 and Table S1). ST131 was the dominant ST among ESBL-positive ECB episodes with a primary urinary (63%) and gastro-intestinal focus (57%), which was higher as compared to other primary foci of ESBL-positive ECB (i.e. 21% among primary hepatic-biliary focus, supplementary Figure S2 and Table S2).

Serotypes

The most common serotype O25:H4 was identified in 19 (9.0%) non-ESBL-Ec and 24 (34.8%) ESBL-Ec isolates, which largely reflected the prevalence of ST131 in each group (Table 2). Multiple serotypes only occurred among non-ESBL-Ec, such as O6:H1 and O6:H31. ST73 was most often of serotype O6:H1 (16/26, 61.5%). Simpson's index for serotype diversity was 96.7% (95% CI 95.8% – 97.6%) and 83.8% (95% CI 76.9% – 90.6%) for non-ESBL-Ec and ESBL-Ec, respectively. Non-ESBL-Ec and ESBL-Ec isolates from ECB episodes with a primary focus in the urinary tract were most often of O-serotype O6 (15/103, 14.6%) and O25 (17/30, 56.7%), respectively (Supplementary Table S3). For ECB episodes with a primary focus in the hepatic-biliary tract, O25 was the most prevalent O-serotype among non-ESBL-Ec (7/46, 15.2%) and O8 (4/14, 28.6%) among ESBL-Ec isolates (Supplementary Table S3). 53 (25.0%) non-ESBL-Ec and 25 (36.2%) ESBL-Ec isolates belonged to either O1, O2, O6 or O25, the serotypes of the 4-valent *E. coli* vaccine that has reached phase 2 development stage [8,24], whereas the majority of non-ESBL-Ec (N = 113; 53.3%) and ESBL-Ec isolates (N = 35; 50.7%) belonged to one of the O-serotypes of the new 10-valent conjugant *E. coli* vaccine (ExPEC-10V) that is currently in development. [15]

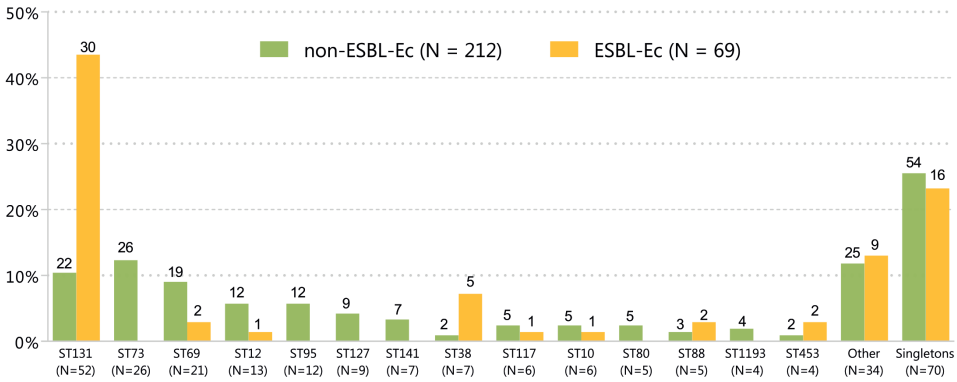


Fig 2. ST distribution among non-ESBL-Ec versus ESBL-Ec[a] in order of frequency[b]. ESBL, extended-spectrum beta-lactamase; ESBL-Ec, ESBL-producing *E. coli*; non-ESBL-Ec, non-ESBL-producing *E. coli*; ST, sequence type. [a]ESBL-positivity based on phenotypic ESBL production. [b]Missing STs and STs that occurred ≤3 times are grouped in "Other". STs that only occurred once are grouped in "Singletons". The height of each individual bars represents the proportion of the ST within the group of non-ESBL-Ec and ESBL-Ec, respectively. The numbers represent the absolute numbers of occurrence.

Table 2. Serotype distribution among *E. coli* blood isolates, stratified for ESBL-positivity

	Non-ESBL-Ec N = 212 (%)	**ESBL-Ec[a] N = 69 (%)**
O25:H4	19 (9.0)	24 (34.8)
O6:H1	16 (7.5)	-
O2/O50:H6	10 (4.7)	-
O6:H31	9 (4.2)	-
O15:H18	7 (3.3)	2 (2.9)
O17/O44/O77:H18	8 (3.8)	-
O4:H5	7 (3.3)	1 (1.4)
O75:H5	8 (3.8)	-
O8:H9	5 (2.4)	2 (2.9)
O16:H5	3 (1.4)	3 (4.3)
O86:H18	1 (0.5)	4 (5.8)
O4:H1	5 (2.4)	-
O1:H7	4 (1.9)	-
O117:H4	4 (1.9)	-
O2/O50:H1	4 (1.9)	-
O23:H16	2 (0.9)	2 (2.9)
O25:H1	4 (1.9)	-
O18/O18ac:H7	3 (1.4)	-
O2/O50:H7	3 (1.4)	-
O45:H7	3 (1.4)	-
O75:H7	3 (1.4)	-
O8:H17	3 (1.4)	-
O9:H17	-	2 (2.9)
O9/O104:H9	-	2 (2.9)
O13/O135:H4	2 (0.9)	-
O18:H1	2 (0.9)	-
O18:H5	2 (0.9)	-
O22:H1	2 (0.9)	-
O24:H4	2 (0.9)	-
O8:H10	2 (0.9)	-
O8:H25	2 (0.9)	-
O8:H30	2 (0.9)	-
Singletons	45 (21.2)	13 (18.8)
Unknown	20 (9.4)	14 (20.3)

ESBL, extended-spectrum beta-lactamase; ESBL-Ec, ESBL-producing *E. coli*, non-ESBL-Ec, non-ESBL-producing *E. coli*. [a]ESBL-positivity based on phenotypic ESBL production.

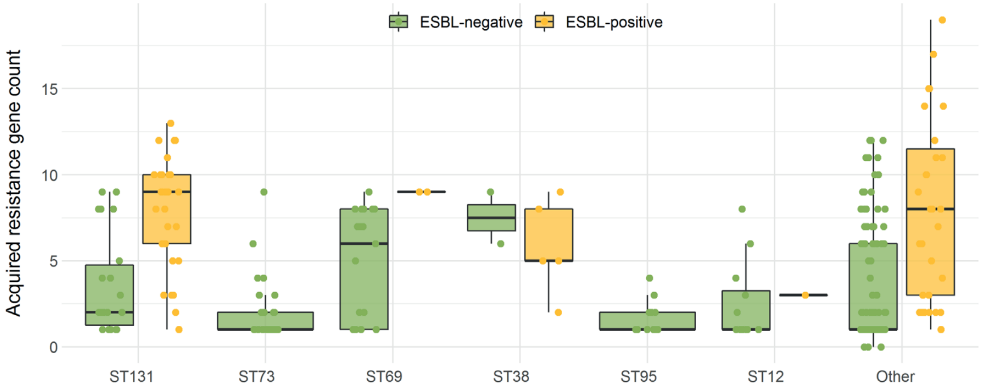


Fig 3. Acquired resistance gene count per ST, stratified for ESBL-positivity[a]. ESBL, extended-spectrum beta-lactamase; ESBL-Ec, ESBL-producing *E. coli*; non-ESBL-Ec, non-ESBL-producing *E. coli*; ST, sequence type. [a]ESBL-positivity based on phenotypic ESBL production. Boxplots display median resistance gene count and inter quartile range (IQR); every dot represents a single isolate. Only STs that occurred >5% within non-ESBL-Ec or ESBL-Ec were grouped into main groups, the rest was categorized as "Other". Results of the pairwise comparisons between STs can be found in the supplementary material.

Antimicrobial resistance genes

In total, 69 unique acquired resistance genes were identified (see Supplementary material). ESBL-genes were detected in 65 (94.2%) of 69 *E. coli* blood isolates with phenotypic ESBL production. $bla_{CTX\text{-}M\text{-}15}$ was most prevalent (N = 28, 43.1%), followed by $bla_{CTX\text{-}M\text{-}9}$ (N = 14, 21.5%) and $bla_{CTX\text{-}M\text{-}27}$ (N = 9, 13.8%). The median acquired resistance gene count for non-ESBL-Ec versus ESBL-Ec was 1 (IQR 1 – 6) versus 7 (IQR 4 – 9) (*P* value < .001). Among non-ESBL-Ec, acquired resistance gene count was highest among blood isolates from a primary gastro-intestinal focus (median 4, IQR 1 – 8). There were significant differences in resistance gene count for different primary foci of non-ESBL ECB, but absolute differences were small (Supplementary Figure S3 and Table S5). Among ESBL-Ec isolates, there were no statistical significant differences in acquired resistance gene counts between epidemiological subgroups. We observed no significant differences among non-ESBL-Ec or ESBL-Ec isolates of different clonal backgrounds (Fig 3 and Supplementary Table S6).

Virulence genes

Of the 49 predefined ExPEC-associated VG, 44 (89.8%) were detected in at least one *E. coli* blood isolate and VG scores ranged from zero (N = 1 non-ESBL-Ec) to 25 (N = 2 ESBL-Ec) (see supplement). The median VG score was 13 (IQR 9 – 20) in non-ESBL-Ec and 12 (IQR 8 – 14) in ESBL-Ec blood isolates (*P* value = .002). There were no significant differences in VG scores of epidemiological subgroups, except that the average VG score of non-ESBL-Ec isolates with a primary urinary focus (median 15, IQR 11 – 21) were higher compared to non-ESBL-Ec isolates with a primary focus in the gastro-intestinal (median 10, IQR 5 – 13) or hepatic-biliary tract

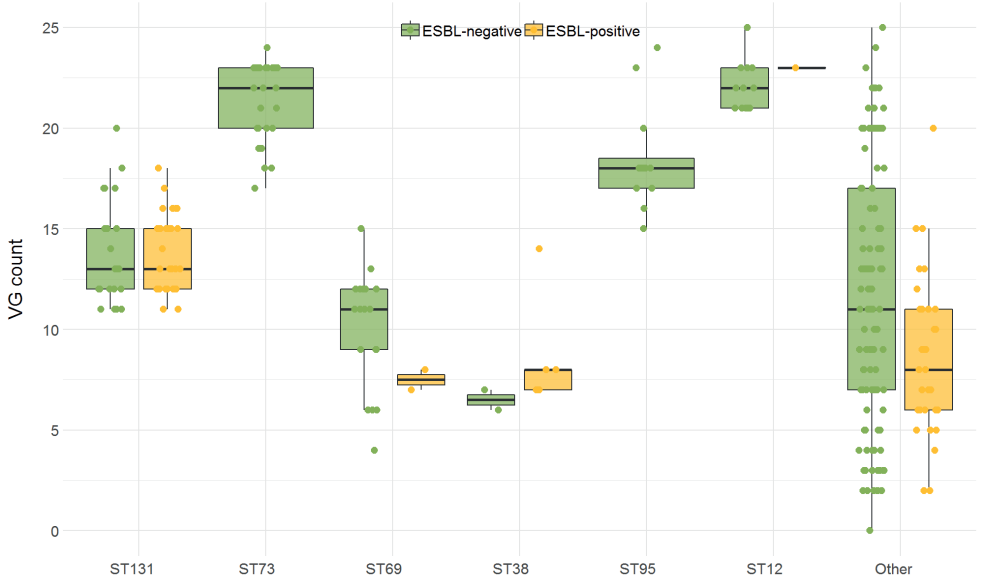


Fig 4. ExPEC-associated VG score in different STs, stratified for ESBL-positivity[a]. ESBL, extended-spectrum beta-lactamase; ESBL-Ec, ESBL-producing *E. coli*; ExPEC, extra-intestinal pathogenic *E. coli*; non-ESBL-Ec, non-ESBL-producing *E. coli*; ST, sequence type; VG, virulence gene. [a]ESBL-positivity based on phenotypic ESBL production. Boxplots display median VG score and inter quartile range (IQR); every dot represents a single isolate. Only STs that occurred >5% within non-ESBL-Ec or ESBL-Ec were grouped into main groups, the rest was categorized as "Other". Results of pairwise comparisons between STs can be found in the supplementary material.

(median 11, IQR 5 – 18) (*P* values = .007 and .04, respectively) (Supplementary Fig S4 and Table S8). There was heterogeneity in VG scores between non-ESBL-Ec of different STs, this was less pronounced for ESBL-Ec isolates (Fig 4 and Supplementary Table S9). ESBL-negative ST38 had the lowest average VG score (median 7, IQR 6 – 7) and ESBL-positive ST12 had the highest VG score (median 23, IQR 23 – 23). Median VG score of both ESBL-negative and ESBL-positive ST131 isolates was 13 (IQR 12 – 15).

DISCUSSION

In this study, we found that ESBL-producing *E. coli* blood isolates were different from non-ESBL-producing *E. coli* causing bacteraemia in terms of clonal distribution, serotype distribution, antimicrobial resistance gene count and VG scores.

In line with previous research, the clonal distribution among ESBL-Ec blood isolates was less diverse compared to non-ESBL-Ec. [25-27] This was mainly caused by the predominance of ST131 within ESBL-Ec, as has been described before. [28-29] In contrast, ST73, a ST that so far is known for its susceptibility to antibiotics [28], was only identified among non-ESBL-Ec blood isolates. Previous studies have shown very different phylogeny of ST73 and ST131, with the first being characterised by a higher level of diversification in to divergent clades. [28,30] The association between ESBL phenotype and STs in *E. coli*, which is repeatedly found, implies that the genetic make-up of strains contributes to the ability to acquire and subsequently maintain plasmids carrying ESBL genes. Indeed, a recent large-scale study that compared the pan-genomes of invasive *E. coli* isolates, including ST131 and ST73, suggested that due to ongoing adaptation to long term human intestinal colonisation and consequent evolutionary gene selection, ST131 might have become able to reduce the fitness costs of long term plasmid maintenance. [31-32] It has been hypothesized that this is also true for other *E. coli* lineages that are associated with multidrug resistance (MDR). Reducing the fitness costs of replicating plasmids encoding MDR will result in having competitive advantage over other intestinal strains. [33]

We hypothesized that the clonal distribution and resistance gene and VG content would differ between ECB episodes of community and hospital onset and between different primary foci, as a result of adaptive evolution of intestinal *E. coli*. We observed some statistical significant differences in resistance gene count and VG scores among non-ESBL-Ec from different primary foci of ECB, such as higher VG scores of blood isolates from a primary urinary focus as compared to isolates from a primary focus in the gastro-intestinal or hepatic-biliary tract. However, absolute differences in gene counts were small and the clinical significance remains unclear. In the current study, we found that differences in molecular content mostly depended on phenotypic ESBL-production and STs. This confirms the findings from a recent study that was performed in Scotland. [34] In that study, there were combinations of VGs as well as a particular accessory gene composition that differentiated between STs rather than between epidemiological factors. The association between ST69 and community onset ECB, as found in the Scottish study, was not identified in the current study. Other differences were the large proportion of *E. coli* isolates from ECB episodes that were deemed hospital-acquired (62%) as compared to our study (18.4% for ESBL-negative and 36.2% for ESBL-positive ECB) and in that study, analyses were not stratified for ESBL-positivity.

Interestingly, in our study, isolates that belonged to ST73 had low resistance gene content but relatively high VG scores as compared to other STs. Furthermore, the average VG score among non-ESBL-Ec was slightly higher than among ESBL-Ec blood isolates, which supports findings of other studies that described an inverse association between antimicrobial resistance and VG content in ExPEC *E. coli*. [35-40] This historical negative association has been challenged, considering

the current predominance of ST131, with its relatively broad VG profile despite being associated with MDR. [41-43] Also in our study, ESBL-positive and ESBL-negative ST131 isolates had equal average VG scores.

We identified serotype O25:H4 as the most prevalent serotype causing ESBL-negative as well as ESBL-positive ECB in the Netherlands, followed by O6:H1. The serotype distribution among non-ESBL-Ec was more heterogeneous compared to ESBL-Ec, similar to the differences in clonal diversity. [44] A large recent European surveillance study that included 1,110 *E. coli* blood isolates from adults between 2011 and 2017 showed that there is heterogeneity in serotype distribution among different countries, which highlights the need for country specific data, such as provided in the current study. [15] We showed that the coverage of the new potential 10-valent vaccine was higher compared to the 4-valent vaccine and was actually doubled for non-ESBL-Ec bacteraemia. Findings of the current study may help further evaluation and implementation of *E. coli* vaccines.

Strengths of the current study are the multicenter design and combination of epidemiological characteristics with highly discriminatory genetic data. There are also important limitations. Firstly, *E. coli* is a heterogeneous species, of which the seven MLST genes only constitute a small proportion of the entire gene content. Because we also only investigated a small fraction of the genes that are commonly part of the accessory genome, such as VGs and acquired resistance genes, we may have missed genomic differences that could have importantly contributed to ecological specialization in the different clinically relevant primary foci. Secondly, we selected *E. coli* isolates from a tertiary care center and teaching hospital from two different regions, which we considered to be representative of the Netherlands. The description of strains that were identified here might not be entirely generalizable to other countries since there could be differences between circulating *E. coli* strains, dependent on local population characteristics and resistance levels. Thirdly, many pairwise comparisons between subgroups were performed, which increases the risk of false-positive findings (i.e. type I errors). Even though we applied a strict *P* value correction for multiple testing, this naturally does not eliminate the risk of false-positive findings. The analyses on resistance gene and VG content should therefore be viewed as hypothesis generating.

In conclusion, associations between clinical characteristics of ECB episodes and molecular content of *E. coli* isolates were limited. However, we did identify important differences in clonality, serotypes, antimicrobial resistance genes and VG scores between non-ESBL-Ec and ESBL-Ec blood isolates that reached beyond their phenotypic ESBL-positivity. Future studies that aim to describe the molecular epidemiology of ECB should therefore preferably focus on *E. coli* without preselection on ESBL-positivity, to limit the risk of inferring characteristics of resistant *E. coli* to the *E. coli* population as a whole.

List of abbreviations

BLAST, basic local alignment search tool; ECB, *E. coli* bacteraemia; ESBL, extended-spectrum beta-lactamase; ESBL-Ec, ESBL-producing *E. coli*; ENA, European Nucleotide Archive; ExPEC, extra-intestinal pathogenic *E. coli*; ICU, intensive care unit; IRB, institutional review board; IQR, interquartile range; MDR, multidrug resistance; MLST, multi-locus sequence type; non-ESBL-Ec, non-ESBL-producing *E. coli*; WGS, whole genome sequencing; VG, virulence gene

Acknowledgements: We would sincerely like to thank Kim van der Zwaluw, Carlo Verhulst and Judith Vlooswijk for their contributions in the laboratory execution of the study. Preliminary results of this paper were presented at the 29th European Congress of Clinical Microbiology & Infectious Diseases, Amsterdam, the Netherlands, 13-16 April 2019 (P1450).

Conflicts of interest: No conflicts of interest to disclose.

Funding: This study was internally funded by the Netherlands National Institute for Public Health and the Environment (RIVM) and the University Medical Center Utrecht (UMCU) and the authors received no specific funding for this work.

REFERENCES

1. Abernethy JK, Johnson AP, Guy R, Hinton N, Sheridan EA, Hope RJ. Thirty day all-cause mortality in patients with *Escherichia coli* bacteraemia in England. ***Clin Microbiol Infect.*** 2015;21: 251.e1–8. doi:10.1016/j.cmi.2015.01.001
2. Fitzpatrick JM, Biswas JS, Edgeworth JD, Islam J, Jenkins N, Judge R, ***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: 244–251. doi:10.1016/j.cmi.2015.10.034
3. Vihta K-D, Stoesser N, Llewelyn MJ, Quan TP, Davies T, Fawcett NJ, ***et al.*** Trends over time in *Escherichia coli* bloodstream infections, urinary tract infections, and antibiotic susceptibilities in Oxfordshire, UK, 1998-2016: a study of electronic health records. ***Lancet Infect Dis.*** 2018;18: 1138–1149. doi:10.1016/S1473-3099(18)30353-0
4. de Kraker MEA, Jarlier V, Monen JCM, Heuer OE, van de Sande N, Grundmann H. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. ***Clin Microbiol Infect.*** 2013;19: 860–868. doi:10.1111/1469-0691.12028
5. Van Der Steen M, Leenstra T, Kluytmans JAJW, Van Der Bij AK. Trends in expanded-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* among Dutch clinical isolates, from 2008 to 2012. ***PLoS One.*** 2015;10: e0138088. doi:10.1371/journal.pone.0138088
6. Schlackow I, Stoesser N, Walker AS, Crook DW, Peto TEA, Wyllie DH. Increasing incidence of *Escherichia coli* bacteraemia is driven by an increase in antibiotic-resistant isolates: electronic database study in Oxfordshire 1999-2011. ***J Antimicrob Chemother.*** 2012;67: 1514–1524. doi:10.1093/jac/dks082
7. Abernethy J, Guy R, Sheridan EA, Hopkins S, Kiernan M, Wilcox MH, ***et al.*** Epidemiology of *Escherichia coli* bacteraemia in England: results of an enhanced sentinel surveillance programme. ***J Hosp Infect.*** 2017;95: 365–375. doi:10.1016/j.jhin.2016.12.008
8. Huttner A, Hatz C, van den Dobbelsteen G, Abbanat D, Hornacek A, Frolich R, ***et al.*** Safety, immunogenicity, and preliminary clinical efficacy of a vaccine against extraintestinal pathogenic *Escherichia coli* in women with a history of recurrent urinary tract infection: a randomised, single-blind, placebo-controlled phase 1b trial. ***Lancet Infect Dis.*** 2017;17: 528–537. doi:10.1016/S1473-3099(17)30108-1
9. Overdevest ITMA, Bergmans AMC, Verweij JJ, Vissers J, Bax N, Snelders E, ***et al.*** Prevalence of phylogroups and O25/ST131 in susceptible and extended-spectrum ß-lactamase-producing *Escherichia coli* isolates, the Netherlands. ***Clin Microbiol Infect.*** 2015;21: 570.e1-e4. doi:10.1016/j.cmi.2015.02.020
10. Van Der Bij AK, Peirano G, Goessens WHF, Van Der Vorm ER, Van Westreenen M, Pitout JDD. Clinical and molecular characteristics of extended-spectrum-ß-lactamase-producing *Escherichia coli* causing bacteremia in the Rotterdam Area, Netherlands. ***Antimicrob Agents Chemother.*** 2011;55: 3576–3578. doi:10.1128/AAC.00074-11
11. van Hout D, Verschuuren TD., Bruijning-Verhagen PCJ., Bosch T., Reuland EA., Fluit AC., ***et al.*** Design of the EPIGENEC Study: Assessing the EPIdemiology and GENetics of *Escherichia coli* in the Netherlands. ***Preprints***. 2019; 2019020066. doi:10.20944/preprints201902.0066.v1
12. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. ***Bioinformatics***. 2013;29: 1072–1075. doi:10.1093/bioinformatics/btt086
13. Simpson EH. Measurement of diversity. ***Nature***. 1949;163.
14. Joensen KG, Tetzschner AMM, Iguchi A, Aarestrup FM, Scheutz F. Rapid and Easy In Silico Serotyping of *Escherichia coli* Isolates by Use of Whole-Genome Sequencing Data. ***J Clin Microbiol.*** 2015;53: 2410–2426. doi:10.1128/JCM.00008-15
15. Geurtsen J, Weerdenburg E, Davies T, Go O, Spiessens B, Geet G Van, ***et al.*** Extraintestinal pathogenic *Escherichia coli* surveillance study to determine O-serotype prevalence and antibiotic resistance in blood isolates collected in Europe, 2011–2017. ECCMID Conference 2019 #P1451.
16. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, ***et al.*** Identification of

acquired antimicrobial resistance genes. ***J Antimicrob Chemother.*** 2012;67: 2640–2644. doi:10.1093/jac/dks261
17. Liu B, Zheng D, Jin Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. ***Nucleic Acids Res.*** 2019;47: D687–D692. doi:10.1093/nar/gky1080
18. Johnson JR, Stell AL. Extended Virulence Genotypes of *Escherichia coli* Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. ***J Infect Dis.*** 2000;181: 261–72. doi:10.1086/315217
19. Johnson JR, Johnston BD, Porter S, Thuras P, Aziz M, Price LB. Accessory Traits and Phylogenetic Background Predict *Escherichia coli* Extraintestinal Virulence Better Than Does Ecological Source. ***J Infect Dis.*** 2019;219: 121–132. doi:10.1093/infdis/jiy459
20. Johnson JR, Porter S, Johnston B, Kuskowski MA, Spurbeck RR, Mobley HLT, ***et al.*** Host Characteristics and Bacterial Traits Predict Experimental Virulence for *Escherichia coli* Bloodstream Isolates From Patients With Urosepsis. ***Open Forum Infect Dis.*** 2015;2: ofv083. doi:10.1093/ofid/ofv083
21. Dale AP, Woodford N. Extra-intestinal pathogenic *Escherichia coli* (ExPEC): Disease, carriage and clones. ***J Infect.*** 2015;71: 615–626. doi:10.1016/j.jinf.2015.09.009
22. Dale AP, Pandey AK, Hesp RJ, Belogiannis K, Laver JR, Shone CC, ***et al.*** Genomes of *Escherichia coli* bacteraemia isolates originating from urinary tract foci contain more virulence-associated genes than those from non-urinary foci and neutropaenic hosts. ***J Infect.*** 2018;77: 534–543. doi:10.1016/j.jinf.2018.10.011
23. Leimbach A. ecoli_VF_collection: V.0.1. Zenodo. 2016. Available at: http://dx.doi.org/10.5281/zenodo.56686.
24. Frenck RW, Ervin J, Chu L, Abbanat D, Spiessens B, Go O, ***et al.*** Safety and immunogenicity of a vaccine for extra-intestinal pathogenic *Escherichia coli* (ESTELLA): a phase 2 randomised controlled trial. ***Lancet Infect Dis.*** 2019;19: 631–640. doi:10.1016/S1473-3099(18)30803-X
25. Mathers AJ, Peirano G, Pitout JDD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. ***Clin Microbiol Rev.*** 2015;28: 565–591. doi:10.1128/CMR.00116-14
26. Hertz FB, Nielsen JB, Schonning K, Littauer P, Knudsen JD, Lobner-Olesen A, ***et al.*** "Population structure of drug-susceptible, -resistant and ESBL-producing *Escherichia coli* from community-acquired urinary tract". ***BMC Microbiol.*** 2016;16: 63. doi:10.1186/s12866-016-0681-z
27. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global Extraintestinal Pathogenic *Escherichia coli* (ExPEC) Lineages. ***Clin Microbiol Rev.*** 2019;32. doi:10.1128/CMR.00135-18
28. Kallonen T, Brodrick HJ, Harris SR, Corander J, Brown NM, Martin V, ***et al.*** Systematic longitudinal survey of invasive *Escherichia coli* in England demonstrates a stable population structure only transiently disturbed by the emergence of ST131. ***Genome Res.*** 2017;27: 1437–1449. doi:10.1101/gr.216606.116
29. Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. *Escherichia coli* ST131, an intriguing clonal group. ***Clin Microbiol Rev.*** 2014;27: 543–574. doi:10.1128/CMR.00125-13
30. Alhashash F, Wang X, Paszkiewicz K, Diggle M, Zong Z, McNally A. Increase in bacteraemia cases in the East Midlands region of the UK due to MDR *Escherichia coli* ST73: high levels of genomic and plasmid diversity in causative isolates. ***J Antimicrob Chemother.*** 2015;71: 339–343. doi:10.1093/jac/dkv365
31. McNally A, Oren Y, Kelly D, Pascoe B, Dunn S, Sreecharan T, ***et al.*** Combined analysis of variation in core, accessory and regulatory genome regions provides a super-resolution view into the evolution of bacterial populations. ***PLoS Genet.*** 2016;12: e1006280. doi:10.1371/journal.pgen.1006280
32. McNally A, Kallonen T, Connor C, Abudahab K, Aanensen DM, Horner C, ***et al.*** Diversification of Colonization Factors in a Multidrug-Resistant *Escherichia coli* Lineage Evolving under Negative Frequency-Dependent Selection. ***MBio***. 2019;10: e00644-19. doi:10.1128/mBio.00644-19
33. Dunn SJ, Connor C, McNally A. The evolution and transmission of multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae*: the complexity of clones and plasmids. ***Curr Opin Microbiol.*** 2019;51: 51–56. doi:10.1016/j.mib.2019.06.004
34. Goswami C, Fox S, Holden M, Connor M, Leanord A, Evans TJ. Genetic analysis of invasive *Escherichia*

coli in Scotland reveals determinants of healthcare-associated versus community-acquired infections. ***Microb genomics.*** 2018;4. doi:10.1099/mgen.0.000190

35. Velasco M, Horcajada JP, Mensa J, Moreno-Martinez A, Vila J, Martinez JA, ***et al.*** Decreased invasive capacity of quinolone-resistant *Escherichia coli* in patients with urinary tract infections. ***Clin Infect Dis.*** 2001;33: 1682–1686. doi:10.1086/323810
36. Vila J, Simon K, Ruiz J, Horcajada JP, Velasco M, Barranco M, ***et al.*** Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? ***J Infect Dis.*** 2002;186: 1039–1042. doi:10.1086/342955
37. Johnson JR, van der Schee C, Kuskowski MA, Goessens W, van Belkum A. Phylogenetic background and virulence profiles of fluoroquinolone-resistant clinical *Escherichia coli* isolates from the Netherlands. ***J Infect Dis.*** 2002;186: 1852–1856. doi:10.1086/345767
38. Johnson JR, Kuskowski MA, Owens K, Gajewski A, Winokur PL. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. ***J Infect Dis.*** 2003;188: 759–768. doi:10.1086/377455
39. Horcajada JP, Soto S, Gajewski A, Smithson A, Jiménez de Anta MT, Mensa J, ***et al.*** Quinolone-resistant uropathogenic *Escherichia coli* strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. ***J Clin Microbiol.*** 2005;43: 2962–2964. doi:10.1128/JCM.43.6.2962-2964.2005
40. Moreno E, Prats G, Sabaté M, Pérez T, Johnson JR, Andreu A. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. ***J Antimicrob Chemother.*** 2006;57: 204–211. doi:10.1093/jac/dki468
41. Johnson JR, Porter S, Thuras P, Castanheira M. Epidemic emergence in the United States of *Escherichia coli* sequence type 131-H30 (ST131-H30), 2000 to 2009. ***Antimicrob Agents Chemother.*** 2017;61: e00732-17. doi:10.1128/AAC.00732-17
42. Johnson JR, Porter S, Thuras P, Castanheira M. The pandemic H30 subclone of sequence type 131 (ST131) as the leading cause of multidrug-Resistant *Escherichia coli* infections in the United States (2011-2012). ***Open forum Infect Dis.*** 2017;4: ofx089–ofx089. doi:10.1093/ofid/ofx089
43. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic *Escherichia coli*. ***EcoSal Plus.*** 2018;8: doi:10.1128/ecosalplus.ESP-0004-2017
44. Ciesielczuk H, Jenkins C, Chattaway M, Doumith M, Hope R, Woodford N, ***et al.*** Trends in ExPEC serogroups in the UK and their significance. ***Eur J Clin Microbiol Infect Dis.*** 2016;35: 1661–1666. doi:10.1007/s10096-016-2707-8

SUPPLEMENTARY MATERIAL

Figure S1. ST distribution among different onset of ECB

Table S1. ST distribution among different onset of ECB

Figure S2. ST distribution among different primary foci of ECB

Table S2. ST distribution among different primary foci of ECB

Table S3. Frequencies of O:serotypes per primary focus of ECB

Table S4. Detected resistance genes with ResFinder 3.1.0 per antibiotic group

Figure S3. Acquired resistance gene count among epidemiological subgroups

Table S5. Pairwise comparisons acquired resistance gene count between epidemiological subgroups

Table S6. Pairwise comparisons acquired resistance gene count between dominant STs

Table S7. Detected ExPEC-associated VG per VG category

Figure S4. ExPEC-associated VG score in different subgroups, stratified for ESBL-positivity

Table S8. Pairwise comparisons VG score between epidemiological subgroups

Table S9. Pairwise comparisons VG score between dominant STs

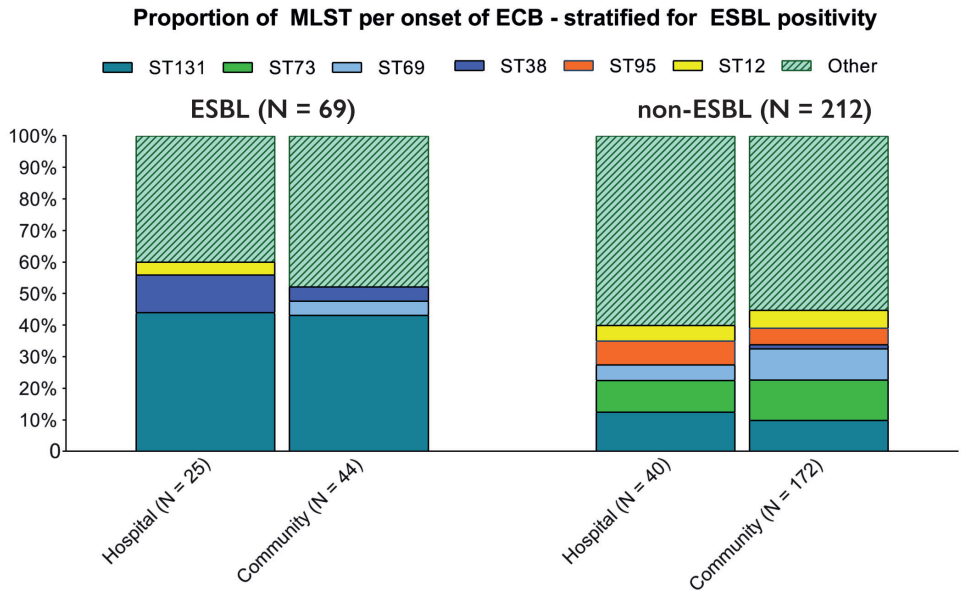


Figure S1. ST distribution among different onset of ECB[a].
[a]ESBL-positivity based on phenotypic ESBL production.
ECB, *E. coli* bacteremia; ESBL, extended spectrum beta-lactamase; ST, sequence type.
Only STs that occurred >5% within non-ESBL-Ec or ESBL-Ec were grouped into main ST groups, the rest is categorized as "Other".

Table S1. ST distribution among different onset of ECB

	ESBL *E. coli*[a]		**Non-ESBL *E. coli*[a]**	
	Hospital (N=25)	Community (N=44)	Hospital (N = 40)	Community (N = 172)
ST131, N (%)	11 (44)	19 (43)	5 (13)	17 (10)
ST73, N (%)	-	-	4 (10)	22 (13)
ST69, N (%)	-	2 (5)	2 (5)	17 (10)
ST38, N (%)	3 (12)	2 (5)	-	2 (1)
ST95, N (%)	-	-	3 (8)	9 (5)
ST12, N (%)	1 (4)	-	2 (5)	10 (6)
Other ST, N (%)	10 (40)	21 (48)	24 (60)	95 (55)

[a] ESBL-positivity based on phenotypic ESBL production.
ECB, *E. coli* bacteremia; ESBL, extended spectrum beta-lactamase; HB, hepatic-biliary; GI, gastro-intestinal; ST, sequence type

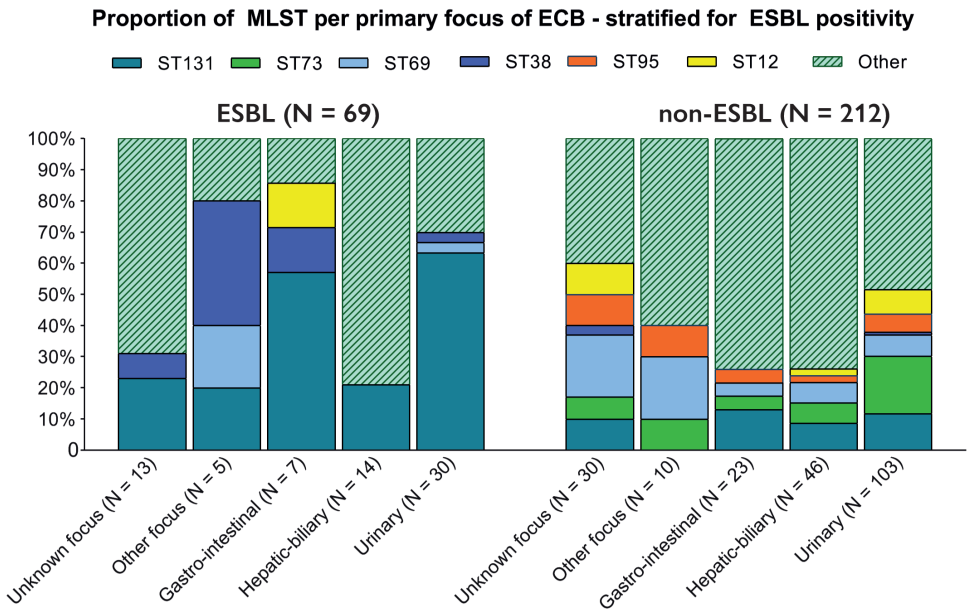


Figure S2. ST distribution among different primary foci of ECB[a]

[a]ESBL-positivity based on phenotypic ESBL production.

ECB, *E. coli* bacteremia; ESBL, extended spectrum beta-lactamase; ST, sequence type. Only STs that occurred >5% within non-ESBL-Ec or ESBL-Ec were grouped into main ST groups, the rest is categorized as "Other".

Table S2. ST distribution among different primary foci of ECB[a]

	ESBL *E. coli*					**Non-ESBL *E. coli***				
	Urinary (N=30)	HB (N=14)	GI (N=7)	Other (N=5)	Unknown (N=13)	Urinary (N=103)	HB (N=46)	GI (N=23)	Other (N=10)	Unknown (N=30)
ST131, N (%)	19 (63)	3 (21)	4 (57)	1 (20)	3 (23)	12 (12)	4 (9)	3 (13)	-	3 (10)
ST73, N (%)	-	-	-	-	-	19 (18)	3 (7)	1 (4)	1 (10)	2 (7)
ST69, N (%)	1 (3)	-	-	1 (20)	-	7 (7)	3 (7)	1 (4)	2 (20)	6 (20)
ST38, N (%)	1 (3)	-	1 (14)	2 (40)	1 (8)	1 (1)	-	-	-	1 (3)
ST95, N (%)	-	-	-	-	-	6 (6)	1 (2)	1 (4)	1 (10)	3 (10)
ST12, N (%)	-	-	1 (14)	-	-	8 (8)	1 (2)	-	-	3 (10)
Other ST, N (%)	9 (30)	11 (79)	1 (14)	1 (20)	9 (69)	50 (49)	34 (74)	17 (74)	6 (60)	12 (40)

[a] ESBL-positivity based on phenotypic ESBL production.

ECB, *E. coli* bacteremia; ESBL, extended spectrum beta-lactamase; HB, hepatic-biliary; GI, gastro-intestinal; ST, sequence type

Table S3. Frequencies of O:serotypes per primary focus of ECB

	ESBL *E. coli* (N = 69)[a]					**Non-ESBL *E. coli* (N = 212)[a]**				
	Urinary (N=30)	HB (N=14)	GI (N=7)	Other (N=5)	Unknown (N=13)	Urinary (N=103)	HB (N=46)	GI (N=23)	Other (N=10)	Unknown (N=30)
O25, N (%)	17 (57)	2 (14)	3 (43)	1 (20)	1 (8)	12 (12)	7 (15)	2 (9)	-	3 (10)
O6, N (%)	-	-	-	-	-	15 (15)	6 (13)	-	2 (20)	2 (7)
O4, N (%)	-	-	1 (14)	-	-	8 (8)	1 (2)	-	-	3 (10)
O2/O50, N (%)	-	-	-	-	-	10 (10)	6 (13)	-	1 (10)	2 (7)
O75, N (%)	-	-	-	-	-	4 (4)	1 (2)	2 (9)	1 (10)	3 (10)
O1, N (%)	1 (3)	-	-	-	-	2 (2)	1 (2)	-	-	1 (3)
O8, N (%)	-	4 (29)	-	-	-	5 (5)	3 (7)	6 (26)	2 (20)	1 (3)
O15, N (%)	1 (3)	-	-	1 (20)	-	5 (5)	-	-	-	4 (13)
O18, N (%)	-	-	-	-	-	5 (5)	-	-	1 (10)	1 (3)
O16, N (%)	2 (6)	1 (7)	1 (14)	-	-	2 (2)	-	2 (9)	-	-
Other serotype	9 (30)	7 (50)	2 (29)	3 (60)	12 (92)	35 (34)	21 (46)	11 (48)	3 (30)	10 (33)

[a] ESBL-positivity based on phenotypic ESBL production.

ESBL, extended spectrum beta-lactamase; HB, hepatic-biliary; GI, gastro-intestinal

frequencies of all serotypes of the 4-valent and new potential 10-valent ExPEC vaccine are reported, the rest (including missing / unknown serotypes) is grouped as "Other serotype". Percentages are column percentages.

Table S4. Detected resistance genes with ResFinder 3.1.0 per antibiotic group

(Broad-spectrum) Beta-lactamases			ESBL and ampC			Macrolides			Fluoroquinolones		
Gene	*N*	%	*Gene*	*N*	%	*Gene*	*N*	%	*Gene*	*N*	%
OXA-1	23	8	*CMY-146*	1	0	*ere(A)*	1	0.4	*qnrA1*	2	1
TEM-1A	8	3	*CMY-2*	2	1	*mph(A)*	48	17	*qnrS1*	5	2
TEM-1B	82	29	*CTX-M-1*	6	2	*mph(B)*	3	1			
TEM-1C	8	3	*CTX-M-102*	8	3						
TEM-1D	2	1	*CTX-M-14*	9	3						
TEM-30	1	0.4	*CTX-M-15*	29	10						
TEM-34	1	0.4	*CTX-M-27*	1	0.4						
TEM-40	1	0.4	*CTX-M-3*	1	0.4						
			CTX-M-55	1	0.4						
			CTX-M-9	2	1						
			SHV-102	5	2						
			SHV-12	1	0.4						
			TEM-28	1	0.4						
			TEM-35	1	0.4						
			TEM-52B	1	0.4						
Aminoglycosides			**Sulfanomides and trimetroprim**			**Tetracyclines**			**Other**		
Gene	*N*	%	*Gene*	*N*	%	*Gene*	*N*	%	*Gene*	*N*	%
aac(3)-Iia	1	0.4	*drfA1*	18	6	*tet(A)*	72	26	*catA1*	12	4
aac(3)-IIa	10	4	*drfA12*	6	2	*tet(B)*	27	10	*cmlA1*	6	2
aac(3)-IId	4	1	*drfA14*	10	4	*tet(D)*	1	0.4	*strA*	19	7
aac(3)-IId	7	2	*drfA17*	44	16	*tet(J)*	1	0.4	*strB*	10	4
aac(3)-IVa	1	0.4	*drfA21*	1	0.4	*tet(M)*	1	0.4	*cat*	1	1
aac(3)-VIa	1	0.4	*drfA5*	12	4	*tet(X)*	1		*mdf(A)*	260	93
aac(6')-Ib-cr	12	4	*drfA7*	9	3				*floR*	8	3
aac(6')-Ib0cr	8	3	*drfA8*	2	1				*lnu(F)*	5	2
aadA1	11	4	*sul1*	64	23						
aadA2	10	4	*sul2*	86	31						
aadA4	1	0	*sul3*	6	2						
aadA5	39	14									
ant(2'')-Ia	4	1									
ant(3'')-Ia	22	8									
ant(3'')-1b	63	22									
aph(3')-Ia	24	9									
aph(3')-Ib	1	0.4									
aph(4')-Ia	1	0.4									
aph(6')-Id	69	25									

In case genes were present twice within a strain, they were only counted once in the resistance gene count.
Indicated proportions are proportions of isolates in which the gene was detected (denominator N=281 isolates).

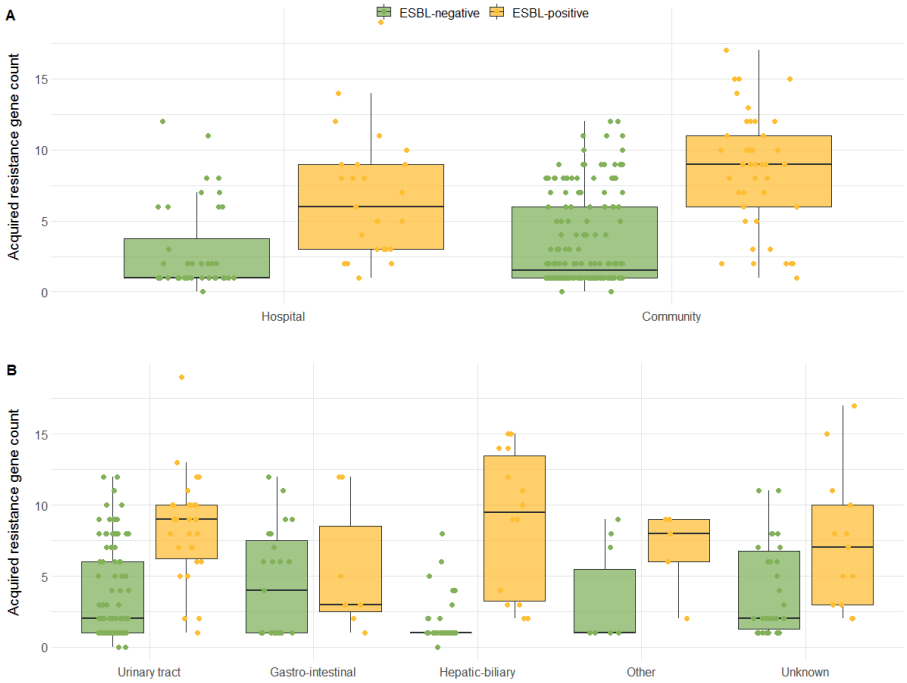


Figure S3. Acquired resistance gene count among epidemiological subgroups.
ESBL, extended-spectrum beta-lactamase. ESBL-positivity was based on phenotypic ESBL-production. Boxplots display median and inter quartile range and every dot represents a single isolate. The ResFinder 3.1.0 database was used to determine acquired resistance genes. **A.** Resistance gene count per onset of infection, stratified for non-ESBL-Ec and ESBL-Ec isolates. **B.** Resistance gene count per primary focus of ECB, stratified for non-ESBL-Ec and ESBL-Ec isolates.

Table S5. Pairwise comparisons acquired resistance gene count between epidemiological subgroups

	Median resistance gene count (IQR)				Pairwise comparisons between groups, within non-ESBL and ESBL[a]	
	Non-ESBL	**ESBL**			**Non-ESBL**	**ESBL**
Onset of infection			**Group 1**	**Group 2**		
Community (N = 216)	2 (1–6)	9 (6–11)	Community	Hospital	NS[b]	NS
Hospital (N = 65)	1 (1–4)	6 (3–9)				
Primary focus			**Group 1**	**Group 2**		
Urinary tract (N = 133)	2 (1–6)	9 (7–10)	Urinary	GI	NS	NS
Hepatic- biliary (N = 60)	1 (1–1)	10 (3–14)	Urinary	HB	2.8e-04***	NS
Gastro-intestinal (N = 30)	4 (1–8)	3 (3–9)	Urinary	Other	NS	NS
Unknown (N = 43)	2 (1–7)	7 (3–10)	Urinary	Unknown	NS	NS
Other (N = 15)	1 (1–6)	8 (6–9)	GI	HB	5.8e-03**	NS
			GI	Other	NS	NS
			GI	Unknown	NS	NS
			HB	Other	NS	NS
			HB	Unknown	3.1e-05****	NS
			Other	Unknown	NS	NS

ESBL, extended-spectrum beta-lactamase; HB, hepatic-biliary; GI, gastro-intestinal; IQR, interquartile range; NA, not applicable; NS, not significant

[a] Groups were compared with Wilcoxon rank sum Test and *P* values were adjusted with the Holm-Bonferroni correction to adjust for multiple testing. ESBL-positivity was based on phenotypic ESBL-production.

[b] *P* value represents the adjusted *P* value for the comparison of the resistance gene count of Group 1 versus Group 2, within the non-ESBLs or ESBLs (i.e. *P* value 2.8e-04 is the *P* value for the comparison in acquired resistance gene count in urinary versus hepatic-biliary primary focus among non-ESBL *E. coli*)

*, **, *** and **** indicate *P* values ≤.05, ≤.01, ≤.001 and ≤.0001.

The ResFinder 3.1.0 database was used to determine presence of acquired resistances genes. Gene counts were rounded to whole numbers if applicable.

Table S6. Pairwise comparisons acquired resistance gene count between dominant STs[a]

	Median resistance gene count (IQR)				Pairwise comparisons between groups, within non-ESBL and ESBL[b]	
	Non-ESBL	ESBL			Non-ESBL	ESBL
			Group 1	Group 2		
Other ST (N = 150)	1 (1–6)	8 (3–12)	ST12	ST131	NS	NS
ST131 (N = 52)	2 (1–5)	9 (6–10)	ST12	ST38	NS	NS
ST73 (N = 26)	1 (1–2)	NA	ST12	ST69	NS	NS
ST69 (N = 21)	6 (1–8)	9 (9–9)	ST12	ST73	NS	NS
ST12 (N = 13)	1 (1–3)	3 (3–3)	ST12	ST95	NS	NS
ST95 (N = 12)	1 (1–2)	NA	ST131	ST38	NS	NS
ST38 (N = 7)	8 (7–8)	5 (5–8)	ST131	ST69	NS	NS
			ST131	ST73	NS	NS
			ST131	ST95	NS	NS
			ST38	ST69	NS	NS
			ST38	ST73	NS	NS
			ST38	ST95	NS	NS
			ST69	ST73	NS	NS
			ST69	ST95	NS	NS
			ST73	ST95	NS	NS

ESBL, extended-spectrum beta-lactamase; NA, not applicable; NS, not significant; ST, sequence type. ESBL-positivity was based on phenotypic ESBL-production. The ResFinder 3.1.0 database was used for determination of acquired resistance genes. Gene counts were rounded to whole numbers if applicable. [a] Comparisons with category "Other" are not shown; because of heterogeneity in STs this comparison was not considered as informative. [b] Pairwise comparisons were made with Wilcoxon rank sum Test and *P* values were adjusted with the Holm-Bonferroni correction to adjust for multiple testing.

Table S7. Detected ExPEC-associated VG per VG category

Adhesins		**Siderophores**		**Protectins and invasins**		**Toxins**		**Other**	
Gene	*N (%)*[a]	*Gene*	*N (%)*[a]	*Gene*	*N (%)*[a]	*Gene*	*N (%)*[a]	*Gene*	*N (%)*[a]
yagZ/ecpA	271 (96.4)	*sitA*	233 (82.9)	*ompA*	235 (83.6)	*usp*	158 (56.2)	*traT*	181 (64.4)
fimH	266 (94.7)	*fyuA*	224 (79.7)	*ompT*	218 (77.6)	*vat*	101 (35.9)	*malX*	164 (58.4)
tia	124 (44.1)	*chuA*	158 (56.2)	*kpsMc*	78 (27.8)	*sat*	91 (32.4)	*iss*	124 (44.1)
iha	111 (39.5)	*iroN*	135 (48.0)	*tcpC*	53 (18.9)	*clbB*	80 (28.5)	*cvaC*	42 (14.9)
papC	103 (36.7)	*iutA*	32 (11.4)	*ibeA*	40 (14.2)	*clbN*	80 (28.5)	*fliC*	19 (6.8)
papH	100 (35.6)	*ireA*	39 (13.9)			*hlyD*	76 (27.0)	*rfc*	13 (4.6)
sfa/focb	87 (40.1)					*hlyA*	72 (25.6)		
agn43	81 (28.8)					*cnf1*	66 (23.5)		
papG	57 (20.3)					*pic*	45 (16.0)		
papF	55 (19.6)					*astA*	29 (10.3)		
afa/drab	43 (15.3)					*cdtB*	13 (4.6)		
nfaE	9 (3.2)								
gafD	8 (2.8)								
bmaE	7 (2.5)								
papE	7 (2.5)								
papA	5 (1.8)								

The following genes were not detected in any of the isolates: *focE*, *hra*, *yfcV* and *tsh* (adhesins) and *hlyF* (toxin).

[a] N indicates numbers of isolates with gene, % of all isolates. [b] The *kpsM*, *afa/dra* and *sfa/foc* operons were considered present if any of the corresponding genes our allelic variants were identified.

Figure S4. ExPEC-associated VG score in different subgroups, stratified for ESBL-positivity[a]
ESBL, extended spectrum beta-lactamase; ExPEC, extra-intestinal pathogenic *E. coli*; VG, virulence genes. Boxplots display median and inter quartile range (IQR) and every dot represents a single isolate. **A.** VG count per onset of infection, stratified for non-ESBL-Ec and ESBL-Ec isolates. **B.** VG count per primary focus of ECB, stratified for non-ESBL-Ec and ESBL-Ec isolates.
[a] ESBL-positivity was based on phenotypic ESBL-production.

Table S8. Pairwise comparisons VG score between epidemiological subgroups

	Median VG score (IQR)				Pairwise comparisons between groups, within non-ESBL and ESBL[a]	
Onset of infection	**Non-ESBL**	**ESBL**	**Group 1**	**Group 2**	**Non-ESBL**	**ESBL**
Community (N = 216)	13 (9–20)	11 (7–13)	Community	Hospital	NS	NS
Hospital (N = 65)	14 (11–20)	12 (8–15)				
Primary focus			Group 1	Group 2		
Urinary tract (N = 133)	15 (11–21)	12 (11–15)	Urinary	GI	.0072**	NS
Hepatic- biliary (N = 60)	11 (5–18)	7 (6–13)	Urinary	HB	.036*	NS
Gastro-intestinal (N = 30)	10 (5–13)	12 (8–13)	Urinary	Other	NS	NS
Unknown (N = 43)	16 (11–20)	12 (8–13)	Urinary	Unknown	NS	NS
Other (N = 15)	13 (8–20)	7 (7-14)	GI	HB	NS	NS
			GI	Other	NS	NS
			GI	Unknown	NS	NS
			HB	Other	NS	NS
			HB	Unknown	NS	NS
			Other	Unknown	NS	NS
Urinary catheter			**Group 1**	**Group 2**		
No (N = 184)	13 (9-20)	10 (7-13)	No catheter	Catheter	NS	NS
Yes (N = 97)	13 (7-18)	13 (11-15)				
30-day mortality			**Group 1**	**Group 2**		
Alive (N = 238)	13 (9-20)	12 (8-15)	Alive	Deceased	NS	NS
Deceased (N = 43)	12 (6-18)	11 (7-14)				
Admission ward			**Group 1**	**Group 2**		
Non-ICU (N = 240)	13 (9-20)	12 (7-15)	Non-ICU	ICU	NS	NS
ICU (N =41)	13 (6-18)	12 (10-14)				

ESBL, expended-spectrum beta-lactamase; HB, hepatic-biliary; GI, gastro-intestinal; IQR, interquartile range; NA, not applicable; NS, not significant; VG, virulence gene.

a Pairwise comparisons were made with Wilcoxon rank sum Test and *P* values were adjusted with the Holm-Bonferroni correction to adjust for multiple testing.

* and ** indicate *P* values ≤.05 and ≤.01. Gene counts were rounded to whole numbers if applicable. ESBL-positivity was based on phenotypic ESBL-production.

Table S9. Pairwise comparisons VG score between dominant STs[a]

	Median VG score (IQR)				Pairwise comparisons between groups within non-ESBL and ESBL[b]	
	Non-ESBL	**ESBL**	**Group 1**	**Group 2**	**Non-ESBL**	**ESBL**
Other ST (N = 150)	11 (7–17)	8 (6 –11)	ST12	ST131	3.2e-05****	NS
ST131 (N = 52)	13 (12–15)	13 (12–15)	ST12	ST38	NS	NS
ST73 (N = 26)	22 (20–23)	-	ST12	ST69	5.5e-05****	NS
ST69 (N = 21)	11 (9–12)	8 (7–8)	ST12	ST73	NS	-
ST12 (N = 13)	22 (21–23)	23 (23–23)	ST12	ST95	.032*	-
ST95 (N = 12)	18 (17–19)	-	ST131	ST38	NS	NS
ST38 (N = 7)	7 (6–7)	8 (7–8)	ST131	ST69	4.4e-03**	NS
			ST131	ST73	1.9e-07****	-
			ST131	ST95	4.9e-03**	-
			ST38	ST69	NS	NS
			ST38	ST73	NS	-
			ST38	ST95	NS	-
			ST69	ST73	2.0e-07****	-
			ST69	ST95	5.8e-05****	-
			ST73	ST95	.032*	-

ESBL, extended-spectrum beta-lactamase; NS, not significant; ST, sequence type; VG, virulence gene.

[a] Comparisons with category "Other" are not shown; because of heterogeneity in STs this comparison is not considered as informative.

[b] Groups were compared with Wilcoxon rank sum Test and *P* values were adjusted with the Holm-Bonferroni correction to adjust for multiple testing. ESBL-positivity was based on phenotypic ESBL-production.

*, **, *** and **** indicate *P* values ≤.05, ≤.01, ≤.001 and ≤.0001.

Gene counts were rounded to whole numbers if applicable.

7

Comparative genomics of ESBL-producing *Escherichia coli* isolates from intestinal colonization and extra-intestinal infection in the Netherlands (2014 - 2017)

Tess Verschuuren, Denise van Hout, Ad Fluit, Ascelijn Reuland, Janetta Top, Anita Schürch, Thijs Bosch, Marc Bonten, Jan Kluytmans and Rob Willems

In preparation

ABSTRACT

Background: The gut microbiota of humans is an important reservoir of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (ESBL-Ec) causing infections. Large community surveillance studies of ESBL-Ec carriage in healthy subjects are used to identify circulating clones and ESBL genes, but such studies are logistically challenging and costly. It is currently unknown if clinical isolates that are more readily available could potentially serve as an alternative to monitor the human community ESBL-Ec reservoir.

Objective: To determine whether ESBL-Ec isolates obtained in routine clinical practice could be used for surveillance of the human gut ESBL-Ec reservoir, by comparing strain, ESBL gene and plasmid replicon relatedness of ESBL-Ec from human faecal carriage with ESBL-Ec strains causing extra-intestinal infection in the Netherlands.

Methods: Whole genome sequencing was performed on 452 ESBL-Ec isolates collected between 2014 and 2017 in the Netherlands consisting of two sample groups: (1) community faeces (n=162), and (2) urine and blood (n=290). Sequence types (ST) were determined using mlst2.0. Acquired ESBL genes were determined with ResFinder v3.1.0 and plasmid replicon types with PlasmidFinder v2.1. Neighbour-joining (NJ) phylogenetic trees were constructed for the core and accessory genome to infer clustering of strains by sample group.

Results: There was only little variation in the ten most prevalent STs, ESBL genes and plasmid replicons between groups. In both groups ST131 was the most prevalent ST, although its proportion was lower in community faeces (23%) compared to clinical samples (39%) (*P* value < .001). The remaining nine most prevalent STs had comparable prevalence among faecal and clinical isolates. The only significant difference in the distribution of ESBL genes was that $bla_{\text{CTX-M-1}}$ was more prevalent in community faeces (18%) than in clinical samples (5%) (*P* value < .01). For plasmid replicons, IncFIA was less frequently found in faeces than in clinical isolates (38% versus 54%, *P* value <.01). NJ trees did not reveal distinct clustering of isolated belonging to the two sample groups.

Conclusions: In the Netherlands, the distribution of the ten most prevalent ESBL-Ec strains, ESBL genes and plasmid replicons colonizing the gut of community-dwelling subjects and recovered from extra-intestinal infection was highly comparable. This suggests that clinical samples can potentially be used for future molecular public health surveillance of ESBL-Ec.

BACKGROUND

In 2018, *Escherichia coli* was the species most frequently identified as a cause of bloodstream infections and urinary tract infections in the Netherlands. [1] A worrisome trend is that the number of bloodstream infections with *E. coli* in Europe is increasing, mainly driven by an increase in community-onset infections. [2–4] In the Netherlands, an increasing part of extra-intestinal *E. coli* infections is caused by extended-spectrum beta-lactamase-producing *E. coli* (ESBL-Ec). [1]

The main human reservoir of ESBL-Ec is the gut of community-dwelling individuals. [5] In the Netherlands, the prevalence of faecal ESBL-Ec carriage in the open population is approximately 5%. [6–8] Sequence type (ST) 131 and ESBL genes of the CTX-M type currently dominate the human ESBL-Ec reservoir, taking over TEM and SHV gene variants that dominated during the 1990s. [5,6,8–11] With possible new variants likely to arise in time, molecular surveillance of the human ESBL-Ec reservoir is fundamental to track temporal changes and to allow early detection of important antibiotic-resistant strains.

Previous large-scale surveillance studies in the Netherlands that assessed ESBL-Ec carriage in the community provided unique and valuable insight into the prevalence and population structure of the human ESBL-Ec reservoir. [6–8,12] Unfortunately, such studies are logistically challenging and costly and, therefore, not performed on a regular basis. Clinical isolates that are routinely obtained in healthcare settings (i.e. in primary care or in hospitals) could potentially serve as a proxy to monitor the population structure of the human ESBL-Ec gut reservoir. However, it is currently unknown whether gut colonization in community-dwelling subjects and extra-intestinal infections are caused by the same ESBL-Ec population. We, therefore, compared molecular characteristics of ESBL-Ec isolates from community faeces to ESBL-Ec isolates from urine obtained in primary care, urine obtained in secondary care, and blood.

METHODS

Study design

Sample collection was fully described previously and included: 1) faecal ESBL-Ec isolates that originated from a Dutch cross-sectional open-population study performed between 2014 and 2016 (n=162), and 2) clinical ESBL-Ec isolates (n=290). [6,13] Clinical isolates were obtained from: (a) patients with community-acquired (CA) urinary tract infection, prospectively collected in primary care in 2017 (n=175); (b) hospitalized patients with nosocomial urinary tract infection between 2014 and 2016 (further referred to as: hospital-acquired (HA) urine isolates), retrospectively collected (n=49); and (c) hospitalized patients with a positive blood culture between 2014 and

2016, also retrospectively collected (n=66). Participating hospitals were the University Medical Center Utrecht and the Amphia Hospital in Breda. Only the first available ESBL-Ec isolate per patient was included in the current study, without a restriction on age (see supplementary material for study flowchart).

Ethics

Individual informed consent was given by subjects participating in the surveillance study providing community faeces isolates (IRB number 14/219-C). [6] For the use of clinical isolates the ethical review board judged this study to be out of the scope of the Medical Research Involving Human Subjects Act and waived the need for official approval (IRB correspondence number 18/056). Based on the 'Code of conduct for health research' individual informed consent was not obtained. [14]

Genotyping

Whole genome sequencing (WGS) was performed on all isolates using Illumina HiSeq 2500, MiSeq, or NextSeq platforms. De novo assembly was performed using SPAdes (v3.6.2). [15] The quality of assemblies was assessed using Quality Assessment Tool for Genome Assemblies (QUAST). [16] STs were inferred with MLST (v2.0) using the Achtman scheme with tseemann/mlst (v2.15.1) (https://github.com/tseemann/mlst). Presence of acquired ESBL genes was determined with ResFinder 3.1.0 and plasmid replicon types with PlasmidFinder 2.1 using abricate (version 0.8.7), both using a minimal coverage length of 80% and minimal sequence identity of 95%. [17,18]

Phylogeny and partitioning in whole genome-based strains

Phylogeny and partitioning of the ESBL-Ec population were estimated using PopPUNK (v1.1.3), where PopPUNK calculated a relative core and accessory distance for each pair in the dataset based on k-mer comparisons. [19] Subsequently, PopPUNK used the produced distance matrix to infer phylogeny and assign whole genome-based strains (wg-b-strains), by defining within- and between- cluster genetic distance thresholds. The adjusted Rand index was used to calculate the congruence between STs and wg-b-strains, where identical population partitioning was one and completely different population partitioning was zero. [19,20] A core genome (cg) neighbour-joining (NJ) tree was constructed with PopPUNK and an accessory genome NJ tree was constructed using fastcluster (v1.1.25) in R, by using the distance matrix produced by PopPUNK. [21] The NJ trees were visually inspected for clustering by sample group.

Statistical analyses

Proportions with 95% confidence intervals (CI) of the ten most occurring ST, ESBL genes and

plasmid replicon types were compared between community faecal and clinical isolates. Only proportion CIs without overlap were tested for statistical significance to obtain *P* values, using a two-proportion z-test. A *P* value < .05 was considered statistically significant. Subgroup analyses were performed for the different types of clinical samples to explore whether certain sample groups would be most useful for potential future molecular surveillance. All calculations were performed in RStudio Version 1.1.456.

RESULTS

Distribution of sequence types (STs)

In total, 108 different STs were identified among the 452 ESBL-Ec isolates. The three most common STs were ST131 (34%), ST38 (10%) and ST1193 (4%). The top ten most frequent STs accounted for 65% of all isolates (Suppl. Table S1). 26 STs were found in both community faecal and clinical isolates, the remaining STs consisted mostly of singletons. The most prevalent ST in both sample groups was ST131, which was significantly more often identified in clinical isolates (39%, n=114) than in community faeces (23%, n=38) (*P* value < .001) (Fig 1). The remaining nine most prevalent STs had comparable prevalence among faecal and clinical isolates. The prevalence of ST131 in the different clinical sample subgroups was 38% (n=66) for CA-urine, 39% (n=19) for HA-urine and 44% (n=29) for blood (Suppl. Table S1 and Figure S2). ST1193 was identified in 15 (9%) CA-urine isolates, 4 (3%) times in faeces but was absent in HA-urine and blood.

Distribution of ESBL genes

In total, 454 ESBL genes were identified, representing 18 different ESBL genes. In two urine isolates two ESBL genes were found ($bla_{CTX\text{-}M\text{-}27}$ and $bla_{CTX\text{-}M\text{-}55}$). Of the 18 different ESBL genes, 13 (72%) belonged to the CTX-M family. The three most frequently identified ESBL genes were $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}27}$, which accounted for 47%, 16% and 16% of all ESBL genes, respectively. Ten ESBL genes were found in both community faecal and clinical isolates, accounting for 98% of all ESBL genes (Suppl. Table S2). The prevalence of $bla_{CTX\text{-}M\text{-}1}$ was lower in clinical (5%) than in community faecal (18%) isolates (*P* value < .0001) (Fig 1). For the occurrence of $bla_{CTX\text{-}M\text{-}1}$, subgroup analyses showed a statistical significant difference for community faeces versus CA-urine (18% versus 4%, *P* value < .0001), but not for the other clinical sample subgroups (Suppl. Figure S3). For the other ESBL genes prevalence did not differ between community faecal isolates and clinical isolates.

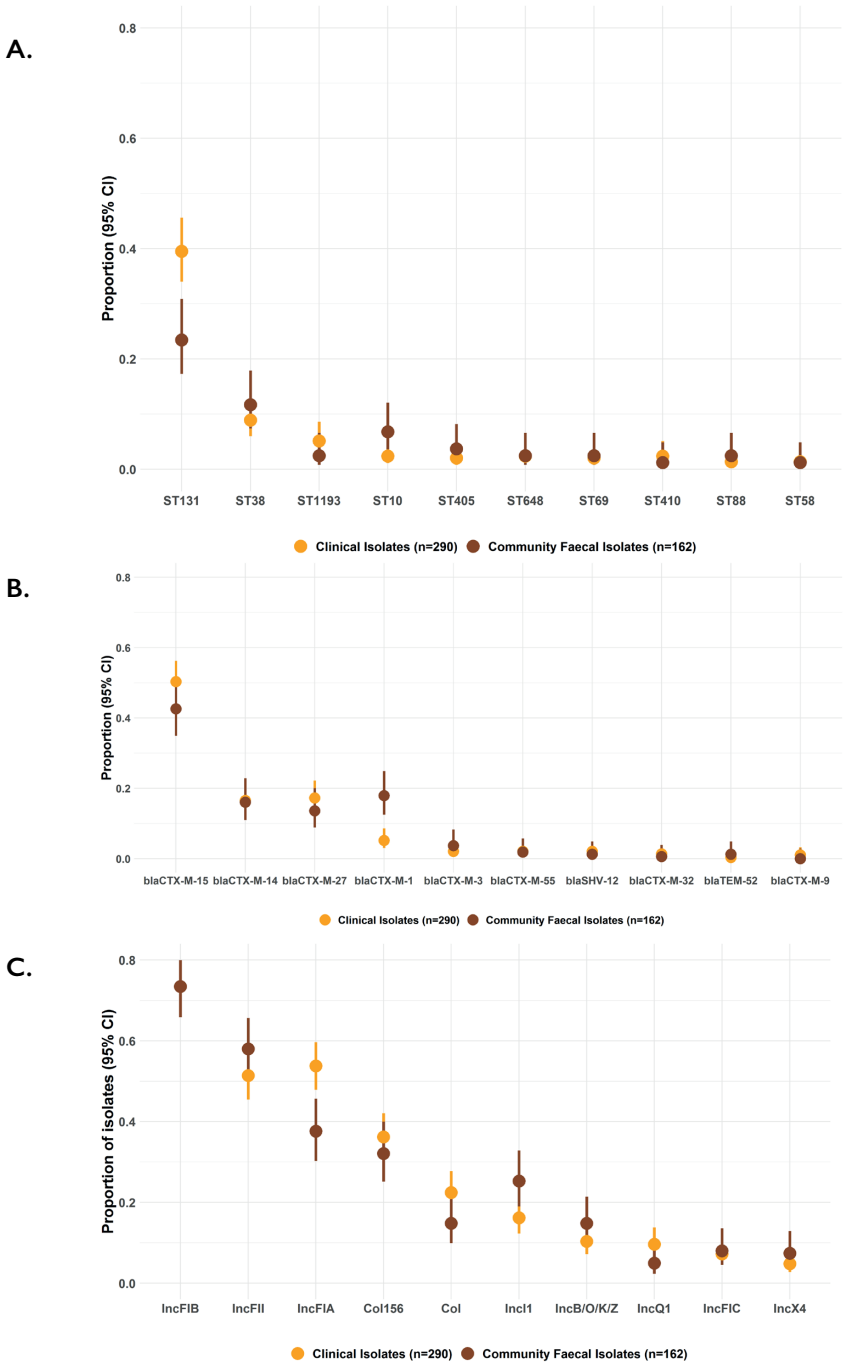


Figure 1. Proportions of the ten most frequently occurring genetic subtypes in community faecal and clinical isolates for: (a) STs, (b) ESBL genes, (c) plasmid replicon types. ESBL, extended-spectrum beta-lactamase; ST, sequence type. a. Exact proportions per sample group are provided in the supplementary material. b. NB. Prevalence and 95% CI of IncFIB in clinical and community faecal isolates overlap.

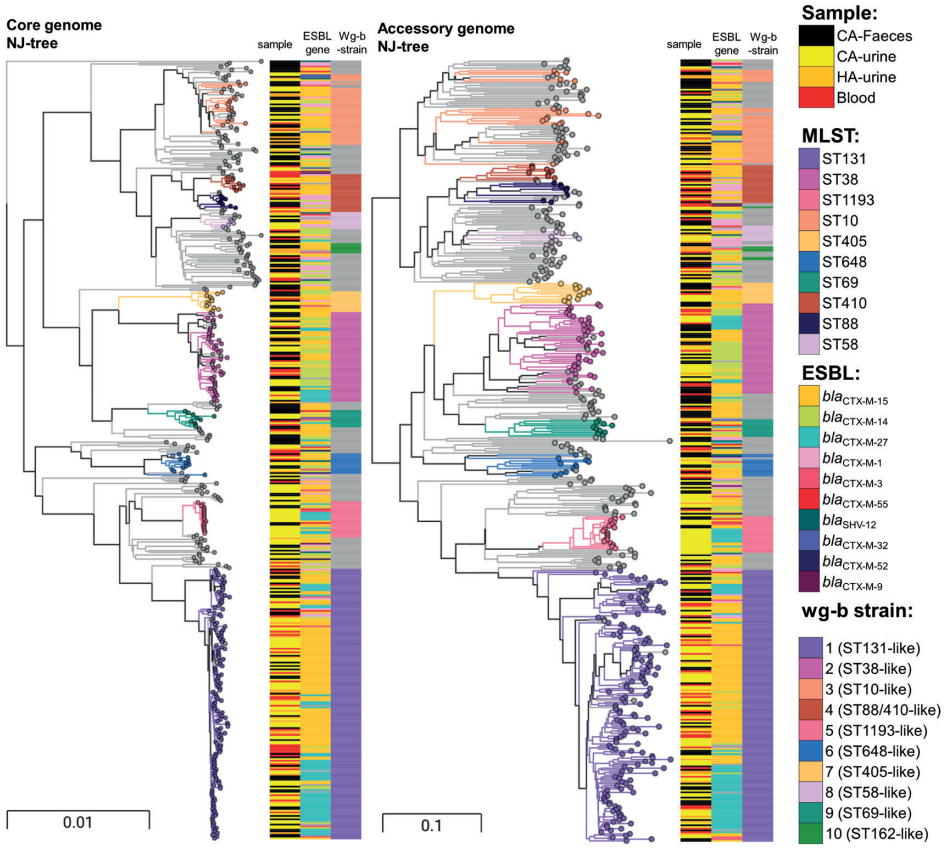


Figure 2. Neighbour-joining (NJ) trees. Core genome and accessory genome phylogeny constructed with PopPUNK

Distribution of plasmid replicons

In 427 (94%) isolates one or multiple plasmid replicons were detected, resulting in 29 different replicon types (Suppl. Table S3). Eight (32%) of the 25 isolates without a predicted plasmid replicon were ST38; the other isolates belonged to less frequent STs (Suppl. Table S4). The median number of plasmid replicon types per isolate was 3 (Q1-Q3 2-5), which was the same for community faecal isolates as for clinical isolates (Suppl. Figure S4). IncF was the most common replicon family, with the three most frequently occurring plasmid replicon types being IncFIB (78% of isolates with plasmid replicon detected), IncFII (57%) and IncFIA (51%). Of the ten most occurring plasmid replicon types, only the prevalence of IncFIA was statistically different between the group of clinical (54%) and community faecal isolates (38%, *P* value < .01) (Fig 1). In subgroup analyses of different clinical sample types, this statistically significant difference was only found for CA-urine (55%) versus faeces (38%) isolates (*P* value < .01) (Suppl. Table S3, Figure S5). IncQ1 was more common in HA-urine (20%) than in faeces (5%) and CA-urine (5%), respectively (both *P* values < .01).

ESBL-Ec phylogenies based on core and accessory genome

In total, 75 wg-b-strains were predicted by PopPUNK (Suppl. Table S5). This strain assignment was congruent with MLST-based ST assignment (adjusted Rand index 0.93 (n=443). The core genome and accessory genome tree demonstrated no distinct clustering of community faecal and clinical isolates, indicating that these two sample groups are not part of distinct *E. coli* sub-populations (Fig. 2).

DISCUSSION

This comparative genetic analysis of 452 ESBL-Ec isolates demonstrated that in the Netherlands, the distribution of STs, ESBL genes and plasmid replicon types was comparable at large between ESBL-Ec from faeces of the open population and from routine clinical samples of extra-intestinal ESBL-Ec infection.

Molecular characteristics of the two sample groups strongly overlapped, as the ten most dominant STs, ESBL genes and plasmid replicons among community faecal ESBL-Ec isolates were also the dominant genetic subtypes among clinical ESBL-Ec isolates. Also, phylogenetic trees of the core and accessory genome did not reveal distinct clustering based on sample source. Still, there was variation in the absolute and relative prevalence of certain subtypes, that could be explained by multiple factors. ST131 (39% versus 23%) and IncFIA (54% versus 38%) were more common in clinical samples compared to community faeces, while $bla_{\text{CTX-M-1}}$ was less common (5% versus 18%) in clinical samples. This is in line with findings of other studies. [10,22–24] A recent epidemiological surveillance study by Day *et al.* in the United Kingdom also found a higher prevalence of ST131 in invasive ESBL-Ec blood isolates (64%) compared to ESBL-Ec from faeces (36%). [10] Notably, the absolute prevalence of ST131 among faecal and blood isolates was considerably higher than in our study, something that is possibly related to differences in local epidemiology and sample collection. For example in the study by Day *et al.*, faeces was included that was submitted for routine diagnostics, that could be for occult blood screening but also for the detection of intestinal pathogens, while in our study a random sample of the Dutch open population was invited to provide a faecal sample.

It has been suggested for ST131 that due to multiple evolutionary events, such as acquisition of certain genes and evolution towards a separate ecological niche, ST131 has a larger pathogenic potential than other STs. [11,23,25–29] Our study shows that ST131 was indeed more prevalent among extra-intestinal infection, but that it was also the dominant ST in community faecal

carriage of ESBL-Ec. ST131 has been associated with the plasmid replicon type IncFIA, possibly causing the higher prevalence of IncFIA among our clinical samples. [6] In the Netherlands $bla_{CTX\text{-}M\text{-}1}$ has previously been described as an important ESBL gene in human intestinal ESBL-Ec carriage (i.e. in strains of lower virulence) but that it is particularly common in non-human reservoirs. [9,10,30–35] All in all, for our study this implies that the differences we identified were mainly the reflection of a relatively higher prevalence of certain, possibly more virulent, strains in our clinical sample collection. Subgroup analyses did not reveal distinct comparability of ESBL-Ec isolates from faecal carriage with a particular clinical sample subgroup.

A limitation of the current study is that due to the limited number of isolates per sample year, our sample collection did not allow an analysis of temporal changes in the molecular epidemiology of community faecal and clinical ESBL-Ec. Also, small subgroup sizes limited the subgroup analyses of the different clinical sample types; in particular the number of ESBL-Ec isolates from HA-urine was small, leading to low precision. Furthermore, due to likely variation in indications to obtain clinical cultures and local epidemiology of different human ESBL-Ec reservoirs, results are not merely generalizable to other countries.

It is important to note that the aim of the current study was to assess the comparability of ESBL-Ec strains from different human reservoirs from the perspective of public health surveillance. In that setting, the key element is to be able to detect and track current (and possible future emerging) important antibiotic-resistant strains. Therefore, we focused on the ten most frequent occurring STs, ESBL genes and plasmid replicon types. Upcoming in-depth genomic analyses will further investigate the genomic similarity of the entire ESBL-Ec population within the different sample groups.

To conclude, our findings indicate that in the Netherlands ESBL-Ec isolates from community faeces and extra-intestinal infection are highly comparable, with the exception of a higher prevalence of ST131 and plasmid replicon subtype IncFIA among clinical isolates and a higher prevalence of $bla_{CTX\text{-}M\text{-}1}$ among community faeces isolates. This indicates that molecular surveillance of clinical ESBL-Ec isolates can reliably estimate the clonal composition of ESBL-Ec clones circulating in the community, with the exceptions mentioned above, thus can potentially be used for future molecular public health surveillance of ESBL-Ec.

List of abbreviations
CA, community-acquired; CI, confidence interval; ESBL, extended-spectrum beta-lactamase; ESBL-Ec, ESBL-producing *E. coli*; HA, hospital-acquired; ICU, intensive care unit; IRB, institutional review board; NJ, neighbour joining; ST, sequence type; WGS, whole genome sequencing

Acknowledgements: We would sincerely like to thank Kim van der Zwaluw, Carlo Verhulst and Judith Vlooswijk for their contributions in the laboratory execution of the study. Preliminary results of this paper were presented at the 29th European Congress of Clinical Microbiology & Infectious Diseases, Amsterdam, the Netherlands (2019).

Funding: This study was internally funded by the Netherlands National Institute for Public Health and the Environment (RIVM) and the University Medical Center Utrecht (UMCU) and the authors received no specific funding for this work.

Competing interests: The authors declare that they have no competing interests.

REFERENCES

1. de Greeff SC, Mouton JW. Nethmap 2019. Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. 2019. Available from: https://rivm.openrepository.com/handle/10029/623134.
2. Public Health England. Annual epidemiological commentary: bacteraemia, MSSA bacteraemia and *C . difficile* infections, up to and including financial year April 2018 to March 2019. 2019:1–88. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/843870/Annual_epidemiological_commentary_April_2018-March_2019.pdf.
3. Vihta K-D, Stoesser N, Llewelyn MJ, Quan TP, Davies T, Fawcett NJ, ***et al.*** Trends over time in *Escherichia coli* bloodstream infections, urinary tract infections, and antibiotic susceptibilities in Oxfordshire, UK, 1998-2016: a study of electronic health records. ***Lancet Infect Dis.*** 2018;18(10):1138–49.
4. de Kraker MEA, Jarlier V, Monen JCM, Heuer OE, van de Sande N, Grundmann H. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. ***Clin Microbiol Infect.*** 2013;19(9):860–8.
5. Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing *Enterobacteriaceae* in Europe. ***Eurosurveillance.*** 2008;13(47).
6. Bunt van den G, Pelt van W, Hidalgo L, Scharringa J, Greeff SC de, Schürch AC, ***et al.*** Prevalence, risk factors and genetic characterization of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* (ESBL-E and CPE): a community-based repeated cross-sectional study in the Netherlands from 2014 to 2016. ***Eurosurveillance***. 2019;24(41): 1800594.
7. Wielders CCH, van Hoek AHAM, Hengeveld PD, Veenman C, Dierikx CM, Zomer TP, ***et al.*** Extended-spectrum beta-lactamase- and pAmpC-producing *Enterobacteriaceae* among the general population in a livestock-dense area. ***Clin Microbiol Infect.*** 2017;23(2):120.e1-120.e8.
8. van Hoek AHAM, Schouls L, van Santen MG, Florijn A, de Greeff SC, van Duijkeren E. Molecular characteristics of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* from humans in the community. ***PLoS One.*** 2015;10(6):e0129085.
9. Mughini-Gras L, Dorado-García A, van Duijkeren E, van den Bunt G, Dierikx CM, Bonten MJM, ***et al.*** Attributable sources of community-acquired carriage of *Escherichia coli* containing ß-lactam antibiotic resistance genes: a population-based modelling study. ***Lancet Planet Heal.*** 2019;3(8):e357–69.
10. Day MJ, Hopkins KL, Wareham DW, Toleman MA, Elviss N, Randall L, ***et al.*** Extended-spectrum ß-lactamase-producing *Escherichia coli* in human-derived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. ***Lancet Infect Dis.*** 2019;3099(19):1–11.
11. Kallonen T, Brodrick HJ, Harris SR, Corander J, Brown NM, Martin V, ***et al.*** Systematic longitudinal survey of invasive *Escherichia coli* in England demonstrates a stable population structure only transiently disturbed by the emergence of ST131. ***Genome Res.*** 2017;27:1437–49.
12. Reuland EA, Overdevest ITMA, Al Naiemi N, Kalpoe JS, Rijnsburger MC, Raadsen SA, ***et al.*** High prevalence of ESBL-producing *Enterobacteriaceae* carriage in Dutch community patients with gastrointestinal complaints. ***Clin Microbiol Infect.*** 2013;19(6):542–9.
13. van Hout D, Verschuuren TD., Bruijning-Verhagen PCJ., Bosch T., Reuland EA., Fluit AC., ***et al.*** Design of the EPIGENEC Study: Assessing the EPIdemiology and GENetics of *Escherichia coli* in the Netherlands. ***Preprints.*** 2019;2019020066.
14. Federation of Medical Scientific Societies. FMWV Code of Conduct for Health Research. 2017.
15. Deng M, Jiang R, Sun F, Zhang X. Research in Computational Molecular Biology. Springer Berlin Heidelberg; 2013. pp. 158-170.
16. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. ***Bioinformatics.*** 2013;29(8):1072–5.
17. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, ***et al.*** Identification of acquired antimicrobial resistance genes. ***J Antimicrob Chemother.*** 2012;67(11):2640–4.

18. Carattoli A, Zankari E, Garciá-Fernández A, Larsen MV, Lund O, Villa L, ***et al.*** In Silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. ***Antimicrob Agents Chemother.*** 2014;58(7):3895–903.
19. Lees JA, Harris SR, Tonkin-Hill G, Gladstone RA, Lo SW, Weiser JN, ***et al.*** Fast and flexible bacterial genomic epidemiology with PopPUNK. ***Genome Res.*** 2019;360917.
20. Hubert L. Comparing Partitions. ***Journal of Classification 2.*** 1985;218:193–218.
21. Argimon S, Abudahab K, Goater RJE, Fedosejev A, Bhai J, Glasner C, ***et al.*** Microreact: visualizing and sharing data for genomic epidemiology and phylogeography. Microb genomics. 2016;2(11):e000093.
22. Ny S, Löfmark S, Börjesson S, Englund S, Ringman M, Bergström J, ***et al.*** Community carriage of ESBL-producing *Escherichia coli* is associated with strains of low pathogenicity: a Swedish nationwide study. ***J Antimicrob Chemother.*** 2017;72(2):582–8.
23. Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchesnokova V, ***et al.*** The epidemic of extended-spectrum-ß-lactamase-producing *Escherichia coli* ST131 is driven by a single highly pathogenic subclone, H30-Rx. ***MBio.*** 2013;4(6):e00377-13.
24. Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. *Escherichia coli* ST131, an intriguing clonal group. ***Clin Microbiol Rev.*** 2014;27(3):543–74.
25. McNally A, Kallonen T, Connor C, Abudahab K, Aanensen DM, Horner C, ***et al.*** Diversification of colonization factors in a multidrug-resistant *Escherichia coli* lineage evolving under negative frequency-dependent selection. ***MBio.*** 2019;10(2):e00644-19.
26. Ben Zakour NL, Alsheikh-Hussain AS, Ashcroft MM, Khanh Nhu NT, Roberts LW, Stanton-Cook M, ***et al.*** Sequential acquisition of virulence and fluoroquinolone resistance has shaped the evolution of *Escherichia coli* ST131. ***MBio.*** 2016;7(2):e00347.
27. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. ***Clin Microbiol Rev.*** 2019;32(3).
28. Stoesser N, Sheppard AE, Pankhurst L, De Maio N, Moore CE, Sebra R, ***et al.*** Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. ***MBio.*** 2016;7(2):e02162.
29. Shaik S, Ranjan A, Tiwari SK, Hussain A, Nandanwar N, Kumar N, ***et al.*** Comparative genomic analysis of globally dominant ST131 clone with other epidemiologically successful extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. ***MBio.*** 2017;8(5).
30. Willemsen I, Oome S, Verhulst C, Pettersson A, Verduin K, Kluytmans J. Trends in extended-spectrum Beta-Lactamase (ESBL) producing *Enterobacteriaceae* and ESBL-genes in a Dutch teaching hospital, measured in 5 yearly point prevalence surveys (2010-2014). ***PLoS One.*** 2015;10(11):e0141765.
31. Kluytmans JAJW, Overdevest ITMA, Willemsen I, Kluytmans-van den Bergh MFQ, van der Zwaluw K, Heck M, ***et al.*** Extended-spectrum ß-lactamase-producing *Escherichia coli* from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. ***Clin Infect Dis.*** 2013;56(4):478–87.
32. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, ***et al.*** Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. ***Clin Microbiol Infect.*** 2011;17(6):873–80.
33. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, ***et al.*** Extended-spectrum ß-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. ***Emerg Infect Dis.*** 2011;17(7):1216–22.
34. Apostolakos I, Franz E, van Hoek AHAM, Florijn A, Veenman C, Sloet-van Oldruitenborgh-Oosterbaan MM, ***et al.*** Occurrence and molecular characteristics of ESBL/AmpC-producing *Escherichia coli* in faecal samples from horses in an equine clinic. ***J Antimicrob Chemother.*** 2017;72(7):1915–21.
35. Reuland EA, Al Naiemi N, Kaiser AM, Heck M, Kluytmans JA, Savelkoul PH, ***et al.*** Prevalence and risk factors for carriage of ESBL-producing *Enterobacteriaceae* in Amsterdam. ***J Antimicrob Chemother.*** 2016;71(4):1076–82.

SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Study flowchart.

Supplementary Table S1. ST proportion per sample group of the 35 most identified STs.

Supplementary Figure S2. Prevalence ten most identified sequence types per clinical sample subgroup of ESBL-positive *E. coli* isolates.

Supplementary Table S2. Prevalence of ESBL genes per sample group of *E. coli* isolates.

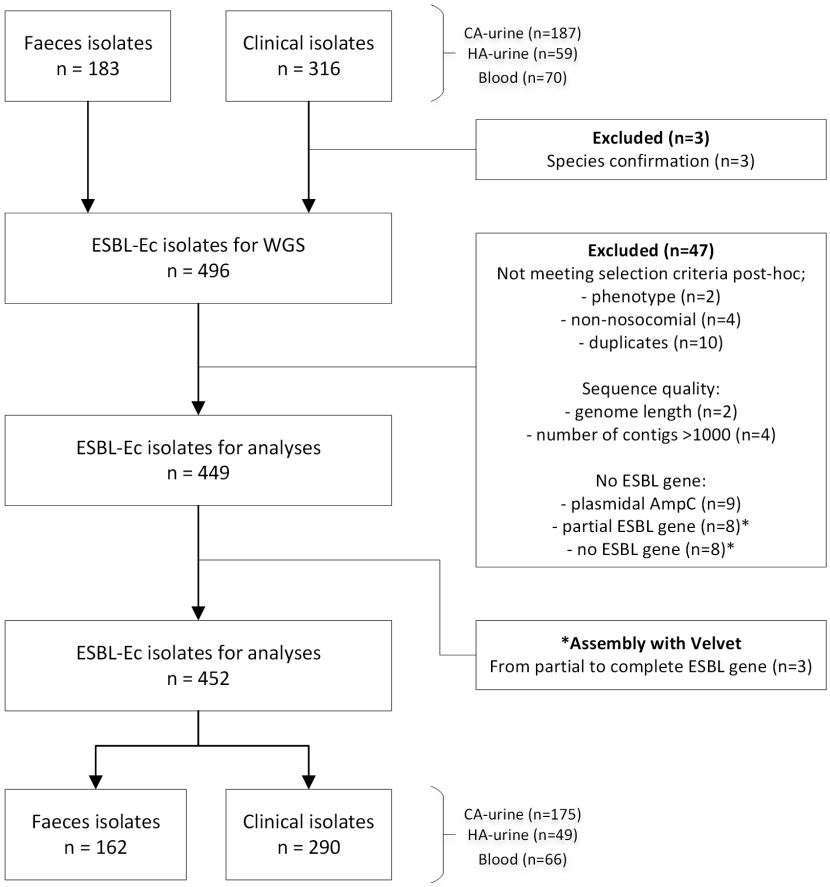
Supplementary Figure S3. Prevalence ten most identified ESBL genes per clinical sample subgroup of *E. coli* isolates.

Supplementary Table S3. Proportions of ten most common plasmid replicon types.

Supplementary Figure S4. Number of plasmid replicons per isolate for community faecal versus clinical ESBL *E. coli*.

Supplementary Figure S5. Prevalence ten most identified plasmid replicons per clinical sample subgroup of ESBL-positive *E. coli* isolates.

Supplementary Table S5. Proportions of the ten most prevalent whole genome based ESBL-Ec strains as predicted by PopPUNK.

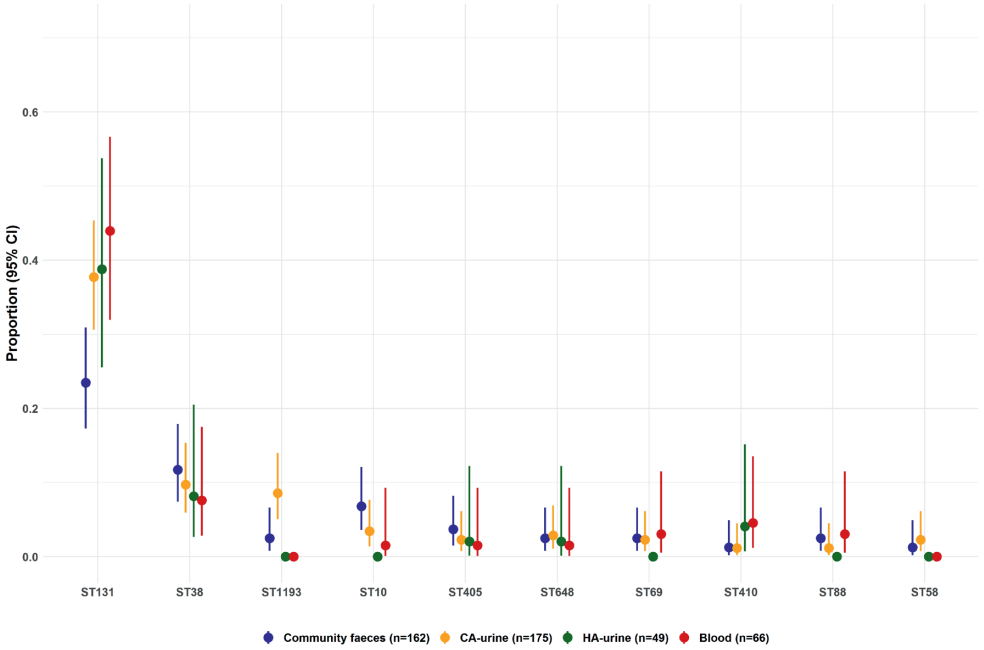


Supplementary Figure S1. Study flowchart

Supplementary Table S1. ST proportion per sample group of the 35 most identified STs

					Clinical							
	All		**Clinical**		*Blood*		*HA-urine*		*CA-urine*		**Faeces**	
ST	n	prop	n	prop	n	prop	n	prop	n	prop	n	prop
Isolates	452		290		66		49		175		162	
Unique ST	108	0.24	72	0.25	27	0.41	23	0.47	49	0.28	62	0.38
Unknown ST	9	0.02	1	0.003	0	0.00	1	0.02	0	0.00	8	0.05
131	152	0.34	114	0.39	29	0.44	19	0.39	66	0.38	38	0.23
38	45	0.10	26	0.09	5	0.08	4	0.08	17	0.10	19	0.12
1193	19	0.04	15	0.05	0	0.00	0	0.00	15	0.09	4	0.03
10	18	0.04	7	0.02	1	0.02	0	0.00	6	0.03	11	0.07
405	12	0.03	6	0.02	1	0.02	1	0.02	4	0.02	6	0.04
648	11	0.02	7	0.02	1	0.02	1	0.02	5	0.03	4	0.03
69	10	0.02	6	0.02	2	0.03	0	0.00	4	0.02	4	0.03
410	9	0.02	7	0.02	3	0.05	2	0.04	2	0.01	2	0.01
88	8	0.02	4	0.01	2	0.03	0	0.00	2	0.01	4	0.03
58	6	0.01	4	0.01	0	0.00	0	0.00	4	0.02	2	0.01
95	5	0.01	5	0.02	0	0.00	0	0.00	5	0.03	0	0.00
636	4	0.01	2	0.01	0	0.00	0	0.00	2	0.01	2	0.01
117	4	0.01	2	0.01	1	0.02	0	0.00	1	0.01	2	0.01
501	4	0.01	3	0.01	1	0.02	1	0.02	1	0.01	1	0.0
744	4	0.01	3	0.01	0	0.00	1	0.02	2	0.01	1	0.01
34	4	0.01	3	0.01	0	0.00	0	0.00	3	0.02	1	0.01
394	4	0.01	1	0.003	0	0.00	1	0.02	0	0.00	3	0.02
224	4	0.01	4	0.01	2	0.03	0	0.00	2	0.01	0	0.00
617	3	0.01	1	0.003	0	0.00	1	0.02	0	0.00	2	0.01
155	3	0.01	2	0.01	0	0.00	1	0.02	1	0.01	1	0.01
141	3	0.01	2	0.01	0	0.00	0	0.00	2	0.01	1	0.01
354	3	0.01	3	0.01	2	0.03	1	0.02	0	0.00	0	0.00
156	3	0.01	1	0.003	1	0.02	0	0.00	0	0.00	2	0.01
12	3	0.01	2	0.01	1	0.02	0	0.00	1	0.01	1	0.01
162	3	0.01	3	0.01	0	0.00	3	0.06	0	0.00	0	0.00
602	2	0.004	1	0.003	0	0.00	1	0.02	0	0.00	1	0.01
44	2	0.004	1	0.003	0	0.00	1	0.02	0	0.00	1	0.01
357	2	0.004	2	0.01	1	0.02	1	0.02	0	0.00	0	0.00
315	2	0.004	1	0.003	1	0.02	0	0.00	0	0.00	1	0.01
73	2	0.004	1	0.003	0	0.00	0	0.00	1	0.01	1	0.01
783	2	0.004	2	0.01	2	0.03	0	0.00	0	0.00	0	0.00
2197	2	0.004	2	0.01	0	0.00	1	0.02	1	0.01	0	0.00
1279	2	0.004	2	0.01	1	0.02	1	0.02	0	0.01	0	0.00
393	2	0.004	0	0.00	0	0.00	0	0.00	0	0.00	2	0.01
209	2	0.004	2	0.01	1	0.02	1	0.02	0	0.01	0	0.00
Other ST	79	0.17	42	0.14	8	0.12	7	0.14	28	0.16	37	0.23

prop, proportion; ST, sequence type

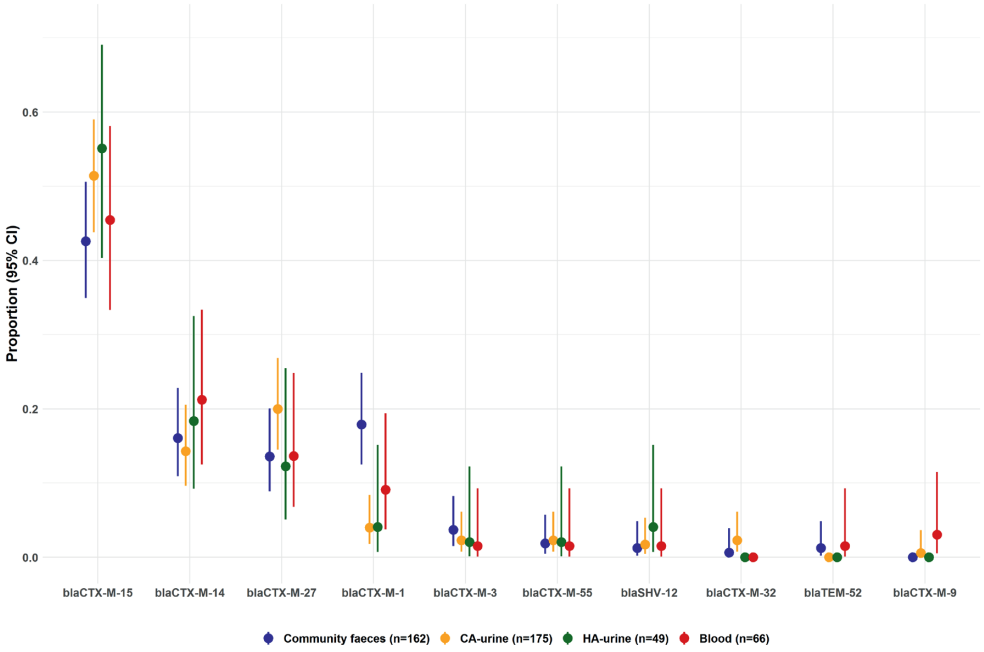


Supplementary Figure S2. Prevalence ten most identified sequence types per clinical sample subgroup of ESBL-positive *E. coli* isolates. CA, community-acquired; ESBL, extended-spectrum beta-lactamase; HA, hospital-acquired

Supplementary Table S2. Prevalence of ESBL genes per sample group of *E. coli* isolates

					Clinical							
	All		**Clinical**		*Blood*		*HA-urine*		*CA-urine*		**Faeces**	
	n	prop	n	prop	n	prop	n	prop	n	prop	n	prop
Isolates	452		290		66		49		175		162	
ESBL genes	18	0.04	16	0.06	11	0.17	9	0.18	12	0.07	12	0.07
$bla_{\text{CTX-M-15}}$	214	0.47	146	0.50	30	0.45	27	0.55	90	0.51	68	0.42
$bla_{\text{CTX-M-14}}$	74	0.16	48	0.17	14	0.21	9	0.18	25	0.14	26	0.16
$bla_{\text{CTX-M-27}}$	72	0.16	50	0.17	9	0.14	6	0.12	35	0.20	22	0.14
$bla_{\text{CTX-M-1}}$	44	0.10	15	0.05	6	0.09	2	0.04	7	0.04	29	0.18
$bla_{\text{CTX-M-3}}$	12	0.03	6	0.02	1	0.02	1	0.02	4	0.02	6	0.04
$bla_{\text{CTX-M-55}}$	9	0.02	6	0.02	1	0.02	0	0.00	3	0.02	3	0.02
$bla_{\text{SHV-12}}$	8	0.02	6	0.02	1	0.02	2	0.04	3	0.02	2	0.01
$bla_{\text{CTX-M-32}}$	5	0.01	4	0.01	0	0.00	0	0.00	4	0.02	1	0.01
$bla_{\text{TEM-52}}$	3	0.01	1	0.00	1	0.02	0	0.00	0	0.00	2	0.01
$bla_{\text{CTX-M-9}}$	3	0.01	3	0.01	2	0.03	0	0.00	1	0.01	0	0.00
$bla_{\text{CTX-M-2}}$	2	0.00	2	0.01	0	0.00	1	0.02	1	0.01	0	0.00
$bla_{\text{TEM-35}}$	2	0.00	1	0.00	1	0.02	0	0.00	0	0.00	1	0.01
$bla_{\text{TEM-28}}$	1	0.00	1	0.00	1	0.02	0	0.00	0	0.00	0	0.00
$bla_{\text{CTX-M-65}}$	1	0.00	1	0.00	0	0.00	0	0.00	1	0.01	0	0.00
$bla_{\text{CTX-M-73}}$	1	0.00	1	0.00	0	0.00	0	0.00	1	0.01	0	0.00
$bla_{\text{CTX-M-192}}$	1	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.01
$bla_{\text{CTX-M-174}}$	1	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	0.01
$bla_{\text{TEM-10}}$	1	0.00	1	0.00	0	0.00	1	0.02	0	0.00	0	0.00
2 ESBL genes	2	0.00	2	0.01	0	0.00	1	0.02	1	0.01	0	0.00
Total genes	454		292		67		49		175		162	

CA, community-acquired; ESBL, extended-spectrum beta-lactamase; HA, hospital-acquired; prop, proportion



Supplementary Figure S3. Prevalence ten most identified ESBL genes per clinical sample subgroup of *E. coli* isolates
CA; community acquired; ESBL, extended-spectrum beta-lactamase; HA; hospital-acquired

Supplementary Table S3. Proportions of ten most common plasmid replicon types[a]

					Clinical							
	All		**Clinical**		*Blood*		*HA-urine*		*CA-urine*		**Faeces**	
	n	prop	n	prop	n	prop	n	prop	n	prop	n	prop
Isolates	452		290		66		49		175		162	
Mean replicons	3.06		3.05		3.29		2.86		3.01		3.07	
IncFIB	332	0.73	213	0.73	51	0.77	35	0.71	127	0.73	119	0.73
IncFII	243	0.54	149	0.51	41	0.62	22	0.45	86	0.49	94	0.58
IncFIA	217	0.48	156	0.54	36	0.55	24	0.49	96	0.55	61	0.38
Col156	157	0.35	106	0.36	16	0.24	14	0.29	75	0.43	52	0.32
Col	89	0.20	65	0.22	15	0.23	7	0.14	43	0.25	24	0.15
IncI1	88	0.19	47	0.16	11	0.17	8	0.16	28	0.16	41	0.25
IncB/O/K/Z	54	0.12	30	0.10	7	0.11	4	0.08	19	0.11	24	0.15
IncQ1	36	0.08	28	0.10	8	0.12	10	0.20	10	0.06	8	0.05
IncFIC	34	0.08	21	0.07	7	0.11	5	0.10	9	0.05	13	0.08
IncX4	26	0.06	14	0.05	4	0.06	3	0.06	7	0.04	12	0.07

CA, community-acquired; HA, hospital-acquired

[a] Prevalence of remaining plasmid replicon types are not included in this supplement but are available upon request.

Supplementary Table S4. Characteristics of ESBL *E. coli* isolates without predicted plasmid replicon.

ST	Sample group	ESBL gene
349	Blood	$bla_{CTX\text{-}M\text{-}14}$
12	Blood	$bla_{CTX\text{-}M\text{-}14}$
328	HA-urine	$bla_{CTX\text{-}M\text{-}14}$
38	Faeces	$bla_{CTX\text{-}M\text{-}15}$
3171	Faeces	$bla_{CTX\text{-}M\text{-}15}$
501	Faeces	$bla_{CTX\text{-}M\text{-}15}$
156	Faeces	$bla_{CTX\text{-}M\text{-}14}$
10	Faeces	$bla_{CTX\text{-}M\text{-}15}$
38	Faeces	$bla_{CTX\text{-}M\text{-}14}$
501	CA-urine	$bla_{CTX\text{-}M\text{-}15}$
38	CA-urine	$bla_{CTX\text{-}M\text{-}14}$
295	CA-urine	$bla_{CTX\text{-}M\text{-}15}$
38	CA-urine	$bla_{CTX\text{-}M\text{-}14}$
1431	CA-urine	$bla_{CTX\text{-}M\text{-}15}$
1147	CA-urine	$bla_{CTX\text{-}M\text{-}15}$
38	CA-urine	$bla_{CTX\text{-}M\text{-}14}$
224	CA-urine	$bla_{CTX\text{-}M\text{-}14}$
38	CA-urine	$bla_{CTX\text{-}M\text{-}15}$
372	CA-urine	$bla_{CTX\text{-}M\text{-}15}$
38	CA-urine	$bla_{CTX\text{-}M\text{-}14}$
648	CA-urine	$bla_{CTX\text{-}M\text{-}1}$
6355	CA-urine	$bla_{CTX\text{-}M\text{-}15}$
131	HA-urine	$bla_{CTX\text{-}M\text{-}15}$
131	HA-urine	$bla_{CTX\text{-}M\text{-}15}$
38	HA-urine	$bla_{CTX\text{-}M\text{-}15}$

CA, community-acquired; HA, hospital-acquired

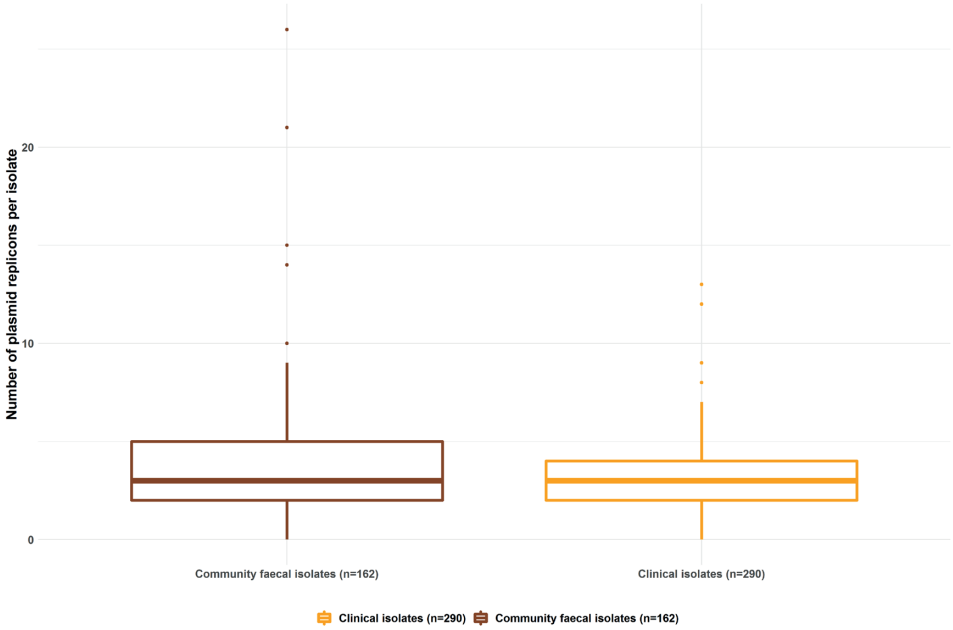
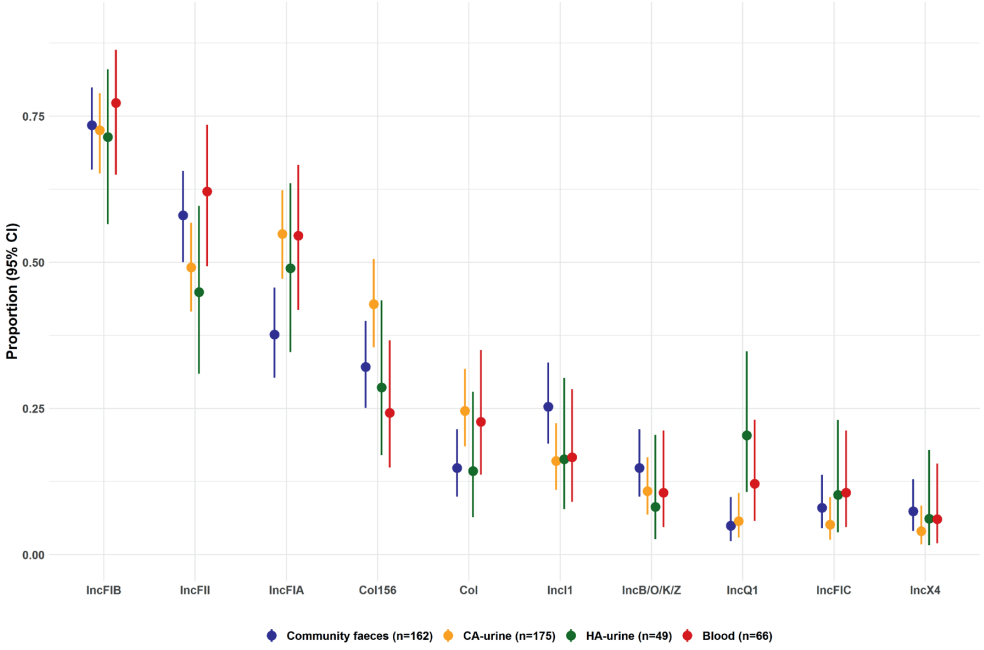


Figure S4. Number of plasmid replicons per isolate for community faecal versus clinical ESBL *E. coli*.



Supplementary Figure S5. Prevalence ten most identified plasmid replicons per clinical sample subgroup of ESBL-positive *E. coli* isolates. CA, community-acquired; ESBL, extended-spectrum beta-lactamase; HA, hospital-acquired

Supplementary Table S5. Proportions of the ten most prevalent whole genome based ESBL-Ec strains as predicted by PopPUNK[a]

					Clinical							
	All		**Clinical**		*Blood*		*HA-urine*		*CA-urine*		**Faeces**	
	n	prop	n	prop	n	prop	n	prop	n	prop	n	prop
Isolates	452		290		66		49		175		162	
Unique wg-b-strains	75	0.17	46	0.16	19	0.29	17	0.35	35	0.20	53	0.33
1 (ST131-like)	157	0.35	117	0.40	29	0.44	19	0.39	69	0.39	40	0.25
2 (ST38-like)	52	0.12	31	0.11	7	0.11	5	0.10	19	0.11	21	0.13
3 (ST10-like)	38	0.08	21	0.07	2	0.03	7	0.14	12	0.07	17	0.11
4 (ST88/410-like)	22	0.05	16	0.06	8	0.12	3	0.06	5	0.03	6	0.04
5 (ST1193-like)	21	0.05	17	0.06	0	0.00	0	0.00	17	0.10	4	0.03
6 (ST648-like)	12	0.03	7	0.02	2	0.03	1	0.02	4	0.02	5	0.03
7 (ST405-like)	12	0.03	6	0.02	1	0.02	1	0.02	4	0.02	6	0.04
8 (ST58-like)	10	0.02	7	0.02	0	0.00	1	0.02	6	0.03	3	0.02
9 (ST69-like)	10	0.02	6	0.02	2	0.03	0	0.00	4	0.02	4	0.03
10 (ST162-like)	6	0.01	4	0.01	0	0.00	3	0.06	1	0.01	2	0.01

CA, community-acquired; HA, hospital-acquired, prop, proportion

8

General discussion

Denise van Hout

INTRODUCTION

An important measure to control the consequences of antibiotic resistance is surveillance. Hospital-based surveillance is an overarching concept that encompasses many different elements, including the detection and tracking of resistant pathogens. Another important part is data collection; surveillance data can guide the development, evaluation and optimization of infection prevention and control strategies. This thesis aimed to contribute to improvement by multiple different projects, which mainly focused on antibiotic-resistant Gram-negative bacteria (GNB). In the first part, we evaluated two different hospital infection prevention strategies, namely selective decontamination in intensive care unit (ICU) patients and the current risk assessment for the detection of multidrug-resistant organism (MDRO) carriers upon hospital admission. In the second part, we investigated surveillance of the current molecular epidemiology of *E. coli* in the Netherlands.

This final chapter synthesizes the evidence from the work described in this thesis to provide an answer to the following questions:

- Should we use **selective oropharyngeal decontamination (SOD)** or **selective digestive decontamination (SDD)** in Dutch ICUs?
- Can we improve the current **laboratory surveillance for acquired colistin resistance** during SDD?
- Should we continue with the current **risk-based screening to detect new MDRO carriers** upon hospital admission?
- What is the current (molecular) epidemiology of ***E. coli* bacteraemia** in the Netherlands?
- Can we use **clinical samples** as a proxy for the molecular epidemiology of intestinal ESBL-producing *E. coli* carriage in the community of the Netherlands?

HOSPITAL-BASED INFECTION PREVENTION

Selective decontamination of the digestive tract (SDD)

Patients that are admitted to an ICU because of critical illness are prone to acquire infections during ICU stay. Selective decontamination in the form of SDD and SOD was developed to prevent these infections and is now a widely used infection prevention strategy in Dutch ICUs. [1] Recent research found that SDD was more effective than SOD in reducing mortality in ICU-

patients in the Netherlands. [2,3] The SDD regimen is more extensive and expensive compared to the SOD regimen with regard to application of antibiotics and microbiological surveillance. As a result, there was uncertainty about which of the regimens should be preferred in terms of balance between costs and effects, as it was questioned whether the yield in effectiveness justified the incremental costs of SDD. In **Chapter 2** of this thesis, we evaluated the cost-effectiveness of SDD versus SOD in an individual patient data cost-effectiveness meta-analysis (IPD-MA) and found that, compared to SOD, SDD significantly reduced in-hospital mortality with no significant difference in healthcare costs. This was mainly driven by the shorter length of stay in the ICU of patients that were treated with SDD. A previous cost-effectiveness analysis (CEA) (2013) that was based on patient-level data of the trial by De Smet *et al.* only (representing 29% of the patients in our IPD-MA) found a beneficial cost-effectiveness profile of SOD compared to SDD, thus reached a different conclusion than our study. [4] This can be explained by multiple things, such as differences between the CEAs in study design, statistical analyses and endpoints. The main difference, however, was our inclusion of individual patient data from the largest and most recent trial that was performed in a low-endemic antimicrobial resistance (AMR) setting – the cluster randomized cross-over trial conducted by Oostdijk *et al.* [3]

Ideally, cost-effectiveness analyses are performed from a societal perspective, by also taking in to account the effects of an intervention on productivity loss or informal care. Unfortunately in our study, cost-effectiveness from a societal perspective could not be determined because we did not have information on such endpoints. However, there are no indications that the effectiveness of SDD from a societal perspective would be opposite to the healthcare perspective – thus more beneficial for SOD – in such a degree that it would have altered our conclusions. We should also recognize that the available data of the individual studies in our IPD-MA did not allow a formal estimation of incremental costs per quality adjusted life year (QALY) gained, which is why we calculated incremental costs of SDD per prevented in-hospital death. Ideally, cost-effectiveness analyses use QALYs to evenly compare interventions across different diseases and to formally compare results to nationally accepted willingness to pay thresholds per QALY. However, in order to measure QALYs, information is needed on the disease attributable change in quality of life (QoL, i.e. morbidity) as well as quantity of life (i.e. mortality). The QoL is usually based on a set of values called health utilities, ranging from 0 (death) to 1 (perfect health). This means that if a person lives in perfect health but only for half a year, that person will have 0.5 QALYs (1 utility * 0.5 years of life = 0.5 QALYs), similar to a patient that lives for one year but in a situation of 'half' of perfect health (0.5 utility * 1 year of life = 0.5 QALYs). Even though the studies in our IPD-MA did not collect information on either QoL or long-term mortality, we still wanted to provide recommendations on the cost-effectiveness of SDD in the context of the Dutch willingness-to-pay threshold of €80,000 (in case of life-threatening illnesses) per QALY gained. [5] Therefore, two additional databases were consulted that contained information on (i) average long-term life-expectancy of former Dutch ICU-patients who were discharged home (from the

Dutch National Intensive Care Evaluation registry [6]), and (ii) average health-related QoL 1 year after ICU-admission (study by Soliman *et al.*). [7] This enabled us to estimate the amount of QALYs that was gained per prevented in-hospital death of an ICU-patient. Our IPD-MA showed that one would need to gain at least 0.61 QALYs for each prevented in-hospital death for SDD to be cost-effective (with 95% probability). Information from the supplementary databases revealed that this amount of QALYs was easily reached: 65% of Dutch ICU-patients that were discharged home was still alive at 4 years after ICU-discharge, and the average QoL index 1 year after ICU admission was 0.71 ± 0.26. A rough calculation, taking a (conservative) average survival of 4 years, would result in 0.71 * 4 = 2.8 QALYs gained per prevented in-hospital death. Because of this – relatively – large amount of QALYs gained by the use of SDD, we concluded that this provided further evidence for implementation of SDD in settings of low levels of AMR.

It is very important to place our results in the context of the setting in which the studies were performed. For our IPD-MA we predefined a specific domain, namely ICU settings with low levels of AMR, in order to limit clinical heterogeneity between trials and to draw meaningful conclusions. This resulted in inclusion of two studies that were both performed in the Netherlands. Consequently, the results of our study are not merely generalizable to countries with moderate or high resistance levels. This is highlighted by the results of a recent cluster-randomized pan-European trial (the R-GNOSIS ICU study), in which selective decontamination was not associated with a statistically significant reduction in ICU-acquired bacteraemia caused by MDRO, nor mortality. [8]

Lastly, there is a general fear of AMR development when antibiotics are used and this is a reason why in some Dutch ICUs, SDD is still not implemented. In 2013, a meta-analysis was performed that included 35 unique studies of SDD or SOD that reported on AMR. [9] There were, compared to no intervention, no statistical significant differences in the prevalence of colonisation or infection with MRSA (methicillin-resistant *S. aureus*), VRE (vancomycin-resistant Enterococci), aminoglycoside-resistant GNB or fluoroquinolone-resistant GNB during selective decontamination. Furthermore, prevalence of third-generation cephalosporin-resistant GNB was lower in recipients of selective decontamination compared to those that received no intervention. A limitation of this meta-analysis was that it did not allow assessment of the long-term use of selective decontamination affecting ICU AMR levels over time. An ecological study that was published a year thereafter (2014) did asses temporal trends and included respiratory isolates of 38 Dutch ICUs using and not using SOD or SDD during 4 years. [10] The use and implementation of selective decontamination was associated with a decreasing trend in AMR levels. The trial by Oostdijk *et al.*, that was included in our IPD-MA, showed a lower prevalence of colonisation and infection by MDRO during SDD than during SOD. [3] Because in that study an increase in aminoglycoside resistance in rectal swabs over time was noticed during both SOD

and SDD, that was more marked during SDD, a post-hoc study was performed, which found no increase in aminoglycoside (or colistin) resistance during a period of 7 years of SOD or SDD use. [11] The most recent longitudinal study (2019) was a single-centre study that found that during 21-years of SDD use, there was no significant increase in ICU-acquired AMR. [12] All in all, the vast majority of evidence to date points toward no increased risk of AMR development during SDD. Fear of resistance development is therefore not based on facts and therefore, not a valid reason to withhold SDD from ICU patients in settings with low endemicity of antibiotic resistance.

> *To conclude*: Should we use selective oropharyngeal decontamination (SOD) or selective digestive decontamination (SDD) in Dutch ICUs?
>
> We should use SDD in ICUs across the Netherlands given the beneficial cost-effectiveness profile of the SDD regimen compared to the SOD regimen and the absence of AMR development.

Directions for future research

- Additional databases can be used to further interpret the results of cost-effectiveness analyses that used trial-based outcomes only, in particular if the interpretation and conclusions on cost-effectiveness are evident. Still, it remains key for future infectious diseases intervention trials to also collect patient-level data on effectiveness on quality of life and if possible, also on long-term life expectancy. This enables proper basic economic evaluation of the interventions in study.
- Similarly, it should be strongly considered during trial design to also collect information on societal costs, such as productivity losses, to ascertain the possibility to perform a CEA from a societal perspective. This is particularly important if the expected magnitude of societal effects of the intervention is large, and if the effects from a societal perspective are likely to be in the opposite direction of the healthcare perspective.

Microbiological surveillance during SDD

Given that one of the topical components of SDD is colistin, microbiological surveillance for the development of acquired colistin resistance is a fundamental part of the SDD regimen. It is therefore recommended in the national SWAB ('Stichting Werkgroep Antibiotica Beleid', Dutch Working Party on Antibiotic Policy) guideline on SDD (2018). [13] There is accumulating evidence that the current routine automated method of testing for colistin resistance is unreliable, since it can fail to detect resistance in case of minimum inhibitory concentration (MIC) values close to the susceptibility breakpoint. [14–18] Currently, the only recommended reference method is

broth microdilution (BMD) using Cation-adjusted Mueller-Hinton broth, a laborious method that requires careful manual preparation. [19] However, in the setting of SDD surveillance, a large amount of samples is processed on a daily basis and routine manual BMD is not feasible. More user friendly (semi-)automated BMD methods have been developed for antibiotic susceptibility testing, such as the Sensititre™ system. In **Chapter 3**, we evaluated the addition of a commercially available selective medium to the conventional inoculation methods in the detection of rectal carriage of GNB with acquired colistin resistance, in a prospective study embedded in routine SDD surveillance. It was shown that the combined use of the conventional methods and selective SuperPolymyxin™ medium had the highest diagnostic yield in detecting GNB with acquired colistin resistance. We wondered to what extent using the new medium would be beneficial in terms of resources, compared to a scenario of implementing routine colistin BMD with the Sensititre™ system. We calculated that implementing SuperPolymyxin™ as a screening medium would be cost-beneficial if the costs of adding SuperPolymyxin™ would not exceed €5.09 per test. Using SuperPolymyxin™ as a screening medium would reduce the number of Gram-negative isolates needing Sensititre™ BMD by 76%.

The practical interpretation of our results partly depends on what one would define as a clinically relevant increase in diagnostic yield. Even though five additional Gram-negative isolates with acquired colistin resistance were identified with the SuperPolymyxin™ medium, the net benefit of adding the medium resulted in only a single extra identified rectal carrier among 428 ICU patients. A scenario in which using SuperPolymyxin™ would add value is if the goal of SDD surveillance would be to identify as many colistin-resistant bacteria as possible, for example to facilitate timely detection of outbreaks or to investigate clonality. In that case, a more than doubling of detected GNB with acquired colistin resistance (i.e. 9 versus 4, as found in the current study) could be considered meaningful. A scenario in which detection of patient-level colistin resistance is deemed more relevant, for example to possibly adapt individual antimicrobial therapy, then the cost aspects of adding SuperPolymyxin™ may be less easily accepted, also given the low prevalence (in our study ~0.7%) of acquired colistin resistance among ICU patients that receive SDD. The current national guideline on SDD (2018) does not provide guidance on this point. [13] Previous analyses suggested that the acquisition of rectal carriage with colistin-resistant GNB may be particularly increased in patients that are already carrier of tobramycin-resistant GNB. [20] This population could therefore be a specific target for additional surveillance. Yet, this distinction is difficult to implement in routine practice. Still, we consider surveillance to be essential during long-term application of SDD – as reflected in national guidelines – and even though the prevalence of acquired colistin resistance may be low, we know that the current routine method of colistin phenotypic susceptibility testing is unreliable. Alternative methods, such as the addition of a selective medium to the laboratory pipeline, should therefore be implemented.

It is important to note that in our study, the commercially available Sensititre™ BMD system with freeze dried colistin was used as a reference test to determine colistin resistance, which has recently been subject of debate on possible false-susceptible and false-resistant results. It was found acceptably reliable in two independent studies from Sweden and Singapore [21,22], intermediate acceptable in a study including carbapenem-resistant *K. pneumoniae* [23] but showed poor results, mainly on major errors (false-resistance), in a study of Enterobacterales that was published after the current study. [18] In that study, the MICRONAUT-S BMD product outperformed Sensititre™, but its performance was still suboptimal with a very major error rate (false-susceptibility) of 3.8%. Because of these results, a step of manual BMD with Mueller Hinton cation-adjusted broth was added to our study to confirm the susceptibility pattern of all identified Gram-negative isolates with acquired colistin resistance. In our analyses of costs and benefits of using SuperPolymyxin™ as a screening medium we still used the scenario that Sensititre™ BMD would be implemented as a routine method, since this was not yet part of our routine laboratory work flow and appeared the only feasible reference test at the time of the study. Nonetheless, if a new, reliable, and perhaps also more straightforward method of phenotypic colistin susceptibility testing would be developed, this may change the recommendations followed by our study.

> *To conclude*: Can we improve the current surveillance for acquired colistin resistance during SDD?
>
> Yes, addition of the selective SuperPolymyxin™ medium to the current laboratory pipeline of SDD surveillance would increase the number of detected Gram-negative isolates with acquired colistin resistance and could be a useful screening medium before the step of BMD. Still, prevalence of acquired colistin resistance during SDD is low. The decision to implement SuperPolymyxin™ should depend on the clinical impact of detecting acquired colistin resistance and may differ locally.

Directions for future research

- The clinical importance of detecting acquired colistin resistance during SDD surveillance should be a point of discussion among experts, since it is currently unclear to what extent additional resources should be invested in the optimization of SDD surveillance.
- There is uncertainty about the reliability of commercially available products for BMD testing of phenotypic colistin susceptibility. Still, the reference method of manual BMD is not feasible to implement in routine practice. Therefore, development and improvement of currently available methods that are appropriate and feasible to implement in a routine laboratory workflow are highly needed, in particular for settings with a large amount of daily surveillance samples.

Detection of multidrug-resistant organism (MDRO) carriers upon hospital admission

Dutch infection prevention guidelines recommend the identification of patients at high risk of MDRO carriage upon hospital admission. [24,25] This strategy has its origin in the Dutch infection prevention of MRSA and over the years has been extended to also detect other types of MDRO. In patients at risk of MDRO carriage, according to this screening, pre-emptive contact precautions should be installed and microbiological cultures should be obtained. In **Chapter 4**, we evaluated this strategy of risk-based screening in an observational study of 144,051 admissions to the University Medical Center Utrecht (UMCU) from 1 January 2015 until 1 August 2019. We found that risk-based screening for MDRO carriage upon admission identified new MDRO carriage in 0.06% (95% CI 0.04% – 0.07%) of all admissions and in 1.8% (95% CI 1.4% – 2.2%) of patients considered to be at high risk of MDRO carriage. In 77.5% of admissions with a positive risk assessment (i.e. in which pre-emptive contact precautions were installed) and screening cultures were obtained, screening cultures were negative for MDRO. The numbers of "MDRO risk assessments needed to perform" and individual "MDRO risk assessment questions needed to ask" to detect one new MDRO carrier upon hospital admission were 1,778 and 10,420, respectively. In a scenario in which risk assessment would have been replaced by the use of existing MDRO labelling in the electronic medical record, an estimated 68% of MDRO carriers – that were identified by risk-based screening – would still be captured.

We compared the prevalence of new identified carriage of different types of MDRO with available data on prevalence upon hospitalisation from other studies (if available, performed during the last 10 years) (Table 1). With these analyses we estimated that the vast majority (between 81.8% and 99.6%) of carriers of different MDRO remained undetected upon admission, despite risk-based screening. Because we compared multiple studies with different study populations and designs, it is important to note the underlying assumptions of these calculations. We assumed that (1) study populations of the other studies represent the same baseline population as our study, i.e. the admission prevalence found in other studies is expected to be the same as in the UMCU, and (2) other studies also report on new identified carriage of MDRO.

This probable large reservoir of MDRO carriers that still remains undetected upon admission, together with the amount of workload required for the identification of one previously unknown MDRO carrier, led us to conclude that our findings support the reconsideration of the current individual risk assessment for MDRO carriage upon hospital admission. One of the counter-arguments that might be raised is the success of the Dutch 'search and destroy' policy against the spread of MDRO over the last years, of which screening upon admission to prevent cross-transmission is one aspect. Still, balance between invested workload and gain remains key. It is important to keep (re-)evaluating existing guidelines and to continuously discuss the relative importance and contribution of current infection prevention practices.

Table 1. Prevalence identified MDRO carriage upon admission in our study and estimation of proportion of MDRO carriers that remains undetected.

	Prevalence of newly identified carriage upon admission by risk-based screening (95% CI) – our study (%)	**Reported prevalence of carriage upon admission in other Dutch studies (%)**	**Estimated proportion detected by risk-based screening (%)**
ESBL-E	0.03 (0.02-0.04)	6.4 to 7.0 [26]	0.4 to 0.5
MRSA	0.02 (0.01-0.03)	0.11 [27] to 0.13 [28]	15.4 to 18.2
CRE	0.001 (0.0002-0.005)	<0.06 [29] to 0.25 [30-32]	0.4 to 1.7
VRE	0.0007 (0.00002-0.004)	1.3 [32] to 1.5[a] [33-34]	0.05

CRE, carbapenem-resistant Enterobacterales, ESBL, extended-spectrum beta-lactamase; ESBL-E, ESBL-producing Enterobacterales; MDRO, multidrug-resistant organism; MRSA, meticillin-resistant *S. aureus*; R, resistant; VRE, vancomycin-resistant Enterococcus

[a] NB. Estimates for VRE carriage derive from point-prevalence surveys in patients during admission and a population-based study on community intestinal carriage.

One of the benefits of screening upon admission over identification of MDRO carriers during hospital stay is the workload associated with finding unexpected MDRO. If a routine (i.e. clinical) culture is positive for MDRO during hospital stay in a patient not yet known to carry such a bacterium, exposed room-mates and/or healthcare personnel of the index patient need to be screened. This contact tracing does not have to be performed when identifying patients through risk-based screening, because in that case, pre-emptive contact precautions were already installed upon admission. Also, until the clinical culture became positive for MDRO, the patient might have already been carrier of an MDRO (not necessarily the entire length of stay) and may have contributed to in-hospital spread unnoticed. In our study, we found that 11% of the new identified MDRO carriers had a clinical culture positive for MDRO during subsequent admission. This means that these patients would else wise be identified as unexpected MDRO carriers during their hospital stay, with contact tracing as a possible consequence. The rest (other 89%) would not have been identified as an MDRO carrier during hospital stay at all, among which were two carriers of carbapenem-resistant Enterobacterales (OXA-48 and OXA-48-like). We do not know how many cross-transmission events were prevented by early identification and (pre-emptive) isolation of these MDRO carriers. Also, it would be interesting to compare all resources needed for the risk-based screening strategy to a scenario in which also costs of unexpected findings of MDRO are included. A description and investigation of this part of infection prevention and control was not part of the scope of the current study.

Lastly, it should be acknowledged that we performed a single-centre study with retrospective observational data, without a control group of no risk-based screening. Ideally, a randomised controlled trial performed in the Netherlands, with current local levels of antibiotic resistance, would have investigated the value of risk-based screening to detect different types of MDRO. This could be a cluster-randomised cross-over trial in multiple hospitals in the Netherlands, with periods of risk-based screening versus no screening upon admission at all. The primary outcome should be transmission events, or even number of infections caused by hospital-acquired MDRO (which can be viewed as a more direct patient-relevant clinical outcome). Simultaneously, bottom-up data on healthcare costs could be collected, so we could investigate which of the strategies would be preferred in terms of balance between resources and effects.

> *To conclude*: Should we continue with the current risk-based screening to detect new MDRO carriers upon hospital admission?
>
> No, findings of this observational study provide multiple arguments to reconsider the current risk assessment for MDRO carriage upon hospital admission. The prevalence of risk factors upon admission is low, as is the number of newly identified MDRO carriers. This leads to a high number of patients that need to be screened in order to identify one new MDRO carrier. At the same time, the majority of influx of MDRO carriers into the hospital probably still remains undetected, despite risk-based screening. We therefore propose to focus on existing labelling of known MDRO carriers instead.

Directions for future research

- The main aim of risk-based screening is to prevent cross-transmission of MDRO by undetected MDRO carriers. In our study, we did not have a real life control group in which we could measure the number of transmissions in case of no risk-based screening. Instead, modelling studies are important to further explore scenarios without risk-based screening upon admission, by using data from this study and estimates from literature on the risk of transmission events in case of unprotected ward stay for the different types of MDRO.
- If the MDRO risk assessment upon admission would be discontinued, hospital-wide surveillance data on microbiological culture results, as used in the current study, should continue to be available in order to follow-up the occurrence of MDRO within the hospital. These data could for example be used in a before-after study.

MOLECULAR EPIDEMIOLOGY OF *E. COLI* IN THE NETHERLANDS

ESBL-negative and ESBL-positive *E. coli* bacteraemia

E. coli bacteraemia (ECB) is a severe infectious disease that affects a vulnerable patient population. This was confirmed in our EPIGENEC study, that found a crude 30-day mortality of 11% and 28% for patients with ESBL-negative and ESBL-positive ECB, respectively. As the incidence of ECB is increasing across Europe, more insight in the (molecular) epidemiology is urgently needed, as this may help identify possible new targets for prevention. [37–40] In **Chapter 6**, we described the current clinical and molecular epidemiology of ECB in the Netherlands. We focused specifically on serotype and genomic differences between ESBL-positive and ESBL-negative *E. coli*, between isolates from different origin (i.e. community versus hospital onset), and between different primary foci of ECB. We found that the most prevalent O-serotypes among non-ESBL-Ec and ESBL-Ec were O6 (12%) and O25 (35%), respectively. Interestingly, O6 was mostly identified in ECB episodes with a primary urinary and hepatic-biliary (HB) focus and was not identified among ESBL-Ec. We also found that the heterogeneity in O-serotypes was larger among non-ESBL-Ec (96.7%, 95% CI 95.8% – 97.6%) than among ESBL-Ec (83.8%, 95% CI 76.9% – 90.6%), reflecting the larger heterogeneity in clonality of non-ESBL-Ec. Based on the serotype distribution, we estimated that the 10-valent ExPEC vaccine candidate (Janssen Vaccines & Prevention, Leiden) would cover 53% of non-ESBL-Ec and 51% of ESBL-Ec bacteraemia isolates. This coverage was slightly lower than the 66% estimated in preliminary results of a recent study (presented at the European Congress of Clinical Microbiology and Infectious Diseases 2019) that included 1,116 (sensitive and resistant) *E. coli* isolates from multiple sites across Europe. [41] That study included 62 isolates from the Netherlands. The results of our study (in which 281 ECB isolates where serotyped) also include serotype distributions across different patient populations in the Netherlands, thereby adding information for further vaccine development. In terms of clinical epidemiology, we found that the urinary tract was the dominant primary focus in both ESBL-negative and ESBL-positive ECB (49% and 44%, respectively), which is similar to findings from previous studies. [39,42,43] The second most important focus of ECB was the hepatic-biliary tract (22% and 20%, respectively), also for both ESBL-negative and ESBL-positive ECB. Importantly, the majority of ECB episodes in the Netherlands were caused by ESBL-negative *E. coli* (~90%) and were of community onset (in our study 81% of ESBL-negative ECB). The open population is therefore a key target population for potential new ExPEC infection prevention strategies. A prospective observational cohort study that is currently ongoing and that will investigate success factors and barriers in preparation of new ExPEC vaccine studies, is therefore recruiting patients in primary care. [44]

Previous literature suggested different levels of pathogenic potential of different *E. coli* strains, implicating that perhaps a subset of *E. coli* strains is particularly prone to cross anatomical and

physiological barriers and enter the bloodstream. [45–51] Clermont *et al.* (2017) found a larger proportion of phylogroup B2, C and D, virulence genes (VG) and antibiotic resistance in ECB strains compared to commensal strains and found that these differences were more marked for bacteraemic strains of urinary origin than of digestive origin. [46] In that study, ECB episodes with a digestive origin most often occurred in patients with risk conditions like immunosuppression, and those strains were not significantly different from commensal strains in terms of phylogroups and VG. It was therefore suggested that these commensal *E. coli* strains were able to translocate into the bloodstream because of hosts with predisposing conditions and not due to increased virulence per se. In line with these findings, a Swedish study by Ny *et al.* (2017) found a higher proportion of phylogroup B2, sequence type (ST) 131 and the ST131 subclone H30-Rx in bacteraemic than in commensal *E. coli* and therefore suggested the existence of certain low-risk and high-risk pathogenic strains. [45] Still, in all these studies molecular characteristics of epidemiological subgroups were never mutually exclusive (i.e. 'commensal' strains were also found to cause ECB and potentially 'pathogenic' isolates were also found as common colonizers). Also in our study in **Chapter 7** (N.B. that included ESBL-positive *E. coli* only), ST131 was the dominant ST in both faecal and clinical samples. Other studies suggested that relative faecal abundance or enhanced colonization abilities may be evenly or more important in the pathogenesis of invasive *E. coli* infection than the more 'classic' virulence traits. [47,52–54] Still, all literature combined, we hypothesized in **Chapter 6** that within invasive strains we would identify molecular differences between epidemiological subgroups, for example between isolates of ECB episodes with a primary focus in the urinary tract compared to those from the gastrointestinal tract. Because we expected that ESBL-positivity could be a potential confounder by being a proxy for host (health) status, we analysed ESBL-negative and ESBL-positive ECB episodes separately. We found some statistically significant differences between epidemiological subgroups (i.e. in resistance gene count and VG scores), but we were hesitant to draw strong conclusions because of small subgroups while absolute differences were limited. So as opposed to previous literature, evidence for molecular specialisation within invasive *E. coli* was not evident from our study. That being said, it should be noted that in that study we did not directly compare invasive strains to commensal strains. The largest molecular differences that we did identify in our study existed between the non-ESBL-Ec and ESBL-Ec population. For example, we found that ST131 was more common among ESBL-positive *E. coli* (ESBL-Ec) than among non-ESBL-producing *E. coli* (non-ESBL-Ec) and that ST73 only occurred among non-ESBL-Ec. This may suggest different evolutionary gene selection of different STs, and that certain strains with a particular 'molecular backbone' might have become able to reduce fitness costs of maintaining plasmids encoding antibiotic resistance (i.e. carrying an ESBL gene). This theory has already been suggested for the successful pandemic *E. coli* clone ST131. [55–57] Finally, it should be recognized that in our study, we only assessed a small part of the entire bacterial genome. Upcoming in-depth molecular research will further elucidate the evolutionary dynamics of bacteraemic non-ESBL and ESBL-Ec, as this was not part of the scope of the current study.

To conclude: What is the current (molecular) epidemiology of *E. coli* bacteraemia (ECB) in the Netherlands?

The dominant part of ECB episodes in the Netherlands is caused by non-ESBL-Ec, is of community onset and has a primary urinary focus, followed by the hepatic-biliary tract. Most frequent O-serotypes are O25 (both non-ESBL-Ec and ESBL-Ec) and O6 (only in non-ESBL-Ec), and the most dominant STs are ST131, ST73 (only in non-ESBL-Ec) and ST69. There are differences in ST and serotype distribution, VG and resistance gene content between ESBL-negative and ESBL-positive bacteraemic *E. coli* isolates.

Directions for future research

- Given the considerable disease burden associated with ECB, more research on potential ExPEC vaccine candidates is urgently needed to develop new prevention programs and to make these available for the open population.
- Molecular epidemiological studies are important to identify main target populations and to assess the potential impact of new preventive strategies.
- Genomic studies that aim to describe and investigate the molecular epidemiology of ECB should focus on the *E. coli* population as a whole, without preselection on ESBL-positivity.

Molecular surveillance of the human intestinal reservoir of ESBL-producing *E. coli*

Molecular surveillance of the human intestinal ESBL-Ec reservoir is important because of multiple reasons. First, insight in the molecular epidemiology of ESBL-Ec may guide clinical decision-making, because insight in local patterns and frequencies of different ESBL-Ec strains can inform the choice of empirical treatment. For example, if it is known from local molecular epidemiology studies that common ESBL-Ec strains are often co-resistant to other types of antibiotics, this will support further broadening of empirical antibiotic therapy in case of suspicion of an ESBL-Ec infection. Second, molecular surveillance is fundamental to track temporal changes in the circulating ESBL-Ec population. As an illustration; large-scale longitudinal molecular surveillance in England, which included the collection of bacteraemic *E. coli* isolates during a systematic 11-year hospital-based survey, enabled a key study on the emergence of important ExPEC *E. coli* lineages. [58] This study was able to demonstrate that the emergence of ST131 as an invasive lineage was most probably due to establishment of a new ecological niche rather than due to its antimicrobial resistant properties, which had thus far been presumed. This study also highlighted the importance of the human commensal niche, as it suggested that the ExPEC population may not form a discrete population but merely represents a spill-over of the intestinal reservoir. This leads to the third argument: the importance of investigating human intestinal carriage as a

reservoir from which ExPEC infections can originate. [58–61] Considering ESBL-positivity is currently the dominant resistance mechanism in *E. coli* in the Netherlands, previous open-population studies have focused on ESBL-Ec and provided unique data on the prevalence and molecular epidemiology of community faecal carriage. [29,31,62,63] Unfortunately, such large-scale studies are both logistically challenging and costly, and therefore not routinely duplicated. For that reason, we aimed to explore whether ESBL-Ec isolates from clinical samples could be used as a proxy for community faecal carriage of ESBL-Ec in **Chapter 7**. We compared the whole genome of ESBL-Ec from clinical samples to ESBL-Ec from faecal samples obtained during the ESBLAT study. [29,64] Some of the main findings of our study were: (1) ST131 was the dominant ST in both sample groups, but the absolute prevalence was higher in clinical samples than in community faecal samples, (2) the other nine most dominant STs had comparable prevalence among faecal and clinical samples, (3) prevalence of ESBL genes was comparable between the two groups, except for $bla_{\text{CTX-M-1}}$, which was more prevalent among faecal samples, and (4) IncFIA was more prevalent in clinical than in faecal samples; other plasmid replicon types had similar prevalence in both groups. This lead to the conclusion that in the Netherlands ESBL-Ec isolates from community faeces and extra-intestinal infection were highly comparable, thus that clinical samples could potentially be used for future molecular public health surveillance of ESBL-Ec. Some important aspects of our study should be recognized in order to further interpret the results.

In our study, we aimed to use a collection of clinical ESBL-Ec isolates from the same years as the ESBLAT open-population study was performed, namely 2014, 2015 and 2016. [29] For community urine isolates only these were unfortunately not available, so these had to be prospectively collected. Consequently, these ESBL-Ec isolates were collected from May to November 2017, resulting in a slight disagreement of sample years. Ideally, in the setting of prospective surveillance of the molecular epidemiology of ESBL-Ec, also time trends in community intestinal carriage would be picked up by surveillance of clinical ESBL-Ec isolates. As the total number of available ESBL-Ec isolates per study year in our final sample collection was relatively low, our study did not enable a comparison of time trends in the two sample groups. The results of the current study should therefore be interpreted as a baseline measurement in comparability of ESBL-Ec from different domains.

The group of clinical ESBL-Ec consisted of isolates that were obtained from (1) urine samples from primary care, (2) urine samples obtained >48 hours after hospital admission, and (3) hospital blood samples. Because this was a first explorative study to assess genomic similarity to community faecal carriage of ESBL-Ec, these different clinical domains were grouped together. However, for future translation of our study results into actual recommendations for surveillance practice, we deemed it important to also investigate the comparability of faecal ESBL-Ec with the different clinical subgroups separately. It could be hypothesized that the isolates from primary

care urine are more similar to faecal ESBL-Ec, given that patients from primary care better reflect the open community than the hospital population. However, it can also be that ESBL-Ec from ECB episodes with a primary focus in the gastrointestinal tract are most similar to faecal isolates, given the underlying translocation from the intestinal tract in to the bloodstream, as was previously suggested. [46] However, subgroup analyses did not reveal distinct comparability of ESBL-Ec isolates from faecal carriage with a particular clinical sample subgroup.

Lastly, an important part of open-population studies is the estimation of prevalence of EBSL-Ec carriage in the community. We did not aim to investigate whether the prevalence of ESBL-carriage in the community could also be inferred from clinical samples. Clinical samples are always obtained because of underlying clinical reasons and diagnostic purposes. For example, urinary samples in primary care are obtained only in case of suspicion of a complicated urinary tract infection (UTI), for example because of therapeutic failure of first line antibiotics. [65] Prevalence of antibiotic resistance in these samples may therefore not accurately represent (i.e. possibly overestimate) the prevalence of antimicrobial-resistant *E. coli* in the community in general. Some studies have sought to assess this discrepancy by investigating clinical urine samples in primary care. A paper from Denmark (2017) suggested similar prevalence of AMR in *E. coli* from study-related consecutive urinary samples in (un)complicated UTI, compared to a national surveillance system that only contained routine urine samples (believed to contain more complicated UTI).[66] A recent Swedish study (2019) suggested otherwise, and found that using routine data from clinical microbiology laboratories may overestimate antibiotic resistance in community UTI. [67] A similar study could well be performed in the Netherlands. For now, open-population studies investigating community carriage of ESBL-Ec will remain necessary if one would want to estimate the prevalence of ESBL-Ec carriage as well as the absolute prevalence of different circulating ESBL-Ec strains in the community.

> *To conclude*: Can we use clinical samples to monitor the molecular epidemiology of intestinal ESBL-producing *E. coli* (ESBL-Ec) carriage in the community of the Netherlands?
>
> Yes, our findings indicate that in the Netherlands ESBL-Ec isolates from community faeces and extra-intestinal infection are highly comparable, with the exception of a higher prevalence of ST131 and plasmid replicon subtype IncFIA among clinical isolates and a higher prevalence of CTX-M-1 among community faeces isolates. This indicates that molecular surveillance of clinical ESBL-Ec isolates could reliably estimate the clonal composition of ESBL-Ec clones circulating in the community, with the exceptions mentioned above, thus could potentially be used for future molecular public health surveillance of ESBL-Ec.

Directions for future research

- Future research could investigate to what extent the prevalence of ESBL-positivity in routine primary care urine samples is comparable to the prevalence of ESBL-Ec carriage in the general population, and thus whether future community prevalence could be inferred by prospective surveillance of routine primary care urine cultures.
- The main aim of our study was to assess the usability of clinical samples for future molecular surveillance of ESBL-Ec from a public health perspective. In that setting, the general purpose is to be able to detect and track current (and possible future emerging) important antibiotic-resistant strains. Therefore, we focused on the ten most dominant STs, ESBL genes and plasmid replicon types. In-depth genomic analyses (not included in the current thesis) that included the entire bacterial genome showed that the ESBL-Ec populations from the two sample groups were not completely identical, possibly related to the dominance of ST131 in the genomic content of the clinical sample group. This will be further explored and is part of ongoing research.

CONCLUDING REMARKS

In this thesis, different aspects of hospital-based surveillance of antibiotic-resistant GNB were evaluated. The main ambition in every project was to translate findings into recommendations for clinical practice and to inform decision making, while keeping an eye on approaching upper limits of healthcare expenditure in the Netherlands. Sometimes cost-effectiveness of an infection prevention regimen was demonstrated (SDD), and sometimes, the balance between invested workload and effects was questioned (MDRO risk assessment upon admission). Populations of antibiotic-resistant bacteria are not static of nature, neither are those of the human host. Local antibiotic resistance levels and dynamics will continue to change. It is my hope that this thesis illustrates the value of hospital-based surveillance as well as the relevance of continuous evaluation of different infection prevention and control practices.

REFERENCES

1. Wittekamp BHJ, Oostdijk EAN, Cuthbertson BH, Brun-Buisson C, Bonten MJM. Selective decontamination of the digestive tract (SDD) in critically ill patients: a narrative review. ***Intensive Care Med.*** 2019;10.1007/s00134-019-05883–9.
2. Plantinga NL, de Smet AMGA, Oostdijk EAN, de Jonge E, Camus C, Krueger WA, ***et al.*** Selective digestive and oropharyngeal decontamination in medical and surgical ICU patients: individual patient data meta-analysis. ***Clin Microbiol Infect.*** 2018;24(5):505–13.
3. Oostdijk EAN, Kesecioglu J, Schultz MJ, Visser CE, de Jonge E, van Essen EHR, ***et al.*** Notice of Retraction and Replacement: Oostdijk et al. Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. JAMA. 2014;312(14):1429-1437. ***JAMA***. 2017;317(15):1583–4.
4. Oostdijk EAN, de Wit GA, Bakker M, de Smet AMGA, Bonten MJM. Selective decontamination of the digestive tract and selective oropharyngeal decontamination in intensive care unit patients: a cost-effectiveness analysis. ***BMJ Open.*** 2013;3(3):e002529.
5. van Rijen AJG. Zinnige en duurzame zorg: advies uitgebracht door de Raad voor de Volksgezondheid en Zorg aan de minister van Volksgezondheid, Welzijn en Sport. Zoetermeer; 2006.
6. Dutch National Intensive Care Evaluation (NICE) registry. Available from: http://www.stichting-nice.nl
7. Soliman IW, de Lange DW, Peelen LM, Cremer OL, Slooter AJC, Pasma W, ***et al.*** Single-center large-cohort study into quality of life in Dutch intensive care unit subgroups, 1 year after admission, using EuroQoL EQ-6D-3L. ***J Crit Care.*** 2015;30(1):181–6.
8. Wittekamp BH, Plantinga NL, Cooper BS, Lopez-Contreras J, Coll P, Mancebo J, ***et al.*** Decontamination Strategies and Bloodstream Infections with Antibiotic-Resistant Microorganisms in Ventilated Patients: A Randomized Clinical Trial. ***JAMA***. 2018;320(20):2087–98.
9. Daneman N, Sarwar S, Fowler RA, Cuthbertson BH. Effect of selective decontamination on antimicrobial resistance in intensive care units: a systematic review and meta-analysis. ***Lancet Infect Dis.*** 2013;13(4):328–41.
10. Houben AJM, Oostdijk EAN, van der Voort PHJ, Monen JCM, Bonten MJM, van der Bij AK. Selective decontamination of the oropharynx and the digestive tract, and antimicrobial resistance: a 4 year ecological study in 38 intensive care units in the Netherlands. ***J Antimicrob Chemother.*** 2014;69(3):797–804.
11. Wittekamp BHJ, Oostdijk EAN, de Smet AMGA, Bonten MJM. Colistin and tobramycin resistance during long- term use of selective decontamination strategies in the intensive care unit: a post hoc analysis. ***Crit Care.*** 2015;19(1):113.
12. Buitinck S, Jansen R, Rijkenberg S, Wester JPJ, Bosman RJ, van der Meer NJM, ***et al.*** The ecological effects of selective decontamination of the digestive tract (SDD) on antimicrobial resistance: a 21-year longitudinal single-centre study. ***Crit Care.*** 2019 Jun;23(1):208.
13. Stichting Werkgroep Antibioticabeleid (SWAB). SWAB Richtlijn: selectieve decontaminatie bij patiënten op de intensive care. 2018;1–29. Available from: https://nvic.nl/swab-richtlijn-sdd
14. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. ***Clin Microbiol Rev.*** 2017;30(2):557–96.
15. Vourli S, Dafopoulou K, Vrioni G, Tsakris A, Pournaras S. Evaluation of two automated systems for colistin susceptibility testing of carbapenem-resistant *Acinetobacter baumannii* clinical isolates. ***J Antimicrob Chemother.*** 2017;72(9):2528–30.
16. Jayol A, Nordmann P, Lehours P, Poirel L, Dubois V. Comparison of methods for detection of plasmid-mediated and chromosomally encoded colistin resistance in *Enterobacteriaceae*. *Clin Microbiol Infect.* 2018;24(2):175–9.

17. Tan TY, Ng SY. Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin. ***Clin Microbiol Infect.*** 2007;13(5):541–4.
18. Pfennigwerth N, Kaminski A, Korte-Berwanger M, Pfeifer Y, Simon M, Werner G, ***et al.*** Evaluation of six commercial products for colistin susceptibility testing in Enterobacterales. ***Clin Microbiol Infect.*** 2019;25(11):1385–9.
19. The European Committee on Antimicrobial Susceptibility Testing. Joint EUCAST and CLSI recommendation - Recommendations for colistin (polymyxin E) MIC testing. 2016. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf.
20. Oostdijk EAN, Smits L, de Smet AMGA, Leverstein-van Hall MA, Kesecioglu J, Bonten MJM. Colistin resistance in gram-negative bacteria during prophylactic topical colistin use in intensive care units. ***Intensive Care Med.*** 2013;39(4):653–60.
21. Matuschek E, Ahman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin - evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter spp*. ***Clin Microbiol Infect.*** 2018;24(8):865–70.
22. Chew KL, La M-V, Lin RTP, Teo JWP. Colistin and polymyxin B susceptibility testing for carbapenem-resistant and mcr-positive *Enterobacteriaceae*: comparison of Sensititre, MicroScan, Vitek 2, and Etest with broth microdilution. ***J Clin Microbiol.*** 2017;55(9):2609–16.
23. Richter SS, Karichu J, Otiso J, Van Heule H, Keller G, Cober E, ***et al.*** Evaluation of Sensititre Broth Microdilution Plate for determining the susceptibility of carbapenem-resistant *Klebsiella pneumoniae* to polymyxins. ***Diagn Microbiol Infect Dis.*** 2018;91(1):89–92.
24. Werkgroep Infectiepreventie. WIP Richtlijn Meticilline-resistente *Staphylococcus aureus* (MRSA). 2012. Available from: https://www.rivm.nl/sites/default/files/2018-11/121205%20MRSA%20v1a%20def.pdf.
25. Werkgroep Infectiepreventie. WIP Richtlijn Bijzonder Resistente Micro-organismen (BRMO). 2013. Available from: https://www.rivm.nl/sites/default/files/2018-11/130424 BRMO.pdf
26. Kluytmans-van den Bergh MFQ, van Mens SP, Haverkate MR, Bootsma MCJ, Kluytmans JAJW, Bonten MJM, ***et al.*** Quantifying hospital-acquired carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* among patients in Dutch Hospitals. ***Infect Control Hosp Epidemiol.*** 2018;39(1):32–9.
27. Bode LGM, Wertheim HFL, Kluytmans JAJW, Bogaers-Hofman D, Vandenbroucke-Grauls CMJE, Roosendaal R, ***et al.*** Sustained low prevalence of meticillin-resistant *Staphylococcus aureus* upon admission to hospital in The Netherlands. ***J Hosp Infect.*** 2011;79(3):198–201.
28. Weterings V, Veenemans J, van Rijen M, Kluytmans J. Prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* in patients at hospital admission in The Netherlands, 2010-2017: an observational study. ***Clin Microbiol Infect.*** 2019;25(11):1428.e1-1428.e5.
29. van den Bunt G, van Pelt W, Hidalgo L, Scharringa J, de Greeff SC, Schurch AC, ***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. ***Eurosurveillance***. 2019;24(41).
30. Dautzenberg MJ, Ossewaarde JM, de Kraker ME, van der Zee A, van Burgh S, de Greeff SC, ***et al.*** Successful control of a hospital-wide outbreak of OXA-48 producing *Enterobacteriaceae* in the Netherlands, 2009 to 2011. ***Eurosurveillance***. 2014;19(9):20723.
31. Reuland EA, Overdevest ITMA, Al Naiemi N, Kalpoe JS, Rijnsburger MC, Raadsen SA, ***et al.*** High prevalence of ESBL-producing *Enterobacteriaceae* carriage in Dutch community patients with gastrointestinal complaints. ***Clin Microbiol Infect.*** 2013;19(6):542–9.
32. Zhou X, Garcia-Cobos S, Ruijs GJHM, Kampinga GA, Arends JP, Borst DM, ***et al.*** Epidemiology of

extended-spectrum beta-lactamase-producing *E. coli* and vancomycin-resistant Enterococci in the northern Dutch-German cross-border region. *Front Microbiol.* 2017;8:1914.
33. van den Braak N, Ott A, van Belkum A, Kluytmans JA, Koeleman JG, Spanjaard L, ***et al.*** Prevalence and determinants of fecal colonization with vancomycin-resistant *Enterococcus* in hospitalized patients in The Netherlands. ***Infect Control Hosp Epidemiol.*** 2000;21(8):520–4.
34. van den Bunt G, Top J, Hordijk J, de Greeff SC, Mughini-Gras L, Corander J, ***et al.*** Intestinal carriage of ampicillin- and vancomycin-resistant *Enterococcus faecium* in humans, dogs and cats in the Netherlands. ***J Antimicrob Chemother.*** 2017:607–14.
35. Centraal Bureau voor de Statistiek. Zorguitgaven stijgen in 2018 met 3,1 procent. (21-6-2019). 2019.
36. van Hout D, Verschuuren TD, Bruijning-Verhagen PCJ, Bosch T, Schürch AC, Willems RJL, ***et al.*** Extended-spectrum beta-lactamase (ESBL)-producing and non-ESBL-producing *E. coli* isolates causing bacteremia in the Netherlands (2014–2016) differ in clonal distribution, antimicrobial resistance gene and virulence gene content. ***PLoSONE*** 2019:;15(1): e0227604.
37. Buetti N, Atkinson A, Marschall J, Kronenberg A. Incidence of bloodstream infections: a nationwide surveillance of acute care hospitals in Switzerland 2008-2014. ***BMJ Open.*** 2017;7(3):e013665.
38. de Kraker MEA, Jarlier V, Monen JCM, Heuer OE, van de Sande N, Grundmann H. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. ***Clin Microbiol Infect.*** 2013;19(9):860–8.
39. Vihta K-D, Stoesser N, Llewelyn MJ, Quan TP, Davies T, Fawcett NJ, ***et al.*** Trends over time in *Escherichia coli* bloodstream infections, urinary tract infections, and antibiotic susceptibilities in Oxfordshire, UK, 1998-2016: a study of electronic health records. ***Lancet Infect Dis.*** 2018;18(10):1138–49.
40. Schlackow I, Stoesser N, Walker AS, Crook DW, Peto TEA, Wyllie DH. Increasing incidence of *Escherichia coli* bacteraemia is driven by an increase in antibiotic-resistant isolates: electronic database study in Oxfordshire 1999-2011. ***J Antimicrob Chemother.*** 2012;67(6):1514–1524.
41. Geurtsen J, Weerdenburg E, Davies T, Go O, Spiessens B, Geet G Van, ***et al.*** Extraintestinal pathogenic *Escherichia coli* surveillance study to determine O-serotype prevalence and antibiotic resistance in blood isolates collected in Europe, 2011–2017. ECCMID Conference 2019 #P1451.
42. Bou-Antoun S, Davies J, Guy R, Johnson AP, Sheridan EA, Hope RJ. Descriptive epidemiology of *Escherichia coli* bacteraemia in England, April 2012 to March 2014. ***Eurosurveillance***. 2016;21(35):30329.
43. Abernethy J, Guy R, Sheridan EA, Hopkins S, Kiernan M, Wilcox MH, ***et al.*** Epidemiology of *Escherichia coli* bacteraemia in England: results of an enhanced sentinel surveillance programme. ***J Hosp Infect.*** 2017;95(4):365–75.
44. Bonten MJM, Verheij T, Cuperus N, den Boer A. Study to collect information about invasive disease caused by extraintestinal pathogenic *Escherichia Coli* (EXPECT-1). NCT04087681.
45. Ny ***et al.*** Community carriage of ESBL-producing *Escherichia coli* is associated with strains of low pathogenicity: a Swedish nationwide study. ***J Antimicrob Chemother.*** 2017;:582–8.
46. Clermont O, Couffignal C, Blanco J, Mentre F, Picard B, Denamur E. Two levels of specialization in bacteraemic *Escherichia coli* strains revealed by their comparison with commensal strains. ***Epidemiol Infect.*** 2017;145(5):872–82.
47. Moreno E, Andreu A, Pigrau C, Kuskowski MA, Johnson JR, Prats G. Relationship between *Escherichia coli* strains causing acute cystitis in women and the fecal *E. coli* population of the host. ***J Clin Microbiol.*** 2008;46(8):2529–34.
48. Johnson JR, Kuskowski MA, Bryan TTO, Maslow JN. Epidemiological correlates of virulence genotype and phylogenetic background among *Escherichia coli* blood Isolates from adults with diverse-source bacteremia. ***J Infect Dis.*** 2002;1439–47.
49. Johnson JR, Owens K, Gajewski A, Kuskowski MA. Bacterial characteristics in relation to clinical source

of *Escherichia coli* Isolates from women with acute cystitis or pyelonephritis and uninfected women. ***J Clin Microbiol.*** 2005;43(12):6064–72.
50. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. ***Int J Med Microbiol.*** 2005;295(6–7):383–404.
51. Köhler CD, Dobrindt U. What defines extraintestinal pathogenic *Escherichia coli*? Int J Med Microbiol. 2011;301(8):642–7.
52. Ruppé E, Lixandru B, Cojocaru R, Clermont O, Denamur E, Armand-lefèvre L. Relative fecal abundance of extended-spectrum-beta-lactamase-producing *Escherichia coli* strains and their occurrence in urinary tract infections in women. ***Antimicrob Agents Chemother.*** 2013;57(9):4512–7.
53. Banerjee R, Johnson JR. A new clone sweeps clean: the enigmatic emergence of *Escherichia coli* sequence type 131. ***Antimicrob Agents Chemother.*** 2014;58(9):4997–5004.
54. Vila J, Saez-Lopez E, Johnson JR, Romling U, Dobrindt U, Canton R, ***et al.*** *Escherichia coli*: an old friend with new tidings. ***Microbiol Rev.*** 2016;40(4):437–63.
55. McNally A, Oren Y, Kelly D, Pascoe B, Dunn S, Sreecharan T, ***et al.*** Combined analysis of variation in core, accessory and regulatory genome regions provides a super-resolution view into the evolution of bacterial populations. ***PLoS Genet.*** 2016;12(9):e1006280.
56. McNally A, Kallonen T, Connor C, Abudahab K, Aanensen DM, Horner C, ***et al.*** Diversification of colonization factors in a multidrug-resistant *Escherichia coli* lineage evolving under negative frequency-dependent selection. ***MBio.*** 2019;10(2):e00644-19.
57. Dunn SJ, Connor C, McNally A. The evolution and transmission of multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae*: the complexity of clones and plasmids. ***Curr Opin Microbiol.*** 2019;51:51–6.
58. Kallonen T, Brodrick HJ, Harris SR, Corander J, Brown NM, Martin V, ***et al.*** Systematic longitudinal survey of invasive *Escherichia coli* in England demonstrates a stable population structure only transiently disturbed by the emergence of ST131. ***Genome Res.*** 2017;27:1437–49.
59. Yamamoto S, Tsukamoto T, Terai A, Kurazono H, Takeda Y, Yoshida O. Genetic evidence supporting the fecal-perineal-urethral hypothesis in cystitis caused by *Escherichia coli*. ***J Urol.*** 1997;157(3):1127–9.
60. Day MJ, Hopkins KL, Wareham DW, Toleman MA, Elviss N, Randall L, ***et al.*** Extended-spectrum ß-lactamase-producing *Escherichia coli* in human-derived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. ***Lancet Infect Dis.*** 2019;19(12):1325–35.
61. Dorado-García A, Smid JH, van Pelt W, Bonten MJM, Fluit AC, van den Bunt G, ***et al.*** Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. ***J Antimicrob Chemother.*** 2018;73(2):339–47.
62. Reuland EA ***et al.*** Prevalence and risk factors for carriage of ESBL-producing *Enterobacteriaceae* in Amsterdam. ***J Antimicrob Chemother.*** 2016:1076–82.
63. Wielders CCH, van Hoek AHAM, Hengeveld PD, Veenman C, Dierikx CM, Zomer TP, ***et al.*** Extended-spectrum beta-lactamase- and pAmpC-producing *Enterobacteriaceae* among the general population in a livestock-dense area. ***Clin Microbiol Infect.*** 201723(2):120.e1-120.e8.
64. van Hout D, Verschuuren TD., Bruijning-Verhagen PCJ., Bosch T., Reuland EA., Fluit AC., ***et al.*** Design of the EPIGENEC Study: Assessing the EPIdemiology and GENetics of *Escherichia coli* in the Netherlands. ***Preprints***. 2019;2019020066.
65. Van Pinxteren B, Knottnerus BJ, Geerlings SE, Visser HS, Klinkhamer S, Van der Weele GM, Verduijn MM, Opstelten W, Burgers JS VAK. NHG-Standaard Urineweginfecties (derde herziening). ***Huisarts Wet.*** 2013;56(6):270–8.
66. Córdoba G, Holm A, Hansen F, Hammerum AM, Bjerrum L. Prevalence of antimicrobial resistant *Escherichia coli* from patients with suspected urinary tract infection in primary care, Denmark. ***BMC***

Infect Dis. 2017;17(1):670.
67. Kornfält Isberg H, Melander E, Hedin K, Mölstad S, Beckman A. Uncomplicated urinary tract infections in Swedish primary care; etiology, resistance and treatment. ***BMC Infect Dis.*** 2019;19(1):155.

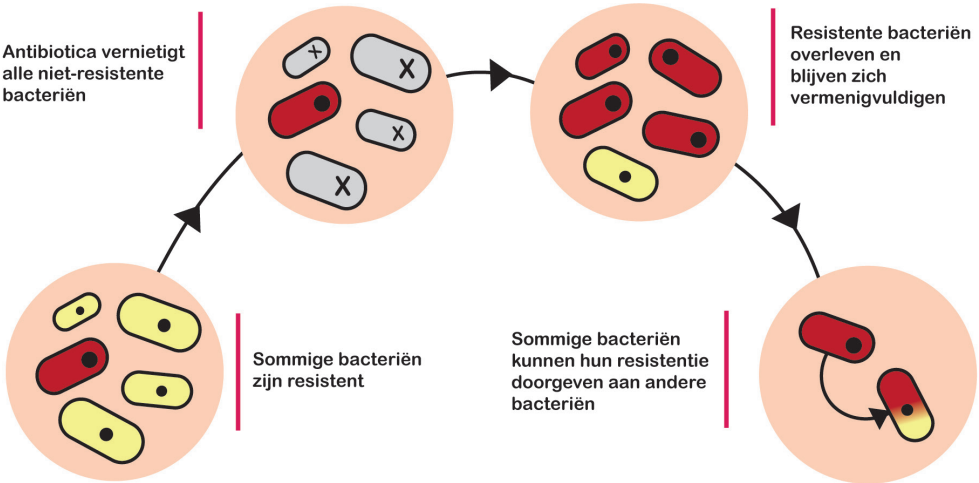
Appendices

DUTCH SUMMARY

NEDERLANDSE SAMENVATTING

ANTIBIOTICARESISTENTIE

Het menselijk lichaam zit vol met micro-organismen, waaronder virussen, schimmels en bacteriën. Bacteriën zijn eencellige micro-organismen zonder celkern. Er wordt over het algemeen onderscheid gemaakt tussen Gram-positieve en Gram-negatieve bacteriën, op basis van de structuur van hun celwand. Ze komen onder andere voor op de huid, in de neus, mond en in onze darmen. We noemen dit ook wel bacteriële 'kolonisatie' van het menselijk lichaam, of 'dragerschap'. De bacteriën in de darmen worden tezamen ook wel de 'microbiota' genoemd (voorheen ook wel bekend als 'darmflora'). Bacteriën zijn doorgaans onschuldig en zijn zelfs belangrijk voor onze stofwisseling en afweer. Maar bacteriën kunnen zich in bepaalde omstandigheden ook vermeerderen en ziektes veroorzaken, zoals huid- of urineweginfecties. Infecties die door bacteriën worden veroorzaakt kunnen we meestal behandelen met antibiotica. Echter kunnen bacteriën resistent (ongevoelig) worden tegen bepaalde typen antibiotica. In dat geval kan een bacterie overleven en zich vermenigvuldigen in de aanwezigheid van een antibioticum dat normaal gesproken ervoor zou zorgen dat de bacterie zou stoppen met groeien, of zelfs de bacterie zou doodmaken. Dit noemen we antibioticaresistentie. We weten dat er meer antibioticaresistentie ontstaat als er veel antibiotica gebruikt worden. Dit komt door het fenomeen van selectiedruk. Onder druk van antibiotica zullen alleen de meest fitte bacteriën overleven en zich vermenigvuldigen. Als er nu binnen een groep bacteriën antibioticaresistente subpopulaties bestaan, en er is blootstelling aan antibiotica, dan zullen de resistente subpopulaties overleven en worden deze hierdoor uitgeselecteerd (Figuur 1).



Figuur 1. Selectiedruk. Aangepast van "De andere kant van de medaille", door Nationaal Farmaceutisch Museum (jaartal onbekend). Beschikbaar via: https://www.nationaalfarmaceutischmuseum.nl/artikelen/de-andere-kant-van-de-medaille.

Bacteriën kunnen antibioticaresistentie aan elkaar doorgeven, bijvoorbeeld door middel van celdelingen. Dat noemen we verticale transmissie. Een andere manier is door het uitwisselen van plasmiden. Plasmiden zijn cirkelvormige strengen DNA waarop zich genen voor antibioticaresistentie kunnen bevinden. Dat noemen we ook wel horizontale transmissie. Een bekend voorbeeld van (voornamelijk) plasmidegemedieerde antibioticaresistentie in bacteriën is de productie van een enzym dat antibiotica kan afbreken, genaamd ESBL ('extended-spectrum beta-lactamase'). Het is niet direct gevaarlijk als een bacterie die wij bij ons dragen resistent wordt tegen bepaalde antibiotica. Echter als men vervolgens een infectie ontwikkelt met een resistente bacterie dan moet deze wel behandeld worden met een ander soort antibioticum (vaak met een breder spectrum). Gelukkig zijn er in Nederland meestal nog genoeg andere soorten antibiotica voorhanden. We doen erg ons best om dit zo te houden. Zo willen we terughoudend zijn in het voorschrijven van antibiotica, zodat we nog zo lang mogelijk alle bacteriële infecties goed kunnen behandelen. Daarnaast hebben we in Nederlandse ziekenhuizen veel infectiepreventiemaatregelen, zodat patiënten zo min mogelijk antibioticaresistente bacteriën met elkaar uitwisselen. Een belangrijke manier om de gevolgen van antibioticaresistentie zoveel mogelijk tegen te gaan is door middel van surveillance. Surveillance is het systematisch verzamelen en analyseren van data over resistentie van bacteriën voor antibiotica. Longitudinale surveillance kan bijvoorbeeld verschuivingen in resistentie in de tijd zichtbaar maken. Deze gegevens zijn belangrijk voor de ontwikkeling, evaluatie en optimalisering van infectiepreventie. In dit proefschrift richten we ons in verschillende projecten op surveillance in het ziekenhuis, en dan met name van antibioticaresistente Gram-negatieve bacteriën. Hiermee willen we een bijdrage leveren aan het verbeteren van surveillance van antibioticaresistentie in Nederland. In de eerste drie hoofdstukken richten we ons op het voorkomen van infecties op de intensive care (IC) door middel van selectieve darm decontaminatie, de detectie van resistente bacteriën in het laboratorium en het herkennen van dragers van resistente bacteriën als zij worden opgenomen in het ziekenhuis. In de laatste drie hoofdstukken richten we ons op moleculaire surveillance van de belangrijke bacterie *Escherichia coli* (*E. coli*) in Nederland.

SAMENVATTING VAN HET ONDERZOEK IN DIT PROEFSCHRIFT

Patiënten die zijn opgenomen op de intensive care (IC) zijn over het algemeen ernstig ziek en hebben daarom een verhoogd risico op infecties. Bacteriën en gisten die de darm en mond-keelholte koloniseren spelen hierin een belangrijke rol en vormen daarom het aangrijpingspunt van decontaminatie strategieën, zoals selectieve darm decontaminatie (SDD) en selectieve orofaryngeale decontaminatie (SOD). De SDD strategie bestaat uit antibioticaprofylaxe door middel van een mondpasta, een suspensie die door de neus-maag sonde wordt gegeven en intraveneuze antibiotica gedurende de eerste vier dagen van IC opname. SOD bestaat alleen uit de mondpasta en intraveneuze antibiotica. De grootste en meest recente studie die is uitgevoerd

in Nederland laat een overlevingsvoordeel zien van IC patiënten die SDD krijgen toegediend in vergelijking met SOD. Echter is de SDD strategie een stuk uitgebreider dan SOD, zowel wat betreft de antibiotica profylaxe als microbiologische surveillance. Er was onzekerheid welke van de twee strategieën er in Nederlandse ICs gebruikt zou moeten worden. In **Hoofdstuk 2** hebben we dit onderzocht door middel van een kosteneffectiviteitsanalyse (KEA). We hebben hiervoor de gegevens gebruikt van de twee cluster-gerandomiseerde studies die in Nederland zijn uitgevoerd en SDD en SOD met elkaar vergeleken en hebben deze gegevens gepoold (in een zogenaamde meta-analyse van data van individuele patiënten). Uit de resultaten bleek dat de SDD strategie kosteneffectief was in vergelijking met SOD: SDD verminderde de ziekenhuissterfte van IC patiënten zonder een significant verschil in ziekenhuiskosten. Dit kwam met name doordat IC patiënten die SDD kregen gemiddeld sneller konden worden teruggeplaatst van de IC naar de reguliere verpleegafdeling. Helaas zijn er nog enkele ICs in Nederland die geen SDD gebruiken vanwege angst voor het ontstaan van antibioticaresistentie. Echter laat de overgrote meerderheid van alle studies die tot nu toe zijn uitgevoerd geen aanwijzingen zien voor een toename in antibioticaresistentie op de korte, middellange en lange termijn. Er zijn in enkele studies zelfs aanwijzingen voor een daling in antibioticaresistentie in IC patiënten die SDD krijgen. Deze angst is dus ongegrond. Op basis van alle gegevens tezamen adviseren we daarom om in Nederlandse ICs de SDD strategie te gebruiken.

Omdat de SDD strategie gebaseerd is op het toedienen van profylactische antibiotica nemen we structureel microbiologische kweken af bij IC patiënten die SDD krijgen. Deze surveillance gegevens gebruiken we om continue in de gaten te blijven houden of er toch geen antibioticaresistentie ontstaat. Een van de antibiotica van SDD is colistine. Het is vanwege de structuureigenschappen van colistine moeilijk om colistineresistentie te detecteren in het laboratorium. We hebben daarom onderzocht of het toevoegen van een selectieve plaat aan het huidige laboratorium proces de detectie van colistineresistente Gram-negatieve bacteriën zou verbeteren. Deze selectieve plaat, het SuperPolymyxine™ medium, bevat het antibioticum colistine en zou bijvoorbeeld als een screening kunnen dienen: alleen de bacteriën die hierop groeien (en dus in theorie ongevoelig zijn voor colistine) worden verder uitgewerkt in het laboratorium. De resultaten van deze studie staan beschreven in **Hoofdstuk 3**. We vonden dat met gebruik van SuperPolymyxine™ er inderdaad meer colistineresistente Gram-negatieve bacteriën werden gevonden. Het is belangrijk om ook eventueel toenemende kosten mee te nemen bij de overweging om een nieuw (diagnostisch) middel in het laboratorium te implementeren. In het geval van SDD surveillance worden er namelijk dagelijks een heleboel kweken afgenomen. Daarom hebben we ook de kosten berekend van het eventueel toevoegen van de Superpolymyxine™ plaat. In verschillende scenario's hebben we dat vergeleken met het implementeren van microdilutie (in het Engels 'broth microdilution' of 'BMD') met het Sensititre™ systeem voor het definitief vaststellen van colistineresistentie. Dit wordt momenteel in ons laboratorium nog niet gebruikt. We vonden dat als de kosten van de nieuwe Superpolymyxine™ plaat niet hoger zouden zijn dan €5.09 per test, het goedkoper zou

zijn om de nieuwe plaat te implementeren als screening medium dan het huidige laboratorium proces voort te zetten. Daarnaast zou het gebruik van SuperPolymyxine™ als screening medium het aantal Gram-negatieve stammen dat BMD zou moeten ondergaan verminderen met 76%.

Bacteriën die resistent zijn geworden voor meerdere soorten antibiotica worden in het Nederlands ook wel bijzonder-resistente micro-organismen (BRMO) genoemd. Als iemand is opgenomen in een Nederlands ziekenhuis en we weten dat iemand drager is van een BRMO, dan verplegen we iemand in isolatie. Er zijn verschillende vormen van isolatiemaatregelen. Het kan bijvoorbeeld betekenen dat iemand een eenpersoonskamer krijgt, artsen en verpleegkundigen speciale kleding aandoen als zij de kamer betreden, dat zij een mondkapje dragen of een combinatie van deze maatregelen. We willen namelijk voorkomen dat de bacterie wordt overgedragen via de zorgverleners naar andere patiënten. Een van de meest bekende BRMO is MRSA (methicilline-resistente *Staphylococcus aureus*). Toen MRSA opkwam in Nederland werd bekend dat er bepaalde risicofactoren waren voor dragerschap. Zo bleek MRSA vaker voor te komen bij patiënten die langdurig in een buitenlands ziekenhuis waren opgenomen of bij patiënten die werkten in de veehouderij. Ter voorkoming van uitbraken en verdere verspreiding van MRSA werd daarom besloten om in nationale richtlijnen op te nemen dat alle patiënten bij opname in het ziekenhuis gescreend moeten worden op aanwezigheid van deze risicofactoren. Dit bekende dat patiënten die bijvoorbeeld werkzaam waren op een veehouderij vanaf opname voor de zekerheid in isolatie werden verpleegd, totdat de microbiologische kweekresultaten bekend werden. Als de kweekuitslagen negatief waren voor MRSA, werden de isolatiemaatregelen weer opgeheven. Dit werd in (internationale) literatuur ook wel bekend als een onderdeel van het succesvolle Nederlandse 'search and destroy' beleid voor MRSA. In de afgelopen jaren is deze screening bij ziekenhuisopname steeds iets verder uitgebreid om ook dragerschap van andere antibioticaresistente bacteriën op te sporen. Het is zelfs een indicator geworden van de Nederlandse Inspectie Gezondheidszorg en Jeugd. Dit betekent dat elk ziekenhuis wordt gecontroleerd of ze aan deze indicator voldoen, als maat voor kwaliteit van zorg. Bij elke ziekenhuisopname wordt de patiënt een lijst van zes vragen gesteld. Dit staat ook wel bekend als de BRMO anamnese. Risicofactoren die bij opname worden uitgevraagd zijn:

1) eerdere aantoning van een ziekenhuisbacterie (d.w.z. BRMO);
2) recente ziekenhuisopname in een ander Nederlands ziekenhuis ten tijde van een uitbraak;
3) recente ziekenhuisopname of behandeling in een buitenlands ziekenhuis;
4) wonen in een opvang of instelling voor asielzoekers;
5) wonen of werken op een bedrijf met levende varkens, vleeskalveren of vleespluimvee;
6) wonen bij of zorgen voor een MRSA positief persoon.

Bij aanwezigheid van één van deze risicofactoren wordt de patiënt in isolatie verpleegd en worden er kweken afgenomen om te kijken of iemand ook echt drager is van een BRMO. Ondanks dit uitgebreide nationale beleid was er tot nu toe nog nooit in kaart gebracht hoeveel BRMO dragers hiermee worden opgespoord. Dit hebben we onderzocht in **Hoofdstuk 4**. We hebben data verzameld van >90% van alle ziekenhuisopnames in het Universitair Medisch Centrum Utrecht (UMCU) gedurende een studieperiode van 4,5 jaar. Er werden 144.051 ziekenhuisopnames geïncludeerd van 84.485 unieke patiënten. We vonden dat de BRMO anamnese voorheen onbekende dragers identificeerde in 0.06% (95% betrouwbaarheidsinterval (BHI) 0.04%-0.07%) van alle ziekenhuisopnames en in 1.8% (95% BHI 1.4%-2.2%) van patiënten met een verhoogd risico op BRMO dragerschap. In 77.5% van alle opnames met een positieve BRMO anamnese waarbij er kweken werden afgenomen waren deze uiteindelijk negatief voor BRMO. Het aantal BRMO anamneses dat moest worden afgenomen om één nieuwe BRMO drager op te sporen was 1.778. Anders gezegd: er moesten 10.420 vragen worden gesteld om één nieuwe drager van BRMO te vinden. Wat belangrijk is om te vermelden is dat we in Nederlandse ziekenhuizen ook een labelsysteem hebben in het elektronisch patiënten dossier, waarmee we patiënten die drager zijn van BRMO kunnen herkennen als zij weer opnieuw worden opgenomen. We berekenden dat als we de huidige BRMO anamnese niet meer zouden uitvoeren en ons in plaats daarvan slechts op deze bestaande labeling zouden richten, we 68% van alle BRMO dragers (gevonden door de BRMO anamnese), nog steeds zouden vinden. Ten slotte hebben we onze bevindingen vergeleken met eerdere Nederlandse studies die onderzoek hebben gedaan naar de prevalentie van BRMO dragerschap bij ziekenhuisopname. We vonden in deze berekeningen dat de overgrote meerderheid (91.8%-99.6%) van de BRMO dragers die wordt opgenomen in het ziekenhuis, waarschijnlijk nog steeds onopgemerkt blijft. Dit komt doordat de risicofactoren die worden uitgevraagd niet goed voorspellen of iemand daadwerkelijk drager is van BRMO (lage positief voorspellende waarde). Daarnaast geldt voor het overgrote deel van de BRMO dragers dat deze risicofactoren niet van toepassing zijn (lage sensitiviteit). Gezien de tijdsinvestering die gepaard gaat met de huidige massale screening bij opname concludeerden we daarom dat onze studie bewijs levert om de huidige BRMO anamnese sterk te heroverwegen.

In **Hoofdstuk 5** staat het studieprotocol van onze EPIGENEC studie (EPIdemiology and GENetics of *E. coli*) beschreven. *E. coli* is een van de bacteriën die we bijna allemaal (onschuldig) bij ons dragen in onze darmen. We noemen dit ook wel het (humane) intestinale reservoir van *E. coli*. Echter is het ook een beruchte bacterie, het is namelijk een frequent pathogeen in infecties. Zo is het de meest voorkomende oorzaak van urineweginfecties en een van de meest belangrijke veroorzakers van bacteriëmie (ook wel 'bloedvergiftiging' genoemd, er horen geen bacteriën te zitten in bloed). Eigenlijk is het lastig praten over 'de' bacterie *E. coli*; er zijn namelijk binnen deze bacterie veel verschillende subtypes. Met behulp van whole genome sequencing (WGS) kunnen we het volledige genetisch materiaal van bacteriën in kaart brengen. Op die manier kunnen we de aanwezigheid van bepaalde resistentiegenen of virulentiegenen vaststellen.

Studies tot nu toe suggereerden mogelijke belangrijke verschillen tussen de verschillende *E. coli* subtypes en hun (pathogeen) vermogen om een bacteriëmie te kunnen veroorzaken. De *E. coli* stammen van mensen bij wie de infectie begon in de urinewegen leken bijvoorbeeld erg anders dan de stammen in de darmen. Dat onderzoek richtte zich met name op antibioticaresistente *E. coli*, ook al wordt het overgrote merendeel van de *E. coli* bacteriëmiën (ECB) in Nederland nog altijd veroorzaakt door antibioticagevoelige *E. coli* (~90%). Er was tot nu toe dus nog weinig bekend in hoeverre dit ook voorkwam bij bacteriëmie door antibioticagevoelige en -resistente *E. coli* in Nederland. Een (relatief) veelvoorkomend resistentiemechanisme in *E. coli* is de productie van ESBL, een enzym dat bepaalde soorten antibiotica kan afbreken. Door middel van grootschalige surveillance studies meten we hoe vaak intestinaal dragerschap van ESBL-positieve *E. coli* (ESBL-Ec) voorkomt (prevalentie, dit wordt momenteel geschat op circa 5%) en welke subtypes er circuleren. Helaas zijn deze studies niet makkelijk om uit te voeren, het kost veel tijd en mankracht (de laatste en grootste studie duurde meer dan vijf jaar van start tot publicatie). Het zou dus mooi zijn als we onze klinische samples, afgenomen tijdens routinezorg, hiervoor konden gebruiken. We hebben de EPIGENEC studie daarom opgezet met twee hoofddoelen: 1) Het beschrijven van de huidige moleculaire epidemiologie in Nederland van *E. coli* bacteriëmie met verschillende klinische kenmerken, en 2) Onderzoeken of *E. coli* uit klinische samples ook gebruikt zou kunnen worden voor surveillance van het intestinale *E. coli* reservoir in de open populatie.

In **Hoofdstuk 6** beantwoorden we de eerste onderzoeksvraag van de EPIGENEC studie (zie alinea hierboven). We hebben hiervoor klinische en genetische kenmerken van 281 ECB onderzocht, waarvan 212 ESBL-negatieve *E. coli* (non-ESBL-Ec) en 69 ESBL-Ec stammen. We vonden dat de meeste (64% tot 81%) episodes van ECB ontstonden vóórdat patiënten waren opgenomen in het ziekenhuis (zogenaamde 'community onset') en meestal een primair focus hadden in de urinewegen (in 44% tot 49% van de gevallen) gevolgd door de lever en/of galwegen. De belangrijkste bevinding van deze studie was dat de non-ESBL-Ec en ESBL-Ec stammen zeer van elkaar verschilden, namelijk in termen van serotypes, subtypes, virulentiegenen en resistentiegenen. De non-ESBL-Ec stammen waren ook een meer heterogene groep dan de ESBL-Ec. Het lijkt er dus op dat gedurende de evolutie bepaalde subtypes van deze stammen beter zijn geworden in het vasthouden van de ESBL plasmiden dan anderen. Uit onze studie bleek verder dat ECB ook in Nederland vaak kwetsbare patiënten betreft; de ruwe 30-dagen mortaliteit was respectievelijk 11% en 28% voor patiënten met non-ESBL-Ec en ESBL-Ec bacteriëmie. Er worden daarom *E. coli* vaccins ontwikkeld die in de toekomst deze infecties hopelijk zoveel mogelijk kunnen voorkomen. De informatie uit deze studie, zoals de kenmerken van de patiënten en de verschillende *E. coli* subtypes, kan gebruikt worden voor verdere ontwikkeling van deze vaccins.

Omdat *E. coli* zo'n belangrijk pathogeen en ESBL een relatief veelvoorkomend resistentiemechanisme is, willen we door middel van surveillance graag continu bijhouden welke ESBL-Ec subtypes er allemaal circuleren in Nederland. Zo kunnen we bijvoorbeeld tijdig veranderingen of nieuwe belangrijke resistente stammen opmerken. We weten dat het darmstelsel van de mens een belangrijk reservoir is voor de *E. coli* bacterie. In **Hoofdstuk 7** onderzochten we daarom of we stammen uit verschillende klinische samples (d.w.z. van infecties) betrouwbaar zouden kunnen gebruiken voor toekomstige surveillance, als proxy voor het humane intestinale ESBL-Ec reservoir. Hiervoor onderzochten we het genoom van 183 ESBL-Ec intestinale dragerschapsstammen uit de ESBLAT-studie en vergeleken deze met ESBL-Ec stammen uit urine verzameld in de huisartsenpraktijk (n=187), ESBL-Ec stammen uit urine verzameld in het ziekenhuis (n=59) en ESBL-Ec stammen uit bloed (n=70). We vonden veel overeenkomsten. Zo was de prevalentie van de tien meest voorkomende subtypes vergelijkbaar tussen de intestinale en de infectieuze stammen, met uitzondering van subtype ST131 (een belangrijk subtype in infecties), die kwam in absolute zin significant vaker voor in klinische samples (39% versus 23%) en was in beide groepen het meest voorkomende subtype. De tien meest voorkomende ESBL-genen waren ook vergelijkbaar in beide groepen. Enige uitzondering hierop was het ESBL-gen CTX-M-1. Dit gen kwam vaker voor in dragerschap dan in klinische samples (18% versus 5%). Belangrijk is wel om op te merken dat we nog niet naar het totale genetisch materiaal van alle stammen hebben gekeken (maar slechts naar bepaalde onderdelen van het genoom). Al met al concludeerden we dat deze eerste vergelijking tussen intestinale en klinische ESBL-Ec stammen veelbelovend is voor de bruikbaarheid van klinische samples in toekomstige moleculaire surveillance van ESBL-Ec. We vonden geen duidelijke klinische sample subgroep die er bovenuit sprong qua vergelijkbaarheid, al werden de klinische subgroepen helaas wel dusdanig klein om hier een definitief antwoord op te geven.

In de algemene beschouwing en tevens het laatste hoofdstuk van dit proefschrift (**Hoofdstuk 8**) worden de belangrijkste bevindingen samengevat en wordt beschreven hoe het onderzoek heeft bijgedragen aan de bestaande literatuur over ziekenhuissurveillance van antibioticaresistente Gram-negatieve bacteriën. Daarnaast wordt uitgebreid besproken welke beperkingen er zaten aan ons onderzoek, welke conclusies we konden trekken en wat dit betekent voor de praktijk. Tot slot worden er per hoofdstuk aanbevelingen gedaan voor toekomstig wetenschappelijk onderzoek.

CONTRIBUTING AUTHORS

Hetty Blok
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Edwin Boel
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Marc Bonten
Julius Center for Health Sciences and Primary Care; Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Thijs Bosch
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Patricia Bruijning-Verhagen
Julius Center for Health Sciences and Primary Care, UMC Utrecht, Utrecht University, Utrecht, The Netherlands
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Ad Fluit
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Sabine de Greeff
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Axel Janssen
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Jan Kluytmans
Julius Center for Health Sciences and Primary Care, UMC Utrecht, Utrecht University, Utrecht, The Netherlands
Microvida Laboratory for Medical Microbiology and Department of Infection Control, Amphia Hospital, Breda, The Netherlands

Evelien Oostdijk
Department of Intensive Care Medicine, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Nienke Plantinga
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Rob Rentenaar
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Ascelijn Reuland
Saltro Diagnostic Center for Primary Care, Utrecht, The Netherlands
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Anita Schürch
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Anne Marie de Smet
Department of Critical Care, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Janetta Top
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Annet Troelstra
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Annemarie van 't Veen
Saltro Diagnostic Center for Primary Care, Utrecht, The Netherlands
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Tess Verschuuren
Julius Center for Health Sciences and Primary Care, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Judith Vlooswijk
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Henri van Werkhoven
Julius Center for Health Sciences and Primary Care, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Rob Willems
Department of Medical Microbiology, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Ardine de Wit
Julius Center for Health Sciences and Primary Care, UMC Utrecht, Utrecht University, Utrecht, The Netherlands
Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

ACKNOWLEDGEMENTS

DANKWOORD

Het is een bijzondere tijd om een promotietraject af te ronden. Ik leg de laatste hand aan dit dankwoord vanuit huis. Het nieuwe coronavirus, SARS-CoV-2, veroorzaakt momenteel een pandemie en er zijn verregaande maatregelen genomen om verspreiding te controleren. Ik heb dit promotieonderzoek verricht bij de afdeling infectieziekten epidemiologie. Mijn supervisoren, collega's en vele anderen met wie ik heb samengewerkt, zijn momenteel ontzettend hard aan het werk om ons door deze periode heen te helpen. Ik wil daar allereerst mijn grote bewondering voor uitspreken.

Wat betreft dit boekje wil ik een heleboel mensen bedanken die op de een of andere manier hebben meegewerkt aan het tot stand komen van dit proefschrift. Sommige mensen proberen kort en bondig te zijn bij hun dankwoord. Maar diegenen die me een beetje kennen, weten dat dit voor mij een vrij onmogelijke opgave is. Vandaar mijn advies om een kopje koffie te pakken en er even voor te gaan zitten.

Prof. dr. M.J.M. Bonten, beste Marc. Hartelijk dank voor deze mogelijkheid en je begeleiding als promotor. Promoveren bij jou staat voor een groot deel in het teken van vrijheid en mogelijkheden en dat heb ik altijd zeer gewaardeerd. Daarnaast wist jij op de juiste momenten knopen door te hakken en te motiveren. Bedankt daarvoor.

Prof. dr. J.A.J.W. Kluytmans, beste Jan. Het is bijzonder hoe jij jouw voorliefde voor epidemiologie, infectiepreventie en patiëntenzorg weet te combineren. Ik weet nog goed dat we het tijdens de ECCMID in Madrid hadden over kleurenprofielen. Bij jouw karakterprofiel horen eigenschappen als gedreven en probleemoplossend, en dat zijn woorden die zeer goed bij jou passen. Toch zie ik ook zeker de stabiliteit en het samenwerken van groen. Het zal jou denk ik niet verbazen dat ik thuis een gele bank heb staan. Eigenlijk is promoveren net als wielrennen een individuele teamsport: heel erg bedankt voor de aanmoediging.

Dr. P.C.J. Bruijning-Verhagen, beste Patricia. Ik kan met alle zekerheid zeggen dat als jij er halverwege niet bij was bijgekomen als copromotor dat mijn boekje nog lang niet af zou zijn. Je kreeg een soort chaotische duizendpoot op je bordje die met van alles tegelijk bezig was maar eigenlijk niks echt afmaakte. Jij hebt me enorm geholpen met prioriteren en structureren. Ik bewonder je eigenschap om elke onderzoeksvraag of analyse opnieuw met een frisse en scherpe blik te kunnen bekijken, met altijd opbouwende feedback. Heel veel dank voor je begeleiding.

Geachte leden van de beoordelingscommissie, prof. dr. ir. M.J.C. Eijkemans, prof. dr. ir. D.J.J. Heederik, prof. dr. P.W.M. Hermans, prof. dr. L.M.O. de Kort en prof. dr. A.M.G.A. de Smet, ik wil u allen hartelijk danken dat u de tijd heeft genomen om dit proefschrift te lezen en te beoordelen.

Een woord van dank aan alle personen met wie ik heb samengewerkt bij de verschillende projecten en die hebben meegeschreven aan de stukken uit dit proefschrift. Beste Anne Marie, Nienke, Henri, Axel, Annet, Hetty, Ascelijn, Ad, Rob Willems, Anita en Thijs: jullie suggesties, toevoegingen en kritische blik zijn erg waardevol geweest. In het bijzonder wil ik graag Ardine benoemen. De kosteneffectiviteitsanalyse was mijn eerste eigen artikel en hierdoor werd mijn interesse nog meer gewekt voor het totaalplaatje van de gezondheidszorg. En ook al bleek het achteraf vooral een papieren functieomschrijving, bedankt voor je bereidheid om deel te nemen in mijn supervisory committee.

Ook nog een specifiek woord van dank aan Rob Rentenaar. Voor het project met de colistineplaten hebben we nauw samengewerkt, ik vond het heel leuk om dat onderzoek samen op te zetten. Daarnaast ken ik maar weinig mensen die zó blij kunnen worden van de ontdekking van een mogelijk nieuw colistine resistentiemechanisme. Je enthousiasme voor de medische microbiologie is heel aanstekelijk.

Beste Kirsten, wat leuk om te merken dat jij je ook graag wilde inzetten voor een goede werkomgeving voor promovendi. Bedankt voor het mede-organiseren van de brainstormsessies over werkbeleving voor de MMB en infectie epi promovendi.

Beste Els, Katja, Eva, en alle dames die ooit onderdeel waren van sec-bonten: ik kan niet in woorden uitdrukken hoe waardevol jullie zijn voor het leven van promovendi. Jullie zijn altijd bereid mee te helpen als er weer eens een en ander geregeld moest worden. Bedankt daarvoor!

Beste Coby en Henk, jullie zijn een waar begrip in het Stratenum en dat is niet voor niets. Jullie zijn altijd vriendelijk en bereid om mee te denken en te helpen bij allerlei praktische zaken. Het Julius Centrum mazzelt maar met jullie. Ook Abdel wil ik bij deze bedanken voor je eindeloze vrolijkheid als wij elkaar tegenkwamen op de gang. Ik denk met plezier terug aan de keren dat je onze kamer hebt ontvangen voor een zomerbarbecue in jouw volkstuin.

Dit dankwoord is niet compleet zonder een woord van dank aan de mensen van COMBACTE. Ook al is het alweer even geleden, het eerste jaar van mijn promotietraject werd ik door jullie

met een warm welkom ontvangen. Nog een apart woord voor Fleur en Darren. Lieve Fleur, jij bent assertief, zorgvuldig en pragmatisch tegelijk en dat maakt jou in mijn ogen een hele goede onderzoeker (en huisarts). Heel leuk om de resultaten van ASPIRE-ICU nu in jouw boekje terug te zien! Lieve Darren, jouw kalmte en mijn chaotische inslag bleken een uitstekende combinatie. Jij bent een integere, harde werker met een buitengewoon groot doorzettingsvermogen. Tegelijkertijd ben je een heel bescheiden persoon en dat siert je enorm. Sinds je het Julius Centrum tijdelijk hebt verlaten mis ik mijn enige echte work husband wel hoor! Bedankt voor je luisterend oor en alle gezelligheid.

Beste infectie epi collega's, beste Wouter, Nienke, Bastiaan, Fleur, Gerrita, Patricia, Stephanie, Henri, Marjolein, Darren, Lufang, Valentijn, Inger, Meander, Tim, Tessa, Fien, Tess, Thijs, Nienke, Meri, Nikki, Emma, Kelly, Janneke, Claudia, Annabel en Thomas: heel erg bedankt voor al jullie inbreng en gezelligheid de afgelopen jaren. De woensdagochtend is een waar ijkpunt midden in de week en een heel goed voorbeeld waar alle elementen van wetenschappelijk onderzoek samenkomen. Discussiëren over studiedesign, bias, ingewikkelde statistische modellen, interpretatie van bevindingen maar ook over allerlei logistieke belemmeringen die je zoal kan tegenkomen (ik denk hier vooral aan multicenter studies). Als iemand mij jaren geleden had verteld dat de XEWMM het format van een weekstart zou aannemen dan had ik waarschijnlijk hard moeten lachen. Dat noemen ze ook wel adaptive design. Helaas is mijn motie om te applaudisseren na de WMM nog steeds niet aangenomen. Ik heb heel erg genoten van de post-WMM koffiemomentjes en alle gezelligheid bij de jaarlijkse ECCMID congressen. Beste Henri, jij bent van onschatbare waarde voor de infectie epi groep. Slim, meedenkend, geduldig en heel collegiaal. Lieve Tess, ik vond het heel leuk om samen het EPIGENEC project op te zetten. Het begon allemaal bij het burrito-overleg in de tuin van Marc en wat volgden waren enkele gezellig ritjes in de Julius mobiel. Ik vraag me wel nog steeds af of het maatschappelijk verantwoord was dat ik een hoop van die bijzonder-resistente *E. coli*'s gewoon mee heb genomen in de lijnbus van het Amphia naar Utrecht. Leuk om te zien dat het project nu zijn vruchten afwerpt! Lieve Tim, wij begonnen zo ongeveer tegelijk aan onze promotie en dat schept een band. Je bent een heel sociale collega; sinds je weg was mistten we in het van Geuns de bulderlach van drie deuren verder heel erg. Hoe leuk dat er iemand was op het werk met wie ik een nieuwe PR op het gebied van een squat clean kon delen. Ik denk overigens dat maar weinig mensen kunnen zeggen dat hun promotie in zekere zin mede mogelijk werd gemaakt door Campina. Door alle omstandigheden sluiten we niet zoals gepland ons promotietraject samen af in het Academiegebouw. Maar we gaan sowieso proosten als dat allemaal weer mogelijk is!

Lieve Diana, Kirsten en Tessa: deze limerick is een autobiografische ode aan onze gezellige avondjes die in het teken stonden van het gesproken woord en poëzie. Opgedragen aan de niet te onderschatten levenswijsheid van Dory uit Finding Nemo ("Just keep swimming").

Zwemdiploma

een vis uit een stad aan de Waal
kreeg in Utrechts water 'n doctoraal
een eigen geluid
zwom nooit achteruit
dus toch niet verzopen in 't academisch kanaal

Lieve kamergenootjes uit het Stratenum en het van Geuns, lieve Tessa, Sabine, Indira, Alies, Gerrita, Roland, Alwin, Josan, Laura, Lisanne, Tim, Katrien, Tess, Inger en Fien. Eigenlijk zijn kamergenootjes de mensen die daadwerkelijk alles meekrijgen tijdens zo'n promotietraject. Van mijlpalen zoals geaccepteerde abstracts, tot flesjes water die uit frustratie tegen de kast worden gegooid. Maar er was vooral ook veel ruimte voor koffiemomentjes en gezelligheid (en niet te vergeten Promovenski met veel legendarische avonden in Club Tenne). Dat ik intens gelukkig word van hysterische kerstversiering werd door jullie allen lieftallig omarmd. Fien, jij bent een van de meest lieve en attente collega's die ik ken. Mensen boffen met een collega zoals jij! Ik hoop dat onze PhD opblaas reddingsboei ooit nog eens van pas komt als mensen vast komen te zitten in het promotiemoeras. Inger, je was een gezellige overbuuf bij onze gezamenlijke laatste loodjes. Tot slot een collectief excuus aan alle diëten die ik in de war heb geschopt de afgelopen jaren met meegenomen baksels.

Lieve geneeskundevriendinnetjes, lieve Anne-Karien, Annelienke, Geerte en Jolanda. Wat bijzonder dat we allemaal uiteindelijk promotieonderzoek zijn gaan doen. Daardoor waren er niet veel woorden nodig om uit te leggen waar je zoal tegenaan kan lopen in de academie. Heel erg bedankt voor alle steun, etentjes en weekendjes weg de afgelopen jaren, die al begonnen tijdens onze studie!

Lieve Linda, wa een leuke stad hejje uutgekoose om te gaan wonen! Inmiddels kennen wij elkaar ook alweer tien jaar. Jij bent niet snel van je stuk te brengen en je nuchtere blik hielp mij meerdere keren relativeren tijdens het schrijven van dit proefschrift. Heel erg bedankt voor alle ontspanning in de vorm van koffietjes, taartjes, etentjes, (nog meer taartjes), borrels, concerten en festivals.

Daarover gesproken: ik heb het geluk om jaarlijks met een bijzonder leuke club mensen blokkenschema's uit te pluizen en kilometers te maken op het festivalterrein. Imke en Bertwin, ik wil ook jullie specifiek nog even noemen: ook al kennen we elkaar nog niet zo lang, we hebben al een hoop memorabele momenten beleefd. Ik kijk uit naar nog veel meer gezelligheid!

Lieve Rian, ik zou eigenlijk wel jouw glimlach willen zien als dit boekje straks bij jou op de mat valt. Heel erg bedankt voor alle inzichten die je me hebt gegeven.

Beste iedereen van Duncan's Training Center (DTC), in het bijzonder Ellen, alle uurtjes crossfit bleken een uitstekende reset knop tussen het schrijven van dit boekje door. Eigenlijk lijken crossfit en promoveren best wel op elkaar: niet teveel aan jezelf twijfelen en blijven doorzetten. Bedankt voor de steun en vooral gezelligheid altijd!

Beste Wendy, bedankt voor al je wijze lessen over zonsondergangen en wiskundige problemen. Zonder de vele kopjes thee was er denk ik geen proefschrift geweest.

Beste Hettie, bedankt voor je doortastende adviezen en hulp bij de laatste loodjes van dit proefschrift.

Lieve Marjan, Janine en Marlies. Ik vind het altijd ontzettend fijn om jullie te zien. Iets met genen denk ik ;-) Jullie zijn echte schatten en zijn altijd oprecht benieuwd hoe het gaat. Pisti, Marcel en Rob, ook jullie heel erg bedankt voor jullie nieuwsgierigheid en interesse altijd voor mijn onderzoek. Lieve Casper, Stijn en Rens: ik hoop dat deze tante jullie trots kan maken met dit boekje!

Lieve Balraz, heel erg bedankt voor al je betrokkenheid, humor en alle gezellige momenten als afleiding van het onderzoek.

Wat een fijne schoonfamilie heb ik erbij gekregen. Cher Pierre et chère Amber, merci beaucoup pour votre toujours présente intérêt de ma thèse. Lieve Yvonne en Rob, jullie hebben bijzonder warme en attente karakters. Bedankt voor jullie betrokkenheid bij alles. Daniel, Rolinka, Luc en Zoé: yes nu nog meer tijd voor gezellige avonden met nieuwe spelletjes! Moge ze te allen tijde helder en duidelijk - zonder interrupties - worden uitgelegd.

Lieve papa, jij hebt me van jongs af aan vrij gelaten in het maken van mijn eigen keuzes en had er altijd vertrouwen in dat het wel goed zou komen. Ik prijs mezelf ontzettend gelukkig dat ik weet dat ik altijd thuis terecht kan (en dat je dan waarschijnlijk ook al een grote pan met eten klaar hebt staan). Lieve mama, jouw warmte en zorgzaamheid kenmerken jou. Bedankt voor je goede adviezen dat het ook belangrijk is om naar gevoel te luisteren en niet alleen naar ons verstand.

Lieve Fee. Onze vriendschap begon stiekem al op de kraamafdeling van het Radboud ziekenhuis, toen we op dezelfde dag geboren werden. Toch kwamen we elkaar pas weer tegen in de brugklas. Inmiddels zijn we vele jaren verder en spelen we niet alleen meer om de beurt snake op de Nokia, maar doen we ook dansjes bij de silent disco in de Hermitage tijdens Museumnacht.Jij staat altijd voor iedereen klaar en dat is echt enorm bewonderenswaardig. Lieve Axel boft met een moeder zoals jij!

Lieve Anne. Inmiddels zijn ook wij alweer achttien jaar vriendinnen. De tijd vliegt! Op de middelbare school hielden we al lange Lord of the Rings marathons. Ik realiseer me nu pas dat de stap naar wetenschappelijke data files niet eens zo groot was, gezien de uitgeprinte en van kleurcode voorziene film scripts. Wat hebben wij eigenlijk niet gemeen, zelfs wat betreft dagelijkse mojito's in Nicaragua zitten we op één lijn. Soms heb ik zelfs het idee dat jij me beter kent dan ikzelf. Zo nam jij me mee naar Lowlands en dat was meteen liefde op het eerste gezicht (hetgeen jij al lang van tevoren wist). Tijdens de pieken en dalen van een promotietraject is het heel fijn om zo'n hechte en evenwichtige vriendin te hebben als jij. Hopelijk ben je trots dat ook ik nu aan de lopende band Ivo Niehe momentjes heb (wat heb ik dit proefschrift geweldig geschreven eigenlijk). Ik kijk uit naar nog vele jaren vriendschap en nog heel veel meer gezelligheid samen met jou en Joris (a.k.a. Gianni).

En dan mijn paranimfen. Lieve Tessa, wij begonnen op precies dezelfde dag aan onze promotie, alsof het zo moest zijn. Ook al zagen we elkaar de hele week op werk, toch gingen we daarnaast weekendjes weg, bezochten we feestjes tussendoor en volgden we een avondcursus Spaans. In de tijd dat ik nog dacht dat hardlopen ook iets voor mij was, liepen we zelfs samen de Singelloop. Voor het eerst kwam ik iemand tegen met dezelfde grote voorliefde voor het RTL Ontbijtnieuws. Dit resulteerde in veel appjes heen en weer op de vroege ochtend met onze verwonderingen over het non-nieuws van die dag. Gelukkig was het nieuwe format - waarin de koffiemok van Jan de Hoop niet langer werd gematcht met de kleur van zijn trui - weer snel voorbij. Wat ik aan jou bewonder is hoe je me altijd helpt om mijn eigen keuzes te durven maken. En je scherpe humor maakte mijn dag méér dan eens weer goed. Was het jouw of mijn idee om gevleugelde uitspraken van coauteurs of supervisors op een tegeltje uit te printen, op te hangen als tegeltjeswijsheid, en deze collectie vervolgens om te dopen tot the Wall of Pain? Ik was met heel veel trots jouw paranimf, en ik ben heel blij dat jij nu die van mij bent.

Lieve Dees, waar zal ik eens beginnen. Iemand omschreef onze band ooit als die van tweelingzussen en zo voelt dat ook echt. Al mijn hele leven zorg je voor je kleine zusje en daar ben ik je heel erg dankbaar voor. Ik hoop dat ik dat inmiddels ook wat meer kan terugdoen. Ik heb nu skills waar ook jij veel aan hebt! Ik kan nu bijvoorbeeld uitrekenen hoe groot de associatie is tussen exposure aan Disney en hoeveelheid gelukshormoon (héél groot). Ik denk dat een vergelijking met de

onafscheidelijke Knabbel en Babbel dan ook wel op zijn plaats is (maar dan de beukennootjes vervangen door toetjes en cocktails). Jouw tomeloze enthousiasme en gedrevenheid om alles uit het leven te halen inspireren mij elke dag. Het is moeilijk in woorden te omschrijven hoe blij en trots ik ben dat jij mijn zus bent. Heel erg bedankt dat je aan mijn zijde wilt staan als paranimf.

Lieve Stéphane. Ik kan wel zeggen dat het op jouw schouders klimmen bij het concert van Tash Sultana tijdens Down the Rabbit Hole een van mijn beste beslissingen ooit was. Bleek ik gewoon op de schouders te zitten van een van de leukste mensen óóit. Ik denk stiekem dat jij van iedereen het meeste geratel hebt moeten aanhoren over het afronden van dit proefschrift. Dankjewel lief, voor jouw altijd aanwezige vertrouwen en relativeringsvermogen. Ik kan er lang of kort over praten, maar samen met jou is het leven gewoon een nóg groter feest. Op naar nog heel veel meer avonturen samen.

ABOUT THE AUTHOR

ABOUT THE AUTHOR

Denise van Hout was born on December 1st 1989, in Nijmegen. She grew up in Nijmegen where she followed secondary education at the Dominicus College, from which she graduated in 2008. Subsequently, she studied Medicine at Utrecht University. She developed a special interest for the Dutch healthcare system which resulted in a senior internship in healthcare management. She also enjoyed combining her medical education with working at Starbucks. After obtaining her medical degree in 2015, Denise took up a PhD position in the field of infectious diseases epidemiology at the Julius Center for Health Science and Primary care (University Medical Center Utrecht) under supervision of Prof. dr. Marc Bonten and Prof. dr. Jan Kluytmans and later also dr. Patricia Bruijning-Verhagen. During the first year she was part of the European COMBACTE consortium and helped set up an international prospective cohort study. She was also member of the NVMM working group that developed clinical guidelines for the treatment of *S. aureus* bacteraemia. After approximately one year she started focusing on Gram-negative infections, of which the work resulted in this thesis. Besides her work as a PhD candidate, she assisted in teaching of epidemiology to medical students, was chairman of the Junior Researchers Meeting (JOB) of the Julius Center and set up several activities to strengthen the working environment of junior researchers. In 2019 she completed the postgraduate Epidemiology master, specializing in clinical epidemiology. As of March 2020, she started working for the EU funded project Rapid European COVID-19 Emergency Research response (RECOVER).

LIST OF PUBLICATIONS

LIST OF PUBLICATIONS

Troeman DPR, **van Hout D**, Kluytmans JAJW. Antimicrobial approaches in the prevention of *Staphylococcus aureus* infections: a review. *J Antimicrob Chemother.* 2019;74(2):281–294. doi:10.1093/jac/dky421

van Hout D, Plantinga NL, Bruijning-Verhagen PC, *et al.* Cost-effectiveness of selective digestive decontamination (SDD) versus selective oropharyngeal decontamination (SOD) in intensive care units with low levels of antimicrobial resistance: an individual patient data meta-analysis. *BMJ Open*. 2019;9(9):e028876. doi:10.1136/bmjopen-2018-028876

van Hout D, Janssen AB, Rentenaar RJ, Vlooswijk JPM, Boel CHE, Bonten MJM. The added value of the selective SuperPolymyxin™ medium in detecting rectal carriage of Gram-negative bacteria with acquired colistin resistance in intensive care unit patients receiving selective digestive decontamination. *Eur J Clin Microbiol Infect* Dis. 2019;39(2):265-271. doi:10.1007/s10096-019-03718-5

van Hout D, Verschuuren TD, Bruijning-Verhagen PCJ, *et al.* Extended-spectrum beta-lactamase (ESBL)-producing and non-ESBL-producing *Escherichia coli* isolates causing bacteremia in the Netherlands (2014 - 2016) differ in clonal distribution, antimicrobial resistance gene and virulence gene content. *PLoS One.* 2020;15(1):e0227604. doi:10.1371/journal.pone.0227604