Community-Onset Bacteraemia and Urinary Tract Infection with Excended-Spectrum - Lactamse-Producing Escherichia Coli and Klebsiella Pneumoniae

risk and prognosis in a population-based study

Richelsen, Rasmus Broge

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

COMMUNITY-ONSET BACTERAEMIA AND URINARY TRACT INFECTION WITH EXTENDED-SPECTRUM β-LACTAMSE-PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE: RISK AND PROGNOSIS IN A POPULATION-BASED STUDY

BY
RASMUS KAPALU BROGE RICHELSEN

DISSERTATION SUBMITTED 2020

AALBORG UNIVERSITY
DENMARK

COMMUNITY-ONSET BACTERAEMIA AND URINARY TRACT INFECTION WITH EXTENDED-SPECTRUM β-LACTAMSE-PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE:* RISK AND PROGNOSIS IN A POPULATION-BASED STUDY

by

Rasmus Kapalu Broge Richelsen

AALBORG UNIVERSITY
DENMARK

Dissertation submitted September 2020

.

Dissertation submitted: 18 Sept. 2020

PhD supervisor: Henrik Nielsen, MD, DMSc, professor
Department of Infectious Diseases
Aalborg University Hospital, Aalborg, Denmark

Assistant PhD supervisors: Henrik Carl Schønheyder, MD, DMSc, professor
Department of Clinical Microbiology,
Aalborg University Hospital, Aalborg, Denmark

Jesper Smit, MD, PhD
Department of Infectious Diseases,
Aalborg University Hospital, Aalborg, Denmark

Collaborators: Jesús Rodríguez-Bãno, MD, PhD, professor
Department of Infectious Diseases,
Hospital Universitario Virgen Macarena, Sevilla, Spain

PhD committee: Clinical Professor Lene Dreyer (chair)
Aalborg University

Associate Professor Pontus Naucler
Karolinska Institutet

Professor Niels Frimodt-Møller
Rigshospitalet

PhD Series: Faculty of Medicine, Aalborg University

Department: Department of Clinical Medicine

ISSN (online): 2246-1302
ISBN (online): 978-87-7210-813-1

Published by:
Aalborg University Press
Kroghstræde 3
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Rosendahls, 2020

CV

Rasmus Kapalu Broge Richelsen, and his identical twin brother Christian, were born in Zambezi, Zambia on 4 August 1983, where his father and mother were employed from 1981 to 1984 at ActionAid Denmark ("Mellemfolkeligt Samvirke"), working as a doctor and a physiotherapist, respectively. He graduated from Secondary School in 2002; and having acquired a Bachelor's Degree in medical engineering, he began his medical studies at Aarhus University in 2007. He graduated as a medical doctor in January 2014 and subsequently completed 12 months of internship in the Central Denmark Region. This position was followed by a six-month internship as a general practitioner. Next, he moved to the North Denmark Region, where he worked as a resident at the Department of Rheumatology and the Department of Infectious Diseases at Aalborg University Hospital. As his interest into infectious diseases grew stronger, he extended the work as a resident by six months at which time his interest in and preparation for conducting a PhD study with Professor Henrik Nielsen took off.

His first scientific work was in the area of cardiology where he had a four-month employment period as a research assistant during medical school. While working as a resident at the Department of Infectious Disease, Aalborg University Hospital, and while being supervised by Professor Henrik Nielsen, he did his first infectious disease project about acyclovir-induced nephrotoxicity based upon a review of nearly 1,000 medical records. He found the work and collaboration inspiring; and in June 2017, he initiated his PhD project on the epidemiology of extended-spectrum β-lactamase in the North Denmark Region at the Department of Clinical Medicine, Aalborg University with Professor Henrik Nielsen as his main supervisor.

He is engaged to Louise Hill-Madsen, with whom he has an almost two year-old-son, Carl Johan.

ENGLISH SUMMARY

Community-onset infections with extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* are increasing throughout the world, including in our region where the prevalence of antimicrobial resistance is traditionally low. Epidemiological data remain the backbone of monitoring temporal disease dynamics, yet current knowledge on community-onset ESBL-producing infections mainly arises from high-endemic countries with different antimicrobial resistance patterns and healthcare settings, making interpretation and generalization to our region difficult.

Therefore, to extend upon current knowledge, **the aims** of the present thesis were to 1) elucidate temporal changes in the incidence of community-onset ESBL-producing *E. coli* and *K. pneumoniae* infections from 2007-2017 in the North Denmark Region, 2) assess risk factors associated with community-onset ESBL-producing bacteraemia, 3) examine the influence of the chosen methodology on its association with exposure to antibiotics as a risk factor of ESBL-producing bacteraemia, and 4) to investigate the impact of ESBL production on mortality and length of hospital stay (LOS).

The thesis is based on a descriptive cohort study, two case-control-control studies, and a cohort study. Microbiological data was acquired from the laboratory information system at the Department of Clinical Microbiology, Aalborg University Hospital. Using the unique Danish civil registration number, we linked this data and obtained information from the following population-based registries; the Danish Civil Registration System (CRS), the North Denmark Bacteremia Research Database (NDBRD), the Danish National Patient Registry (DNPR) and the Danish National Prescription Registry (DNPR*).

S**tudy I** included 3,741 episodes of community-onset ESBL *E. coli* or *K. pneumoniae*. In this study, we demonstrated an increase in community-onset ESBL-producing *E. coli* and *K. pneumoniae* infections from 7.5 to 105 per 100,000 person years from 2007 to 2017. This increase was driven primarily by an increase in *E. coli* urinary tract infection, of which a growing part became community-acquired rather than healthcare-associated during the study period. In **Study II,** including 223 patients with community-onset ESBL *E. coli* or *K. pneumoniae* bacteraemia matched with 2,214 non-ESBL controls and 2,228 population controls, we found that recent and numerous hospitalization and antibiotic exposure, especially use of fluoroquinolones (adjusted odds ratio (aOR) 3.56 [95% confidence interval (CI); 2.52-5.05]), inferred the highest risk compared with non-ESBL controls, hereby confirming that traditional risk factors also pertain to our region of low antimicrobial resistance. Several of the risk factors were merely associated with bacteraemia. In **Study III,** using the same cases as in Study II, we showed that the impact of the chosen methodology varied across special antibiotics and antibiotic classes. We also demonstrated that the association between non-β-lactam antibiotics (e.g. fluoroquinolone) and ESBL production was particularly prone to confounding by indication, and that a shortening of antibiotic exposure

generally increased the associated ORs. Finally, in **Study IV,** we were not able to demonstrate an excessed mortality associated with ESBL production in first-time community-onset *E. coli* or *K. pneumonia* bacteraemia or urinary tract infection. Thus, the 30-day mortality was 15.8% (95% CI; 11.3-21.8) for ESBL *E. coli* bacteraemia (n=190) and 14.0% (95% CI; 12.9-15.2) for non-ESBL *E. coli* bacteraemia (n=3,641); likewise, the 30-day mortality for *E. coli* urinary tract infection was 9.5% (95% CI; 7.5-12.1) compared with 8.7% (95% CI; 8.3-9.2) for ESBL (n=634) and non-ESBL (n=16,517), respectively. Healthcare-associated infection, comorbidity, age, polybacteraemia and a non-urinary tract focus of infection appeared to be predictors of death. Still, ESBL *E. coli* bacteraemia seemed to be associated with an increased LOS.

In conclusion, we confirmed an increasing incidence of community-onset ESBL *E. coli* and *K. pneumoniae* incidence from 2007 to 2017, and temporal dynamics in risk factors supported a dissemination of ESBL into the community. We confirmed that traditional risk factors like numerous and recent hospitalization and exposure to antibiotics characterized our low-ESBL-prevalence region and demonstrated the impact of control group selection and certain in/exclusion criteria on the exposure to antibiotics as a risk factor. Interestingly, mortality in first-time community-onset *E. coli* or *K. pneumoniae* infections was virtually unaffected by ESBL production in our cohort.

DANSK RESUME

Samfundserhvervet infektion med extended-spectrum β-lactamase (ESBL)-producerende *Escherichia coli* og *Klebsiella pneumoniae* er stigende i antal i hele verden, og også i vores område, hvor prævalensen af antibiotikaresistent traditionelt er lav. Epidemiologiske data er en af hjørnestenene i monitorering af sygdomsudvikling i samfundet. Til trods herfor stammer vores viden om samfundserhvervet ESBL-producerende infektion primært fra høj-endemiske områder med en anden antibiotika resistens profil og opbygning af sundhedsvæsenet end i Danmark, hvilket vanskeliggør fortolkning og generalisering af disse studier til vores område.

Formålet med denne afhandling er derfor at udvide vores viden på området ved 1) at belyse udviklingen i samfundserhvervede infektioner med ESBL *E. coli* og *K. pneumoniae* fra 2007 til 2017 i Nordjylland, 2) at undersøge risikofaktorer for ESBL *E. coli* bakteriæmi, 3) at undersøge hvordan valget af analysemetode influerer på resultatet af antibiotikaforbrug som en risikofaktor for at udvikle ESBL infektioner, og 4) at undersøge betydningen af ESBL-produktion for dødeligheden og længden af hospitalsindlæggelse.

Denne **afhandling bygger på** to kohortestudier og to case-control-studier. Det mikrobiologiske laboratorie system på Klinisk Mikrobiologisk Afdeling, Aalborg Universitetshospital, dannede grundlag for vores kohorte, og ved hjælp af det unikke cpr-nummer koblede vi disse data med date fra CPR-Registeret, den Nordjyske Bakteriæmidatabase, Landspatientregisteret og Lægemiddeldatabasen.

I **studie I** inkluderede vi 3,741 episoder af samfundserhvervet ESBL *E. coli*- eller *K. pneumoniae*-infektion, og påviste en stigning i forekomsten heraf fra 7.5 til 105.0 per 100.000 person-år, hvilket hovedsageligt var drevet af en øget forekomst af *E. coli* urinvejsinfektioner, hvoraf de rent samfundserhvervede (dvs. ”community-acquired”) infektioner blev mere hyppige end de sundhedsrelaterede (”healtcare-associated”) infektioner op igennem studieperioden. I **studie II** inkluderede vi 223 patienter med en førstegangs-samfundserhvervet ESBL *E. coli*- eller *K. pneumoniae*-bakteriæmi matchet med 2.214 non-ESBL kontroller og 2.228 populationskontroller. Her fandt vi, at hyppig og nylig hospitalskontakt og antibiotika forbrug, i særdeleshed brug af fluoroquinolon (aOR 3.56 [95% CI; 2.52-5.05]), var risikofaktorer for ESBL bakteriæmi, når man sammenlignede med non-ESBL-kontrollerne. Talrige risikofaktorer var associeret til risikoen for blot bakteriæmi sammenholdt med populationskontrollerne. I **studie III** tog vi udgangspunkt i den samme population som i studie 2 og viste, hvordan valget af analysemetode påvirkede estimaterne forskelligt for de forskellige antibiotika og antibiotikaklasser. Herunder demonstrerede vi, hvordan sammenhængen imellem brug af non-β-lactam antibiotika (f.eks. fluoroquinolone) og udviklingen af ESBL-infektion var særligt sårbar over for ”confounding by indication”, og hvordan en forkortelse af perioden for antibiotika forbrug generelt øgede associationen imellem antibiotika forbrug og risikoen for

ESBL-infektion. Slutteligt kunne vi i **studie IV** ikke påvise en overdødelighed ved ESBL-produktion i samfundserhvervede *E. coli* eller *K. pneumoniae* bakteriæmi eller urinvejsinfektioner, idet vi fandt en 30-dagesmortalitet for ESBL *E. coli*-bakteriæmi (n=190) på 15.8% (95% CI; 11.3.-21.8) sammenholdt med 14.0% (95% CI; 12.9-15.2) for non-ESBL *E. coli* bakteriæmi (n=3,641), og ligeledes for *E. coli*-urinvejsinfektion, dvs.. 9.5% (95% CI; 7.5-12.1) sammenholdt med 8.7% (95% CI; 8.3-9.2) for henholdsvis ESBL (n=634) og non-ESBL (n=16,517). Sundhedsrelateret infektion, komorbiditet, alder, polybakteriæmi, og et ikke-urinvejsrelateret infektionsfokus syntes at være associeret med død. ESBL *E. coli*-bakteriæmi var dog associeret med en længere indlæggelse på hospital.

Sammenfattende bekræftede vi en kraftig stigning i incidensen af samfundserhvervet ESBL *E. coli*- og *K. pneumoniae*-infektion fra 2007 to 2017, og dette understøttes af udviklingen i risikofaktorer, der også viser en udbredelse af ESBL i samfundet. Vi bekræftede, at traditionelle risikofaktorer som hyppig og nylig hospitalskontakt og antibiotikaforbrug, også var risikofaktorer i vores lavendemiske ESBL-region, og vi demonstrerede hvordan valget af kontrol gruppe og specifikke in/exclusions kriterier påvirkede resultatet af antibiotika forbrug som en risikofaktor for ESBL-bakteriæmi. Til vores undren var dødeligheden af samfundserhvervet *E. coli*- og *K. pneumoniae*-infektion i vores kohorte tilsyneladende uafhængig af, om bakterien producerede ESBL.

ACKNOWLEDGEMENTS

The work presented in this thesis was carried out during my employment as a PhD student at the Department of Clinical Medicine, Aalborg University, Denmark, and in affiliation with the Department of Infectious Diseases, Aalborg University Hospital, 2017-2020.

I am profoundly grateful to a number of persons who made this thesis possible. First of all, I would like to thank my supervisors; Henrik Nielsen for introducing me to the field of infectious disease research, for believing in me, and for your never-ending support and positive attitude towards my numerous ideas and proposals; Henrik Schønheyder for the support and your enthusiastical and meticulous feedback; Jesper Smit for teaching me scientific writing, and for enthusiastic and impatient sharing of your epidemiological knowledge and for being the go-to-guy when the going got rough.

Special thanks to Pavithra Laxsen Anru and Kim Oren Gradel for significant contributions to this project, including your programming skills and data management support; also to Martin Nygård Johansen for discussions and help on statistical matters. A great thanks to Jesús Rodríguez-Bãno and Belen Gutiérrez-Gutiérrez for boarding this project right away, for enthusiastic and fruitful discussions and for generously sharing your experience with ESBL Gram-negative bacteria. Thank you for your hospitality in Seville and for opening your homes for my family. Finally, a big thanks to everyone in the "Caracola", especially Reinaldo, Natalia and Luisa for welcoming me and for supportive coffee drinking served by the most dedicated barista in Seville, Antonio Quijote.

I would like to thank all my friends and colleagues at the Department of Infectious Disease and the Department of Clinical Microbiology, Aalborg University Hospital; Freddy Nielsen for impatiently helping me out with the identification of ESBL producers; Hans Linde, David Fuglsang-Damgaard, Marc Trunjer Kusk Nielsen, Kirstine Kobberøe Søgaard, Frederikke Dam Larsen and Erik Otte for support and discussions on microbiological issues; and the AIR (Aalborg Infection Research) group including Jacob Bodilsen, Viggo Holten, Marta Aagard, Theis Mariager, Michael Dalager-Pedersen, Karina Frahm Kirk, Sandra Ovesen and Hannah Nielsen for great discussions, advice, encouragement and countless cups of coffee.

I would also like to express my gratitude to all my friends and family for everyday help and support. Most of all, I would like to thank my beloved partner in life Louise Hill-Madsen and our son Carl Johan for your never-ending support, patience and love – and especially Carl Johan for carrying my computer around the house encouraging me to "Arbie" ("work") when I am trying to relax.

Rasmus Richelsen, September 2020

Thesis papers

I. Richelsen R, Smit J, Anru PL, Schønheyder HC, Nielsen H. Incidence of community-onset extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae infections: an 11-year population-based study in Denmark. Infect. Dis. (Auckl). 2020;52:547–56.

II. Richelsen R, Smit J, Anru PL, Schønheyder HC, Nielsen H. Risk factors of community-onset extended-spectrum β-lactamase Escherichia coli and Klebsiella pneumoniae bacteraemia: an 11-year population-based case-control-control study in Denmark. Clin. Microbiol. Infect. 2020 [In press].

III. Richelsen R, Smit J, Nielsen H. Impact of chosen methodology when evaluating antibiotic exposure as a risk factor of community-onset extended-spectrum β-lactamase-producing *Escherichia coli* or *Klebsiella pneumoniae* bacteraemia: A population-based Danish case-control-control study. Int J Antimicrobial Agents [submitted].

IV. Richelsen R, Smit J, Schønheyder HC, Laxsen Anru P, Gutiérrez-Gutiérrez B, Rodríguez-Bãno J, Nielsen H. Outcome of community-onset ESBL-producing Escherichia coli and Klebsiella pneumoniae bacteraemia and urinary tract infection: a population-based cohort study in Denmark. J. Antimicrob. Chemother. 2020 [In press].

Abbreviations

CI: Confidence interval

CPR: Civil personal registration

CRS: Civil Registration System

DNPR: Danish National Patient Registry

DNPR*: Danish National Prescription Registry

ESBL: Extended-spectrum β-lactamase

EUCAST: European Committee on Antimicrobial Susceptibility Testing

GCR: Generation cephalosporin-resistant

HCA: Healthcare-associated

LOS: Length of hospital stay

MRR: Mortality rate ratio

NDBRD: North Denmark Bacteremia Research Database

OR: Odds ratio

TABLE OF CONTENTS

CHAPTER 1. THESIS OUTLINE

Concern over antibiotic resistance is growing, and the World Health Organization has named antibiotic resistance as one of the biggest threats to global health. Bacteria producing extended-spectrum β-lactamases (ESBLs) are becoming more prevalent in Denmark, and *Escherichia coli* and *Klebsiella pneumoniae* account for most of the isolates. Different risk factors have been associated with the current rise in ESBL-producing infections, including an increasing elderly population with frequent comorbidities, inappropriate prescribing practices and overuse of antibiotics, international travelling and global distribution of contaminated foodstuffs. Infection with ESBL-producing bacteria has been associated with a worse prognosis and places a considerable financial burden on the health care system. Nevertheless, data elucidating risk factors and prognosis of community-onset ESBL-producing *E. coli* and *K. pneumoniae* infections from low-prevalence countries like Denmark is scarce. This information is important to improve our knowledge on these resistant bacteria and their relation to infections in humans. Moreover, such information contributes to better interventions that may serve to prevent antibiotic resistance, improve empirical antibiotic therapy and better the prognosis of patients suffering from these infections. Therefore, by use of medical databases and population-based registries, we conducted this PhD project to investigate the increase in community-onset ESBL *E. coli* and *K. pneumoniae* infections, to elucidate risk factors of these infections and to investigate how infections with ESBL-producing isolates influence the prognosis.

The thesis is based upon four papers, which are referred to in the text by their Roman numerals (I-IV)[1–4]. The first paper is a descriptive study highlighting the increase in community-onset ESBL *E. coli* and *K. pneumoniae* infections in the North Denmark Region from 2007 to 2017.[1] Study II is an exploratory study investigating risk factors of bacteraemia with ESBL *E. coli* and *K. pneumoniae*.[2] Study III is a methodological study investigating the impact of the chosen methodology, when examining antibiotic use as a risk factor of ESBL-producing *E. coli* and *K. pneumoniae* bacteraemia.[3] Finally, Study IV ascertains the prognostic impact of infections with these resistant bacteria.[4]

The background material (Chapter 2) gives a short introduction into ESBLs and their epidemiology, emphasizing the shift from nosocomial to community-onset infections with a focus on *E. coli* and *K. pneumoniae* bacteraemia and urinary tract infections, including a literature review in relation to the aims of the thesis. The following chapters summarizes the methods used (Chapter 3) and the results obtained (Chapter 4), while Chapter 5 discusses the main findings in relation to the current knowledge, methodological considerations and ends with a conclusion leading to clinical implications and future perspectives (Chapter 6). References and appendices including the full versions of the four studies conclude this thesis.

CHAPTER 2. BACKGROUND

2.1. INTRODUCTION TO ANTIBIOTIC RESISTANCE

"The question arises. Where do these penicillin resistant strains come from? …

… in any hospital using large quantities of penicillin (and what hospital is not nowadays?) bacteria resistant to its action are probably increasing at the expense of those that are sensitive, and it seems not impossible that in time the resistant organisms will be the sole survivors", Mary Barber, 1947.[5]

Antibiotic resistance is almost as old as the remarkable discovery of penicillin by sir Alexander Fleming in 1929.[6] Thus, not long after the introduction of penicillin into clinical care, Mary Barber[5] predicted a worrisome future for antimicrobial resistance, which indeed turned out to be the Achilles heel of antibiotics in the years to come. In fact, any introduction of new antibiotics has been followed by clinically significant resistance to that antibiotic in a few years [7]. And not only did the worrisome future predicted by Mary Barber preclude to the hospitals; indeed, antibiotic resistance is becoming of increasing concern in the community outside the hospitals as well. Thus, in 2017, 3rd-generation cephalosporin-resistant (GCR) Enterobacteriaceae were ranked a "priority 1 critical pathogen" by the World Health Organization,[8] and it was modelled that 3rd-GCR *E. coli* and *K. pneumoniae* attributed nearly 13,000 deaths in the EU and the European Economic Area in 2015.[9] The vast majority of 3rd-GCR pathogens are due to ESBL-producing *E. coli* and *K. pneumoniae*, of which an increasing proportion is acquired in the community.[10]

Finally, while the deployment of new antibiotics accelerated from the 40s to the 70s, the development of new antibiotics has lagged far behind the evolution of resistance in the recent decades…

"Given the current gap between our ability to develop novel antibiotics and the real need for such drugs, the threat of a postantibiotic era is looming large on the horizon", Anne E. Clatworthy et al., 2007 [7]

2.2. E. COLI AND *K. PNEUMONIAE*

E. coli and *K. pneumoniae* might be harmless commensals of the human gastrointestinal tract. Nevertheless, they are also the two major Gram-negative bacteria, causing a broad range of clinical diseases from simple cystitis to life-threatening sepsis. *E. coli* is by far the most frequent cause of urinary tract infections,[11]

accounting for around 79% of cases of acute cystitis[12] and 70-80% of cases of acute pyelonephritis,[13] while *K. pneumoniae* accounts for around 2-10% of urinary tract infections depending on age and gender.[12,13] In addition, *E. coli* is the main aerobic bacteria in intraabdominal infections.[11] If a urinary tract infection is not contained, it might progress to bacteraemia. *E. coli* is the leading cause of bloodstream infections in the industrialized world,[14] accounting for 22-25% of bloodstream infections.[15,16] In population-based studies, *E. coli* bloodstream infections are reported at an incidence of 42.2-47.7 per 100,000 person years.[15,16] Furthermore, in Europe the reported frequency of *E. coli* bacteraemia increased annually by 8.1% from 2002 to 2008.[17]

The majority of *E. coli* bloodstream infections are community-onset infections,[14] i.e. the infection is evident or incubating at the time of hospital admission, usually defined by a positive blood culture obtained within the first 48 hours of admission.[18] Population-based studies focusing explicitly on community-onset *E. coli* bloodstream infections report incidence rates of 23-39 per 100,000 person years from the industrialized world.[19–21] *Klebsiella* species are the fourth most frequent cause of community-onset bloodstream infection with an community-onset incidence of *K. pneumoniae* at 5.2-5.7 per 100,000 person years.[14] In Denmark, the overall incidence of bacteraemia has risen from 114 to 166 per 100,000 person years from 1992 through 2006 with *E. coli* being the leading cause, accounting for roughly one-third of community-onset bacteraemias.[22]

2.3. EXTENDED-SPECTRUM β-LACTAMASES

2.3.1. INTRODUCTION TO β-LACTAMASES

β-lactamases are enzymes produced by diverse bacteria. They have the ability to hydrolyse chemical compounds containing a β-lactam ring, e.g. the β-lactam-containing antibacterial agents, including the penicillins, cephalosporins, carbapenems and monobactam.[23] Bacteria producing β-lactamases have existed for millions of years and have evolved hand in hand under the selective pressure exerted by the naturally occurring β-lactam biosynthesis of neighbouring bacteria.[23] However, the first β-lactamase was "discovered" in 1940 by Abraham and Chain, reporting an enzyme capable of destroying penicillin.[24] Since then, along with the evolving history of antibiotics, dozens of new β-lactamases have been discovered.[23]

2.3.2. DEFINITION OF EXTENDED-SPECTRUM β-LACTAMASES

Extended-spectrum β-lactamases are enzymes produced by certain Gram-negative bacteria capable of hydrolysing the β-lactam ring of β-lactam-antibiotics, rendering

the bacteria resistant to most β-lactam antibiotics.[25] In contrasts to *broad*-spectrum β-lactamases, *extended*-spectrum β-lactamases also confer resistance to higher-generation cephalosporins with an oxyimino sidechain (cefotaxime, ceftazidime, ceftriaxone and cefepime); and hence ESBL-producing bacteria usually shows resistance to penicillins, first-, second- and 3rd-generation cephalosporins and the oxyimino monobactam aztreonam. In addition, the genes transferring the resistance often show considerable co-resistance. This limits the treatment options; however, ESBLs are not able to break down the β-lactam-antibiotic cephamycins (cefoxitin) or carbapenems (e.g. meropenem and ertapenem). ESBLs are inhibited by β-lactamases inhibitors *in vitro*, e.g. clavulanate acid and tazobactam; however, the efficiency of agents containing these substances *in vivo* (piperacillin-tazobactam or amoxicillin-clavulanate) is controversial, and most guidelines therefore do not recommend these substances in the treatment of severe infections.[26–28]

2.3.3. CLASSIFICATION OF ESBL

Traditionally, the classification of β-lactamases has been based on the structure of β-lactamases, i.e. amino acid sequences as proposed by Ambler et al. in 1980.[29] The Ambler classification scheme is useful for taxonomic grouping; however, it is based upon amino acid similarity, and not on phenotypic characteristics, making it less useful in the clinical setting.[25] In 1995, a functional classification scheme of β-lactamases was proposed by Bush, Jacoby and Medeiros.[30] Rather than using the amino acid sequence, they defined the enzymes by their substrate and inhibitory profile. A rapid increase in the genes encoding β-lactamases has been observed since these classification schemes were proposed, and in 2009 Giske et al. proposed a new classification scheme (Figure 1).[26] The aim of this scheme was to redefine ESBLs from a clinical perspective, acknowledging that the current definition of ESBLs was narrow and excluded a wide range of bacteria that, indeed, were resistant to cephalosporins, while reducing the taxonomic complexity. Giske et al. proposed a classification of clinically important β-lactamases into the three categories; $ESBL_A$, $ESBL_M$ and $ESBL_{CARBA}$ and subclasses. These main categories should be sufficient for infection control and clinical use, while subclasses should allow for increased precision among scientists. The $ESBL_A$ enzymes are characterized by non-susceptibility to extended-spectrum cephalosporins and clavulanate synergy.[26] Also, it was recommend to use breakpoints according to the current European Committee on Antimicrobial Susceptibility Testing (EUCAST) strategy, so that isolates were categorized with clinically significant resistance rather than based on enzymatic activity of the involved β-lactamases.[26] In this thesis, we solely considered $ESBL_A$, hereby excluding a minor proportion (3.0%) of plasmid-mediated AmpC.

Figure 1. Classification of $ESBL_{A,}$ $ESBL_{M}$, and $ESBL_{CARBA,}$ as proposed by Giske et al., J. Antimicrob. Chemother, 2009 [26] (reproduced with permission from Oxford University Press).

Acquired β-lactamases with hydrolytic activity against extended-spectrum cephalosporins and/or carbapenems			
β-Lactamase classes	$ESBL_{A}$	$ESBL_{M}$	$ESBL_{CARBA}$
	High prevalent $ESBL_{A}$ CTX-M TEM-ESBLs SHV-ESBLs VEB PER	$ESBL_{M-C}$ (Plasmid-mediated AmpC) CMY FOX MIR MOX DHA LAT BIL ACT ACC	$ESBL_{CARBA-A}$ KPC GES-2, -4, -5, -6, -8 NMC SME IMI-1, -2
	Low prevalent $ESBL_{A}$ GES-1, -3, -7, -9 SFO-1 BES-1 BEL-1 TLA IBC CMT[a]	$ESBL_{M-D}$ (OXA-ESBL) OXA-10-group OXA-13-group OXA-2-group OXA-18 OXA-45	$ESBL_{CARBA-B}$ (MBL) IMP VIM SPM-1 GIM-1 SIM-1 AIM-1
			$ESBL_{CARBA-D}$ (OXA-carbapenemases) OXA-23-group OXA-24-group OXA-48[b] OXA-58-group
Operational definition	Non-susceptibility to extended-spectrum cephalosporins AND clavulanate synergy	Non-susceptibility to extended-spectrum cephalosporins AND phenotypic detection ($ESBL_{M-C}$) OR genotypic detection ($ESBL_{M-D}$)	Non-susceptibility to extended-spectrum cephalosporins and at least one carbapenem AND $ESBL_{CARBA}$ detected with phenotypic and/or genotypic methods

2.3.4. EPIDEMIOLOGY OF ESBL

The first *extended*-spectrum β-lactamases were detected in Germany in 1983 recovered from *K. pneumonia* isolates of patients.[31] Soon after, other SHV- and TEM-type ESBLs were reported, primarily from *Klebsiella* species in French hospitals, and by 1989 ESBLs were reported from France, Argentina, Chile, China, Greece, Switzerland, England, Tunisia and Japan, all found to be correlated with extensive use of cefotaxime.[32,33] The majority of these ESBLs differed from their *broad*-spectrum derivates by only few amino acid sequences, and the resistance was transmissible by plasmid mediation.[33] Meanwhile, in 1989, another ESBL family was discovered in Germany (Munich) and Argentina, followed by Italy and France. This ESBL family was characterized by mainly conferring resistance to cefotaxime rather than ceftazidime, and hence it was named CTX-M type ESBL (for cefotaximase-Munich).[10] Since the millennium, the CTX-M type has become the most widespread ESBL. CTX-M ESBL differed from previous ESBL types by mainly being found in *E. coli* and by extending the spreading from primarily being within the hospitals into

the community.[34–39] In contrast to TEM and SHV ESBL, where ESBL variants arose by mutations of their broad-spectrum TEM and SHV predecessor, the genes encoding the CTX-M family of enzymes likely originated from the chromosome of various species of the genus *Kluyvera.* These genes have been captured by mobile genetic elements and mobilized to *E. coli* plasmids, leading to their rapid dispersal among Enterobacteriaceae.[40] Also, CTX-M-encoding plasmids have been associated with highly successful virulent clones of *E. coli* and *K. pneumoniae*, for example the pandemic *E. coli* ST131 clone identified in 2008,[41,42] further contributing to the rapid dissemination throughout the world.[43]

Currently, five major CTX-M gene families (group 1, 2, 8, 9 and 25) are circulating worldwide, each with several subtypes. The groups differ by >10% amino acid sequences.[43] Different gene families dominate in different places; thus, CTX-M-15 (group 1) is most prevalent in Europe, North America, India and the Middle East; CTX-M-14 (group 9) in China, Southeast Asia and Spain; and CTX-M-2 (group 2) in Israel, Japan and Argentina.[11] In 2017, According to Danish national surveillance data,[44] the most prevalent ESBL enzymes in 3rd-GCR *E. coli* bacteraemias (n=337) were CTX-M-15 (49%), CTX-M-27 (15%) and CTX-M-14 (15%) [44], while 77.4% (48/62) of *K. pneumoniae* isolated from bacteraemias belonged to the $bla_{\text{CTX-M-15}}$ genotype in 2018.[45]

Worldwide

Although great regional differences exist, Asia is the continent reporting the highest prevalence of ESBL-producing bacteria. Recent studies from Cambodia and India report approximately half of the bacteria isolated from blood cultures to be ESBL-producing Enterobacteriaceae.[46,47] A recent meta-analysis examining gut colonization with ESBL-producing organisms among healthy individuals found a prevalence of 46% in the West Pacific, 22% in Southeast Asia, 22% in Africa, 15% in the Eastern Mediterranean, 2% in America and 4% in Europe; and globally the trend was characterized by a 5.4% annual increase.[48]

Europe

In Europe, the number of *E. coli* bacteraemias increased by 8.7% annually from 2002 to 2008, and this increase was mainly driven by antibiotic-resistant strains[17]; however, considerable geographical differences exist in European countries.[25] The European Antimicrobial Resistance Surveillance Network (EARS-Net) monitors resistance among seven major pathogenic bacteria, including *E. coli* and *K. pneumoniae*. Isolates are obtained from blood or cerebrospinal fluid, and the clinical relevance is accordingly indisputable. In general a worrying trend of increasing resistance is observed in Europe, with a north-to-south and west-to-east gradient; the lowest resistance is seen in the northern and western parts of Europe.[49] In 2015, overall 13.1% of all *E. coli* isolates in Europe were resistant to 3rd-generation-cephalosporins. Most of these isolates (88.6%) were ascertained as ESBL-positive. For *K. pneumoniae*,

30.3% of the isolates were 3rd-GCR and 85.3% of these were ascertained as ESBL-positive.[49]

The Nordic countries

In Norway, the proportion of ESBL-producing *E. coli* and *K. pneumoniae* in isolates of clinical relevance increased from 0.6% in 2006 to 4.3% in 2012/2013.[50] In Denmark, 3rd-GCR *E. coli* and *K. pneumoniae* blood isolates rose to a peak in 2009-2011. Hereafter, *E. coli* resistance stagnated and in 2017, 6.7% of *E. coli* isolated from blood was 3rd-GCR. Resistance among *K. pneumoniae* has levelled off since 2009; and in 2017, 7% of the *K. pneumoniae* isolates obtained from blood were 3rd-GCR.[44] These studies do not differentiate between community-onset or hospital-acquired infections. However, an increasing proportion of ESBL-producing bacteria in the community has been documented in a number of cross-sectional studies reporting around 5% of healthy asymptomatic participants to be colonized with ESBL-producing bacteria (Table 1).

Table 1. Colonization with ESBL-producing bacteria in aymptomatic participants.

Author Year	Design Setting Period	Study population Culture types Number included	ESBL proportion, % Dominating genotypes (if reported)
Ny S., et al. [51] **2018**	- **Cross-sectional multinational study** - **Sweden (Stockholm Area) /Finland (Turku and Helsinki Region)** - **2015-2017**	- **"Healthy" asymptomatic volunteering recruited at primary health care centres, among patients for elective surgery, university students and by internet advertisement** - **Faecal samples** - **287 (Sweden) / 172 (Finland)**	- **Sweden:** - ***E. coli:* 6.6%** - ***K. pneumoniae:* 0.3%** - **Finland** - ***E. coli:* 4.7%** - ***K. pneumoniae: 1.1*%**
Ny S., et al. [52] **2017**	- **Prospective** - **Sweden (nationwide)** - **2012-2013**	- **Faecal samples of asymptomatic community carriers** - **Faeces samples** - **2134**	- ***E. coli:* 4.7%** - **CTX-M-15: 45.3%; CTX-M-14: 20.0%; CTX-M-27: 8.4%**
Ulstad C., et al. [53] **2016**	- **Cross-sectional study** - **Norway** - **2014-2016**	- **"Healthy" asymptomatic volunteering recruited in general practices, universities and health institutions.** - **Rectal swabs** - **284**	- ***E. coli* / *K. pneumoniae:* 4.6% (14/284)** - **CTX-M-15: 40.0%; CTX-M-3: 13.3%; CTX-M-27:13.3%**
Strömdahl H., et al. [54] **2011**	- **Cross-sectional study** - **Sweden (Malmö Area)** - **2008-2010**	- **"Healthy" asymptomatic volunteering recruited from primary health care units** - **Rectal swabs** - **196**	- ***E. coli:*** - **2008: 2.1% (2/96)** - **2010: 3.0% (3/100)**
Hammerum A.M., et al. [55] **2011**	- **Cross-sectional study** - **Denmark** - **2008**	- **"Healthy" asymptomatic volunteering recruited among army recruits** - **Faecal samples** - **84**	- ***E. coli;*** 3.6% (3/84)

Likewise, a recent Danish cross-sectional study found that 4.5% (230/5,517) of patients presenting to the emergency department in 2018 were carriers of ESBL-producing bacteria (nose, throat and rectal swabs).[56]

2.4. INCIDENCE, RISK FACTORS AND PROGNOSIS

The risk factors and prognosis of ESBL-producing infections have been assessed in several previous studies as reviewed by Trecarichi et al.[57] However, there is less evidence in terms of studies focusing exclusively on community-onset ESBL-producing infections. To identify and summarize the current knowledge of community-onset ESBL-producing infections, a literature review was conducted to 1) assess the prevalence and incidence of community-onset ESBL-producing infections within the Nordic countries (defined as Denmark, Norway, Sweden, Finland and Iceland), 2) identify risk factors of community-onset ESBL-producing bacteraemia and 3) assess the influence of ESBL-production on the outcome of community-onset infections.

2.4.1. LITTERATURE REVIEW

We searched PubMed and Embase from inception to July 2020. A search strategy was constructed in collaboration with an experienced medical librarian, individualized to each database and including both Mesh terms and text words. The complete search strategy is available in Appendix C. We restricted the search to English and Scandinavian language (Danish, Norwegian and Swedish) and excluded conference abstracts. We assessed the title and abstract of all relevant papers. All relevant studies fulfilling the PICO criteria (population/patients, intervention, comparator, outcome) were included.[58] Finally, the reference lists of all relevant papers were reviewed and additional relevant work identified. We excluded studies that did not provide sufficient information of exposure/outcome variables with respect to community-onset infections and studies that included children. For Study II-IV, we also excluded studies that did not include a comparison group. Predictions studies were included if they provided sufficient information of risk factors in the derivation cohort. The results of the literature review are shown in Table 2 (incidence), Table 3 (risk factors), supplementary material Table S1 Study III (methodological)[3] and Table 4 (outcome).

Table 2. Studies reporting on prevalence of community-onset ESBL-producing infections in the Nordic countries.

Author Year	Design Setting (catchment population) Data source (microbiology) Period	Study population Culture types Number included	Summary of ESBL prevalence (proportion and/or incidence) Dominating genotypes (if reported)
Isendahl J., et al. [59] 2019	- Retrospective population-based case-control study - Sweden (nationwide) - National surveillance register (SmiNet) - 2007-2012	- Patients hospitalised with community-onset Enterobacteriaceae bacteraemia - Blood - 945 ESBL cases	- Incidence - Overall: 1.7 per 100,000 person years - 2012: 2.9 per 100,000 person years - 2016: 6.0 per 100,000 person years (reported in the study based on national surveillance data)
Jansåker F., et al. [60] 2019	- Retrospective cohort study - Denmark (Copenhagen Area ~ 1.8 million) - 2 microbiology departments (referral) - 2010-2016	- Patients from primary care with *E. coli* bacteriuria empirically treated with an oral antibiotic for UTI prior to microbiology results - Urine - 37,917 (of 125,270 episodes identified)	- *E. coli:* 4.3% (1,624/37,917)
Isberg H., et al. [61] 2019	- Prospective observational study - Sweden (Skåne county ~ 1.3 million) - Volunteering patients recruited at PHCCs - 2014-2016	- Women attending PHCCs with a suspected uncomplicated UTI - Urine - 304 patients	- *E. coli:* 2.3% (4/176) - *E. coli:* 3.5% (807/23,197) (resistance reported from the referral laboratory)
Søgaard M., et al. [62] 2017	- Retrospective case-control study - Denmark (The North Denmark Region ~ 580,000) - 1 microbiology department (referral) - 2007-2012	- *E. coli* bacteriuria collected from patients visiting general practitioners - Urine - 339 ESBL cases	- *E. coli* - 2007: 0.43%* - 2012: 4.5%* *Available from supplementary material
Helldal L., et al. [63] 2013	- Retrospective prevalence study - South-western Sweden (~ 750,000) - 1 microbiology laboratory (referral) - 2004-2008	- Urine cultures collected from patients in the community (long-term care and outpatient clinics) - Urine - Not reported	- *E. coli* - 2004: 0% - 2008: 1.6% (142/8669) - 2008/2009: CTX-M: 96%; CTX-M-15: 75% (166/221)* *Derived from table
Søraas A., et al. [64] 2013	- Case-control study - South-Eastern Norway (~ 450,000) - 2 microbiology laboratories (referral) - 2009-2011	- Patients with a positive *E. coli* or *K. pneumoniae* urine culture and without recent health care contact - Urine - 28,000 samples (15,000 unique patients)	- *E. coli* or *K. pneumoniae* - 1.6% (359 samples/28,000)
Hansen D., et al. [65] 2012	- Prospective point prevalence study - Denmark (nationwide)	- Urine culture isolates collected from patients visiting general practitioners - Urine	- *E. coli:* 1.5% - *K. pneumoniae*: 2.7% - *P. mirabilis:* 0%

	- **National microbiology departments (13/15 participated)** - **2007**	- **47,504**	
Lindbäck H., et al. [66] **2010**	- **Prospective cohort study** - **Sweden (Uppsala county ~ 323,000)** - **Volunteering patients recruited at PHCCs** - **2008**	- **Patients with clinical symptoms/signs of lower UTI presenting to PHCCs** - **Urine** - **360 samples/205 culture positive**	- ***E. coli*: 0.6% (1/156)*** ***Derived from table**

Abbreviations: PHCC, Primary healthcare centre; UTI, Urinary tract infection.

Table 3. Studies reporting on risk factors of community-onset ESBL-producing *Enterobacteriaceae* bacteraemia. A table featuring the same studies with respect to the methodological issues examined in Study III is available in supplementary material Table S1, Study III [3].

Author Year	Design Setting Data source Period ESBL proportion, %	Study population Exposures	Results[1] Limitations
Isendahl J., et al. [59] 2019	- Retrospective population-based case-control study - Sweden (nationwide) - National registries, medical charts - 2007-2012 - Not reported	- 945 ESBL-Enterobacteriaceae vs. 9350 population controls - Explorative risk factors	- *Adjusted model:* - Urological disorder (OR 4.32 [3.41-5.47]), immunological disorder (OR 3.54 [2.01-6.23]), Haematological malignancy (OR 2.77 [1.57-4.87]), solid tumours (OR 2.28 [1.76-2.94]), diabetes (OR 2.03 [1.58-2.61]), dementia (OR 1.89 [1.22-2.94]), chronic obstructive pulmonary disease (OR 1.61 [1.07-2.43]), ≥6 CCI Score OR 12.63 [8.36-19.10]**, urological procedure (OR 2.98 [2.45-3.53]), hospitalization <30 days (OR 6.68 [5.02-8.88])**, fluoroquinolones* (OR 5.52 [2.8-11.0]), non-EPE-active antibiotics with selective Gram-negative spectrum* (OR 3.8 [1.9-7.7]) * within 3 months ** Only selected results presented - No non-ESBL comparison group existed
Kim M., et al. [67] 2019	- Retrospective cohort study - South Korea, 1 hospital - Medical records - 2012-2015 - *E. coli:* 27.2%; *Klebsiella* species 14.9%	- 140 ESBL *E.* coli/*Klebsiella* species vs. 454 non-ESBL *E.* coli/*Klebsiella* species (derivation cohort) - Prediction study (derivation 2/3, validation 1/3)	- *Multivariate:* - ESBL identification within previous year (OR 7.8 [3.2-19.1]), beta-lactam or fluoroquinolone treatment <30 days (with 2 or more courses <90 days) (OR 6.82 [2.31-20.16])**, hospitalization within previous year (OR 2.5 [1.6-4.0]), urinary catheter (OR 2.3 [1.0-5.2]) ** Only selected results presented
Lee Y., et al. [68] 2019	- Prospective cohort study - South Korea (6 sentinel hospitals) - Medical records, National Insurance Health data claims - 2016-2017 - 17%	- 70 ESBL *K. pneumoniae* vs. 338 non-ESBL *K. pneumonia* - Explorative risk factors	- *Multivariate:* - Previous history of admission (OR 2.23 [1.20-4.15]), TMP/SMT (OR 8.66 [2.05-36.65]), urinary catheterization (OR 2.21 [1.11-4.39])
Fröding I., et al. [69] 2019	- Retrospective case-control study - Sweden (3 hospitals) - Medical charts - 2012-2015 - 0.53% (of patients hospitalised with a blood	- 277 ESBL Enterobacterales vs 400 controls with suspected community-onset sepsis (i.e. both cases and controls were hospitalised, had a blood culture drawn and treatment with Gram-negative active antibiotics initiated <24 hours)	- *Multivariate:* - Prior EPE-positive culture (OR 19.1 [9.2-39.8]), prostate biopsy ≤30 days (OR 22.2 [5.0-97.3]), healthcare abroad ≤6 months (OR 3.9 [3.9 [1.3-11.6]), 60-69 years (OR 3.3 [1.3-8.4])**, suspected diagnosis of UTI at admission (OR 2.6 [1.6-4.3]). ** Only selected results presented

	culture drawn and treatment with Gram-negative active antibiotics initiated <24 hours)	- Prediction study	- "Only" Stockholm area, the scores requires validation in other areas before implementation
Gottesman B., et al. [70]	- Retrospective cohort study - Israel (1 hospital) - Medical records - 2007-2011 - 4.5% (strictly community-acquired)	- 12 ESBL *E. coli* vs. 255 non-ESBL *E. coli* (with strictly community-acquired bacteraemia, i.e. excluding HCA associated bacteraemias) - Explorative (ESBL subgroup analysis)	- *Multivariate* - Quinolone use (OR 7.0 [1.7-29.4]), older age (OR 1.10 [1.02-1.18]) - ESBL production not the primary outcome, but only addressed in subgroup analysis. Very few ESBL cases, and focusing strictly on community-acquired infections
Quan ,J. et al. [71] 2017	- Prospective cohort study - China (28 hospitals) - Face-to-face interview - 2013-2014 - *E. coli:* 55.5%; *K. pneumoniae:* 16.5%	- 355 ESBL *E. coli* and 46 ESBL *K. pneumoniae* vs. 285 non-ESBL *E. coli* and 233 non-ESBL *K. pneumoniae*) - Explorative risk factors	- *Multivariate:* - *E. coli* - HCA infection (OR 1.57 [1.06-2.33]), obstructive urinary tract disease (OR 1.87 [1.13-3.12]), previous surgical history (OR 1.78 [1.01-3.12]), use of a cephalosporin antibiotic within 3 months (OR 3.22 [1.63-6.38]) - *K. pneumoniae* - Heart failure (OR 4.08 [1.08-15.43]) - Very high prevalence area and limited generalisability
Lee CH., et al. [72] 2017	- Retrospective cohort study - Southern Taiwan (2 hospitals) - Medical records - 2008-2013 - 5.7%	- 65 ESBL EKP* vs 1,076 non-ESBL EKP* - Prediction study **E. coli, K. pneumoniae, K. oxytoca, and P. mirabilis*	- *Multivariate:* - Nursing home residents (OR 27.8 [12.6-61.4]), recent hospitalization* (OR 3.8 [1.6-9.0]), recent invasive procedures* (OR 12.3 [5.6-27.2]), recent antimicrobial use* (OR 15.3 [7.7-61.4]), frequent ED use (≥3 times within a year) (OR 10.0 [4.9-20.2]), diabetes mellitus (OR 2.1 [1.1-4.1]), urological disease (OR 3.4 [1.1-10.2]) *within 4 weeks - The prediction model is calculated and validated using the same cohort
Zahar JR., et al. [73] 2017	- Prospective cohort study - France/Switzerland (49/1 hospital) - Medical records (N/A) - 2013 - 8.5%	- 58 ESBL-Enterobacteriaceae vs. 624 non-ESBL-Enterobacteriaceae - Explorative risk factors (ESBL subgroup analysis)	- *Multivariate:* - A previous hospital stay >5 days (*P=0.01*) - The study primarily addresses risk factors between HCA and strict community-acquired bacteraemias and only address ESBL vs non-ESBL in a small subgroup analysis
Park YS., et al. [74] 2014	- Retrospective (N/A) case-control study - South Korea (1 hospital) - Medical records (N/A) - 2005-2009 - 1%->21% (7%)	- 60 ESBL *E. coli* vs. 180 patients who had a blood culture drawn without ESBL *E. coli* - Explorative risk factors	- *Multivariate analysis:* - Independent risk factors: HCA infection (OR 8.3 [2.4-28.7]), malignancy (OR 4.6 [1.3-16.3]), urinary tract infection (OR 139.1 [24.6-788.2]), hepatobiliary infection (OR 79.1 [13.5-463.8]), 3rd-generation cephalosporin use during (within 3 months) (OR 16.4 [2.0-131.8]), severe sepsis/septic shock (OR 73.7 [12.4-438.5]) - Small study size. Selected control group provides very wide estimates
Park S., et al. [75] 2011	- Retrospective case-control-control study - South Korea (1 hospital) - Medical records	- 50 ESBL *E. coli* vs. 100 non-ESBL *E. coli* vs. 100 bacteraemia of all cause - Explorative risk factors	- *Multivariate analysis:* - Recent use of antibiotics (OR 4.3 [1.5-12.3]). *(Comparison with both control groups and reported cases compared with non-ESBL controls)*

	- 2005-2010 - 1.2%-> 10.2% (6.7%)		- Small, single centre
Lee JA., et al. [76] 2011	- Retrospective case-control study - South Korea (1 hospital) - Medical records - 2002 – 2009 - 7.6%	- 33 ESBL *K. pneumoniae* vs. 219 non-ESBL *K. pneumoniae* - Explorative risk factors	- *Multivariate analysis:* - Corticosteroid use (OR 13.7 [1.9-97.6]), percutaneous tube (OR 7.3 [2.4-22.1]), prior antibiotics (OR 5.7 [2.4-13.2]) - Small, single center. Not clear how they choose the 219 non-ESBL controls (out of 402 eligible)
Rodríguez-Bañõ, J. et al. [77] 2010	- Prospective case-control-control study - Spain (13 hospitals) - Medical charts, patient questionnaires - 2004-2006 - 7.3%	- 95 ESBL *E. coli* vs. 190 sepsis patients vs. 188 non-ESBL *E. coli* - Explorative risk factors	- *Multivariate analysis:* - HCA infection (OR 2.1 [1.2-3.8]), urinary catheter use (OR 3.1 [1.5-6.5]) and previous antimicrobial use (OR 2.7 [1.5-4.9]). (*Comparison with both control groups and reported compared with non-ESBL controls)*
Hsieh CJ., et al. [78] 2010	- Retrospective case-cohort study - South Taiwan (1 hospital) - Medical records - 2005-2006 - 4.7%	- 19 ESBL *E. coli* vs. 385 non-ESBL *E. coli* - Explorative risk factors	- *Only univariate analysis performed*: - Long-term care facility residence (p<0.002), cellulitis (p<0.010), catheter use (p<0.007), antibiotic use within 30 days (p<0.044) - Low number of EBSL patients, single centre. Did not perform adjusted analysis
Kang CI., et al. [79] 2010	- Retrospective cohort study - South Korea (18 hospitals) - Medical records (N/A) + surveillance database - 2008-2009 and 2006-2007 - 9.5%	- 82 ESBL *E. coli* vs. 783 non-ESBL *E. coli* - Explorative risk factors	- *Multivariate analysis:* - Primary bacteraemia (OR 2.99 [1.84-4.88]), underlying liver disease (OR 2.03 [1.12-3.86]), HCA infection (OR 1.86 [1.44-2.39]) - Post-hoc analysis of surveillance data and risk of ESBL misclassification. Patients mainly from large referral centres (and having severe underlying disease) with limited generalisability to community hospitals
Yang Y., et al. [80] 2010	- Retrospective cohort study - Taiwan (1 hospital) - Medical records - 2006-2008 - 20.7%	- 12 ESBL *E. coli* and *K. pneumoniae* UTI bacteraemia vs. 46 *E. coli* and *K. pneumoniae* UTI bacteraemia - Explorative risk factors	- *Multivariate analysis:* - Male gender (OR 9.2 [1.7-50.6]) and healthcare facility residency (OR 15.5 [2.4-98.9]) - Very small study, single centre, high prevalence area

Abbreviations: CCI, Charlson Comorbidity Index; HCA, Healthcare-associated; N/A, Not applicable (i.e. exact information not available); OR, Odds ratio; UTI, Urinary tract infection.

[1] Only multivariate/adjusted results presented when available. Risk factors reported as ORs with 95% CIs if nothing else stated.

Table 4. Studies reporting on outcome (mortality and/or length of hospital stay) of community-onset ESBL-producing infections.

Author Year	Design Setting Data source Period ESBL proportion, %	Population Cases Controls	Mortality Length of hospital stay (LOS) Independent predictors of death
Isendahl J., et al. [59] 2019	*See Table 3.*	- Enterobacteriaceae bacteraemia - ESBL (n=945) - Matched population controls (n=9350)	- 30-day mortality: 11.3% vs. 0.3% - Not addressed - Increasing age, CCI score, solid tumour, haematological malignancies, chronic heart disease, <12 years of education
Kim M., et al. [67] 2019	*See Table 3.*	- *E. coli*/*Klebsiella* spp. bacteraemia - ESBL (n=140) - Non-ESBL (n=454)	- 30-day mortality: 8.5% vs. 5.6% (p=0.131) - Days, median (IQR): 15 (10-24) vs. 10 (7-16) (p<0.001) - High-risk source, Pitt bacteraemia score≥3, CCI≥3.
Zahar JR., et al. [73] 2017	*See Table 3.*	- Enterobacteriaceae bacteraemia - ESBL (n=58) - Non-ESBL (n=624)	- 14-day mortality: Overall 9.8% "comparable outcome between ESBL and non-ESBL" - Not addressed - Age, clinical severity (SOFA score)
Park S., et al. [75] 2011	*See Table 3.*	- *E. coli* bacteraemia - ESBL *E. coli* (n=50) - non-ESBL all-cause bacteraemia (n=100)	- 30-day mortality:18% ESBL vs 8% non-ESBL, OR=2.5 (0.9-7.0), aOR 6.4 (0.3-145.5) - Not addressed - Higher APACHE II score, severe sepsis, septic shock, malignancy
Lee JA., et al. [76] 2011	*See Table 3.*	- *K. pneumoniae* bacteraemia - ESBL (n=33) - Non-ESBL (n=219)	- 30-day mortality: 12.1% vs. 16.0% (p=0.429) - Days, mean: 26.5±39.0 vs. 19.9±42.4 (p=0414) - Not addressed
Rodríguez-Bañõ, J. et al. [77] 2010	*See Table 3.*	- *E. coli* bacteraemia - ESBL (n=95) - Non-ESBL (n=188) / sepsis patients (n=190)	- 14-day mortality: 17% ESBL vs. 8% non-ESBL *(p=0.02)*, association disappeared when adjusting for IEAT - Not addressed - Pitt score>1, non-UTI source of infection, IAET
Hsieh CJ., et al. [78] 2010	*See Table 3.*	- *E. coli* bacteraemia - ESBL (n=19) vs. - non-ESBL (n=385)	- 30-day mortality: 21.1% vs. 10.9% (p=0.25) - Days, mean: 21.0 vs. 13.1 (*p=0.22*) - Not addressed
Kang CI., et al. [79] 2010	*See Table 3.*	- *E. coli* bacteraemia - ESBL (n=82) - Non-ESBL (n=783)	- 30-day mortality: 15.0% vs. 7.6% (p=0.096) - Not addressed - Severe sepsis, higher Pitt score. Primary bacteraemia, pneumoniae, underlying liver disease
Yang Y., et al. [80] 2010	*See Table 3.*	- *E. coli / K. pneumoniae* UTI bacteraemia - ESBL (n=12) - Non-ESBL (n=46)	- 21-day mortality: 8.3% vs. 4.4% *(p=0.403)* - Days, mean (SD) 16.3±9.3 vs. 7.9±5.2 (*p=0.010*) - Not addressed
Studies not reported in Table 2.			
Lee C., et al. [81] 2018	- Retrospective cohort study - Southern Taiwan (1 hospital) - Medical records	- *EKP** bacteraemia - ESBL (n=65) - Non-ESBL (n=1076)	- 28-day mortality: 32.3% vs. 9.2% (*p<0.001*), 36.7% vs. 21.7%* (*p=0.031*) - Days, mean (SD): 16±9.7 vs 14.5±18* (p=0.55) - IEAT, Pitt bacteraemia score> 4, underlying fatal comorbidities, liver cirrhosis, pneumonia or urosepsis focus of infection

	- 2008-2013 - 5.7%	**E. coli, K. species, and P. mirabilis*	***Following propensity score matching 60 ESBL to 180 non-ESBL**
Park S., et al. [82] **2015**	- Retrospective case-control study - South Korea (1 hospital) - Medical records - 2007-2013 - 9.9%	- CA* *E. coli* APN - ESBL (n=75) - Non-ESBL (n=225) *Excluding HCA infections	- Mortality period not specified: 1.33% vs. 1.78% (*p-value not provided*) - Days, median (IQR): 11 (6-16) vs. 7 (6-9) (*p*=0.001) - Not addressed. But clinical failure (death or recurrence of symptoms) was associated with septic shock and immunocompromisation
Jean SS., et al. [83] **2014**	- Prospective, observational - Thailand, Taiwan, The Phillippines, Colombia and Portugal - Electronic CRF - 2010-2011 - Not reported	- *E. coli* / *Klebsiella* spp. complicated intraabdominal infections. - ESBL (n=17) - Non-ESBL (n=88)	- Mortality period not specified: 11.8% vs. 4.5% (*p*=0.455) in mortality related to complicated intraabdominal infections (6/12) - Days, median: 17.6 vs. 11.6 (*p*=0.011) - Not addressed
Kim B., et al. **2013** [84]	- Prospective cohort study - South Korea (11 hospitals) - Electronic CRF, patient questionnaires - 2010-2011 - 8.7%	- Enterobacteriaceae APN - ESBL (n=46) - Non-ESBL (n=88)	- Mortality not addressed. Clinical cure (recurrence of UTI symptoms/signs, or death) was 9.5% vs. 5.5% (*p*=0.294) - Days, median: 17.6 vs. 11.6 (*p*=0.011) - Not addressed
Marchaim D., et al. [85] **2010**	- Prospective cohort - Israel (10 hospitals) - CRF (?) - 2006-2008 - *E. coli* 14%, *K. pneumoniae* 45% (referenced in article)	- Enterobacteriaceae bacteraemia - ESBL (n=205) - Non-ESBL (n=242)	- In-hospital mortality: 30% vs. 11% (*p*=0.001), OR=3.5 (2.1-6.1) - Days, mean (SD): 25±156.4 vs. 13.25±48.7 (*p*=0.27)
Apisarnthanarak A., et al. [86] **2008**	- Prospective case-control study - Thailand (1 hospital) - Medical records - 2003-2007 - 6 %	- *E. coli/ K.pneumoniae* bacteraemia - ESBL (n=36) - Non-ESBL (n=108)	- Mortality period not specified: 36% vs. 15% (*p<0.05*) - Days, median (range): 8 (1-43) vs. 6 (3-15) (*p*=0.001) - *K. pneumoniae* BSI, failure to receive empirical regimen including either a carbapenem or β-lactam/ β-lactamase inhibitor combinations
Apisarnthanarak A., et al. [87] **2007**	- Case-control-control - Thailand (1 hospital) - Medical records - 2003-2004 - 9%	- *E. coli* all-clinical cultures or hospitalised patients - ESBL (n=46) - Non-ESBL (n=46), non-infectious hospitalised patients (n=46)	- Mortality period not specified: 30% vs. 6% vs. 0% (*p*<0.001) - Days, median (range): 8 (1-43) vs. 6 (3-14) vs 5 (3-13) (*p*<0.001) - Not addressed
Borer A., et al. [88] **2002**	- Cohort study - Israel (1 hospital) - Medical records - 1997 - 5%	- Enterobacteriaceae bacteraemia - ESBL (n=6) - Non-ESBL (n=112)	- Mortality period not specified: 83.3% vs. 14.4% (*p*<0.001)* - Not addressed - Not addressed *Significant more patients with *K. pneumoniae* bacteraemia among ESBL, and another population.

Abbreviations: APN, Acute pyelonephritis; CA, community-associated (i.e. excluding HCA); CCI, Charlson Comorbidity Index; cIAI, Complicated intrabdominal infection; HCA, Healthcare-associated; IEAT, Inappropriate empirical antibiotic therapy; IQR, Interquartile range; UTI, Urinary tract infection; SD, standard deviation;

2.4.2. RESULTS OF THE LITTERATURE REVIEW

Prevalence and incidence studies (Table 2)

Overall, we identified eight studies reporting on the prevalence of community-onset ESBL-producing infections from the Nordic countries (Table 2). Seven studies focused on urine cultures collected from primary healthcare centres, outpatients or general practitioners, while one study included hospitalized patients with community-onset bacteraemia.[59] Among the seven studies focusing on urine cultures, two prospective studies included clinical signs and symptoms into the definition of urinary tract infection,[61,66] and one study applied a simultaneously prescription of an antibiotic for treatment of urinary tract infection to strengthen the likelihood of capturing a "true" urinary tract infection.[60] The remaining four studies relied exclusively of positive urinary samples collected by the referral laboratory.[62–65] Almost all studies reported the prevalence as a proportion, while Isendahl et al.[59] reported an incidence rate (i.e. person years). In general, an increasing proportion of ESBL-producing isolates was observed from 2004 onwards, e.g. from 0.43% in 2007 to 4.5% in 2012 for *E. coli* urinary tract infections in Denmark.[62] Likewise, in the one study reporting an incidence rate, the overall incidence rate of ESBL-producing Enterobacteriaceae bacteraemia in Sweden was 1.7 per 100,000 person years between 2007-2012, which increased to 6.0 per 100,000 person years in 2016.[59] This study, however, did not report on specific incidence rates in different subpopulations. Thus, population-based studies assessing the incidence rate as well as studies of patients admitted to hospital with community-onset ESBL-producing infections are limited within the traditional low antimicrobial resistance regions of the Nordic countries.

Risk factors studies (Table 3)

We identified 15 studies investigating risk factors of community-onset ESBL-producing Enterobacteriaceae bacteraemia **(**Table 3**)**. Eleven studies were from traditionally "high-prevalence areas" (six from Korea, three from Taiwan, one from China and one from Israel), while only two studies were from Scandinavian countries (Sweden).[59,69] Considerable heterogeneity with respect to study design and selected risk factors existed. The retrospective case-control design was the most utilized design and *E. coli* the most widely investigated bacteria. In general, the studies were small, with only four studies including >100 ESBL cases;[59,67,69,71] and many of the studies were single-centre studies. Following adjustment, the most common identified risk factors were recent (usually within 3 months) use of antibiotics (reported in 11 studies), healthcare-associated (HCA) infection (four studies) or recent admission to hospital (from 30 days to one year) (four studies), use of catheter (four studies) or urological disorder (three studies) and healthcare facility or nursing home residency (three studies) (Table 3). The risk factors that inferred the highest odds ratios (ORs) included prior ESBL-positive culture (OR 7.8 to 19.1),[67,69] recent prostate biopsy ≤30 days (OR 22.2)[69] and nursing home residency (OR 27.8).[72] The study by Isendahl et al.[59] was by far the largest, including 945 ESBL cases, and it was also the only population-based (nationwide) study. However, their inclusion of a background

population as control group makes it difficult to disentangle specific risk factors of "ESBL-production" from risk factors of "bacteraemia".[2]

Antibiotics as a risk factor (Table S1 supplementary material, Study III [3])

The methodological approach utilized by the risk factor studies when examining antibiotic use as a risk factor of community-onset ESBL-producing bacteraemia was the specific aim of Study III. Thus, a detailed discussion including a summarizing table of the methodological approach applied in the different studies is available in the supplementary material (Table S1 supplementary material) for Study III.[3]

Outcome studies (Table 4)

Overall, 17 studies investigating outcomes (mortality and/or length of hospital stay [LOS]) of community-onset ESBL infections were identified (Table 4). Nine of these studies were also included in table 3. Thirteen studies focused exclusively on patients with bacteraemia, two studies addressed patients with acute pyelonephritis,[82,84] one study concentrated on patients with intraabdominal infections[83] and one study included all clinically relevant samples.[87] Almost all of the studies used a non-ESBL control group for comparison, while the only population-based study included a control group of randomly selected population controls.[59] Ten studies included all Enterobacteriaceae, *E. coli*/*K. pneumoniae* or *E. coli*/*K. pneumoniae*/*P. mirabilis*, while *E. coli* was the sole bacterium included in six studies[75,77,78,81,82,87] and *K. pneumoniae* the sole bacterium in one study.[76] Seven studies were prospective, and the remaining were retrospective. Most studies were hospital based; six were multi-centre studies, ten were single-centre studies, and one was population based.[59] Mortality was assessed by varying time-intervals; 30-day/28 day mortality in seven studies, 21-day mortality in one study,[80] 14-day mortality in two studies,[73,77] in-hospital mortality[85] in one study while the remaining studies did not provide a specific period. Among patients with bacteraemia, with the exception of one minor study,[88] mortality varied between 7.5-36% for ESBL producers compared with 2-15% for non-ESBL producers. However, the differences only rarely reached statistical significance, and generally any excess mortality in the ESBL group tended to be reduced following adjustment. Moreover, adjustment procedures varied considerably, which might impact the result (to be discussed in the methodological section). In patients with acute pyelonephritis the mortality was much lower.[82,84] Eleven studies addressed LOS. The mean LOS varied between 16-26.5 days for ESBL producers compared with 7.9-19.9 days for non-ESBL producers, and in 73% (8/11) of the studies ESBL production was found to be statistically significant associated with an increased LOS in crude analysis. None of these studies examined LOS between survivors or non-survivors individually.

2.4.3. LIMITATIONS OF THE EXISTING LITTERATURE

Population-based studies are generally considered the optimal means of defining the epidemiology of community-onset infectious diseases and establishing the burden of disease by providing complete numerator and denominator data to estimate age and sex-standardized incidence rates.[89] Despite this, in the Nordic countries, population-based studies examining the incidence *rate* (e.g. as person years) of ESBL-producing community-onset infections are almost absent. Indeed, no studies have addressed the incidence rate within specific population groups (e.g. age, gender) in the Nordic countries. Among the studies addressing risk factors of community-onset ESBL-producing bacteraemia and the studies focusing on outcome following community-onset ESBL-producing infection (mainly bacteraemia), most studies included a limited number of ESBL cases, almost all studies were hospital-based, many of which were single centre studies, and very few studies were from traditional low-prevalence antimicrobial areas. Prior antibiotic exposure was consistently reported a risk factor; however, different specific antibiotics and antibiotic classes were reported as risk factors across the studies, and heterogeneity existed in the methodological approach when examining antibiotic. Overall, it appeared that EBSL-producing infections were associated with an increased crude mortality; however, significance was rarely reached. Moreover, following adjustment, ESBL-production was rarely associated with mortality. It is likely, though, that the small sample sizes precluded any association. In conclusion, large population-based studies from low-prevalence regions addressing risk factors and mortality are urgently needed, as are studies addressing LOS that properly account for survivors and non-survivors, and studies assessing outcomes of patients with urinary tract infection.

2.5. AIMS OF THE THESIS

The aims of the PhD study were:

I. To estimate the incidence of community-onset ESBL *E. coli* and *K. pneumoniae* bacteraemia and urinary tract infection in the North Denmark Region from 2007 onwards. We hypothesized that the increase in ESBL-producing isolates would be driven mainly by an increase in strictly community-acquired ESBL-producing *E. coli.*

II. To investigate risk factors associated with community-onset ESBL-producing *E. coli* and *K. pneumoniae* bacteraemia in our region where the antimicrobial resistance is traditionally low.

III. To examine the influence of methodology, including in/exclusion criteria of antibiotic exposure, when investigating antibiotic exposure as a risk factor of ESBL production in community-onset *E. coli* and *K. pneumoniae* bacteraemia.

IV. To examine the impact of ESBL production on outcome following *E. coli and K. pneumoniae* bacteraemia and urinary tract infection. We hypothesized that bacteraemia and urinary tract infection with ESBL-producing *E. coli* and *K. pneumoniae* were associated with a worse clinical outcome than infection with non-ESBL-producing isolates.

CHAPTER 3. METHODS

3.1. SETTING

The four studies were conducted in the North Denmark Region during the period 2007-2017. The catchment population counted approximately 580,000 citizens in a mixed rural and urban area. The region consisted of one 800-bed university hospital, while the number of district hospital decreased from eight in 2007 to five in 2017. All specialities are covered within the region; however, highly specialized treatment, e.g. high-dose stem cell transplantation and solid organ transplantation are referred outside the region.[2,90] Denmark has a tax-supported public health care system, ensuring free healthcare access (primary, secondary and tertiary) to care for every citizen.[91]

3.2. DATA SOURCES

The Danish Civil Registration System (CRS)

The CRS was established as an administrative tool in 1968, and since then every citizen in Denmark has been assigned a unique 10-digit civil personal registration (CPR) number upon birth or immigration.[91] The CPR number is used in all Danish administrative and medical registers and databases, and it is the unique key component allowing for unambiguous linkage at the level of the individual citizen between healthcare systems and other public registries. The CRS holds information on marital status, place of birth, emigration and vital status updated at a daily level, allowing for nationwide cohort studies with virtually complete long-term follow-up.[91] Figure 2 shows the data sources utilized in the studies and the key role of the CRS in facilitating linkage among these sources.

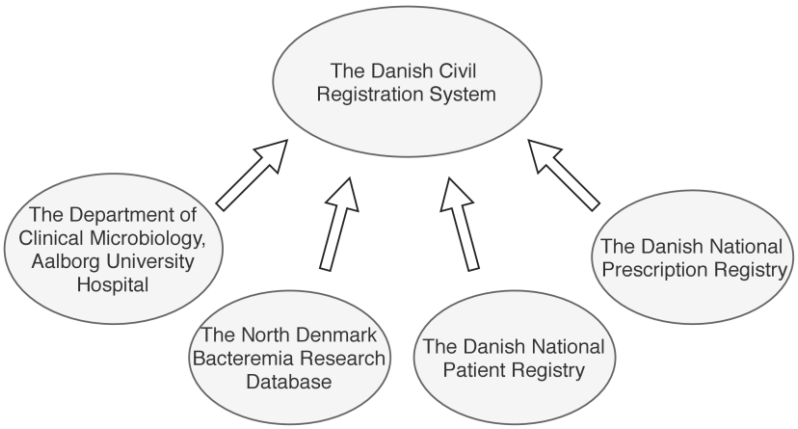


Figure 2. Data sources used in the studies. The CPR number assigned in the Danish Civil Registration System is the unique key allowing individual-level linking of the data sources.

The Laboratory Information System

The laboratory information system (wwLab/ADBakt, Autonik, Sweden), hosted at the Department of Clinical Microbiology, Aalborg University Hospital, Denmark, has registered all diagnostic specimens (blood, urine, etc.) requested in the region. Data obtained from this system included the name of the requestor, date of collection, and data on bacteria and susceptibility testing. This database provided the basis for identification of *E. coli* and *K. pneumoniae* from blood and urine cultures, including phenotypic identification of ESBL.

The Department of Clinical Microbiology, Aalborg University Hospital, served as referral laboratory for all hospitals and general practitioners in the North Denmark Region from 2010 onwards. In 2006, a reform of local government merged the two former counties into one health region; consequently, in a transition period from 2006 to the end of 2009, the Department of Clinical Microbiology did not serve a minor part (catchment population ~ 57,000) of the current North Denmark Region (Appendix A).[1]

The North Denmark Bacteremia Research Database (NDBRD)

Since 1992, patients with bacteraemia have been registered prospectively in the NDBRD.[90] Data recorded include bacteria, focus of infection, empirical antibiotic therapy and polymicrobial bacteraemia.[90] The focus of infection was based on all available evidence, e.g. clinical, microbiological and imaging studies. We defined empirical antibiotic therapy as antibiotic therapy before first notification of a positive blood culture, and it was considered as active if administered intravenous and if blood isolates were susceptible *in vitro* to at least one of any given antibiotics.[4]

The Danish National Patient Registry (DNPR) - ("Landspatientregisteret")

The DNPR contains data on all hospital admissions (since 1977) and outpatient contacts (since 1995).[92] Data includes dates of admission and discharge, and up to 20 diagnosis or procedure codes classified according to the World Health Organisation's International Classification of Disease (ICD)-8 until 1993 and the ICD-10 thereafter. By 2015, at least 114 studies had examined a wide range of codes; and in the majority of studies, the positive predictive values was >80%, including codes used to estimate the Charlson Comorbidity Index score.[92] This data played a pivotal role in categorizing our cohort into community-onset or hospital-acquired infection by linking admission dates from the registry with the date the sample was obtained ("index date").

The Danish National Prescription Registry (DNPR*) - ("Lægemiddeldatabasen")

The DNPR* is a sub-register of the Register of Medicinal Products Statistics, maintained by the Danish Medicines Agency.[93,94] The DNPR* is administered by Statistics Denmark. Since 2003, information from the DNPR* has been available to researchers. This information consists of individual-level information on all redeemed prescriptions dispensed from Danish community pharmacies, including date, dosage and Anatomical Therapeutic Classification (ATC) code. Of importance, the database does not include in-hospital dispensed antibiotics. In Denmark, antibiotics are not available over-the-counter.

3.3. DEFINITIONS

Place of acquisition

We adopted the probably most widely used classification scheme for acquisition of bacteraemia as defined by Friedman et al. in 2002.[18] However, we modified the definitions according to the epidemiology of *E. coli* and *K. pneumoniae,* the data available in our registers and by reviewing the most widely used definitions in studies of community-onset ESBL-producing Enterobacteriaceae bacteraemia (Appendix B). For example, we were unable to reliably identify residents of nursing homes or long-term care facilities from our registers; thus, this criterion was excluded from the classification of HCA infection. Also, for sound reasons, some studies consider the infection to be nosocomial if positive blood samples are obtained within a certain time window of discharge from hospital, e.g. from ≤3 days[59] and up to 1 month.[78] However, to compare our results with the results in the majority of studies within the field (Appendix B), we decided not to include duration of time since discharge from hospital in the definition of community-onset. Consequently, the following definitions were applied:

Community-onset: Community-onset infection was defined as a positive blood sample collected within two days of admission, while patients with a positive blood sample obtained >2 days after admission were considered to have a nosocomial infection. If a patient was transferred from another hospital or department, the LOS was calculated from the date of admission to the first hospital.

Healthcare-associated: Community-onset infection was classified as a HCA infection if prior to the index date the patient had (1) been admitted to the hospital within 90 days for ≥2 days or (2) attended a hospital or outpatient clinic of haematology, nephrology or oncology (e.g. for haemodialysis or chemotherapy) within the past 30 days or (3) had undergone any surgical procedures related to the genitourinary tract within the past 30 days.[1] Criterion 3) was included to take into

account that the urinary tract is the most common source of *E. coli* and/or *K. pneumoniae* infection. The coding of these specific variables is available in the supplementary material commencing Study I.[1]

Community-acquired: Patients with a community-onset infection who did not fulfil the criteria for HCA infection were classified as having a community-acquired infection.

Bacteraemia

Bacteraemia was defined as the presence of one or more positive blood cultures. Since 1996, blood culturing at the Department of Clinical Microbiology, Aalborg University Hospital, has been performed using the automated BacT/Alert® system (bioMérieux, Marcy l'Etoile, France). For adult patients, each blood culturing contains three bottles; two aerobic and one anaerobic. Polybacteraemia was defined as two or more blood culture isolates of different species or antibiogram obtained within 48 hours.[90]

Urinary tract infection

A urinary tract infection was defined as the presence of $>10^4$ colony-forming units of *E. coli* and *K. pneumoniae*. We decided to use a high cut-off of $>10^4$ colony-forming units to reduce the likelihood of probable contamination.[4] Nonetheless, a "true" diagnosis of urinary tract infection requires additional information on patient symptoms. This information was not available but would have to be retrieved individually from the medical records, which was not feasible due to the high number of patients, and because of data legislation issues. Thus, it must be acknowledged that some of these episodes might represent asymptomatic bacteriuria rather than urinary tract infection.

Identification of ESBL

The identification of EBSL-producing *E. coli* and *K. pneumoniae* has changed during the study period from 2007 to 2017. A detailed description of the identification of ESBL during the study period is available in the supplementary material of Study I.[1] Of importance to this thesis, the Department of Clinical Microbiology, Aalborg University Hospital, initiated screening of ESBL-producing isolates in both blood and urine specimen in 2006. Since 2011, the identification of ESBL-isolates has been in accordance with the EUCAST. Identification of ESBL was based on phenotypic analysis during the whole study period, as no systematic genotypic identification has been performed at the Department of Clinical Microbiology, Aalborg University Hospital.

Registration of an isolate as an ESBL producer was manually conducted, and hence prone to bias. If an ESBL-producing isolate had been identified, any subsequent isolate of the same species with a similar antibiogram obtained from the same patient

within 180 days was considered identical and no additional ESBL screening (or confirmation testing) was performed. In this case, the biomedical laboratory technician should remember to register the isolate as being an ESBL producer. This procedure, we discovered, was especially prone to erroneous registration. To ensure correct identification of ESBL production, we constructed algorithms in Stata 15.0 (Stata Corp, College Station, TX) based upon the susceptibility testing and the screening and confirmation criteria of the ESBL identification, and compared our findings with the ESBL status registered in the laboratory information system. We systematically assessed disagreements; when necessary, we reviewed the complete microbiological records, which included complete susceptibility testing, and any prior samples collected within 180 days.

3.4. STUDY DESIGNS

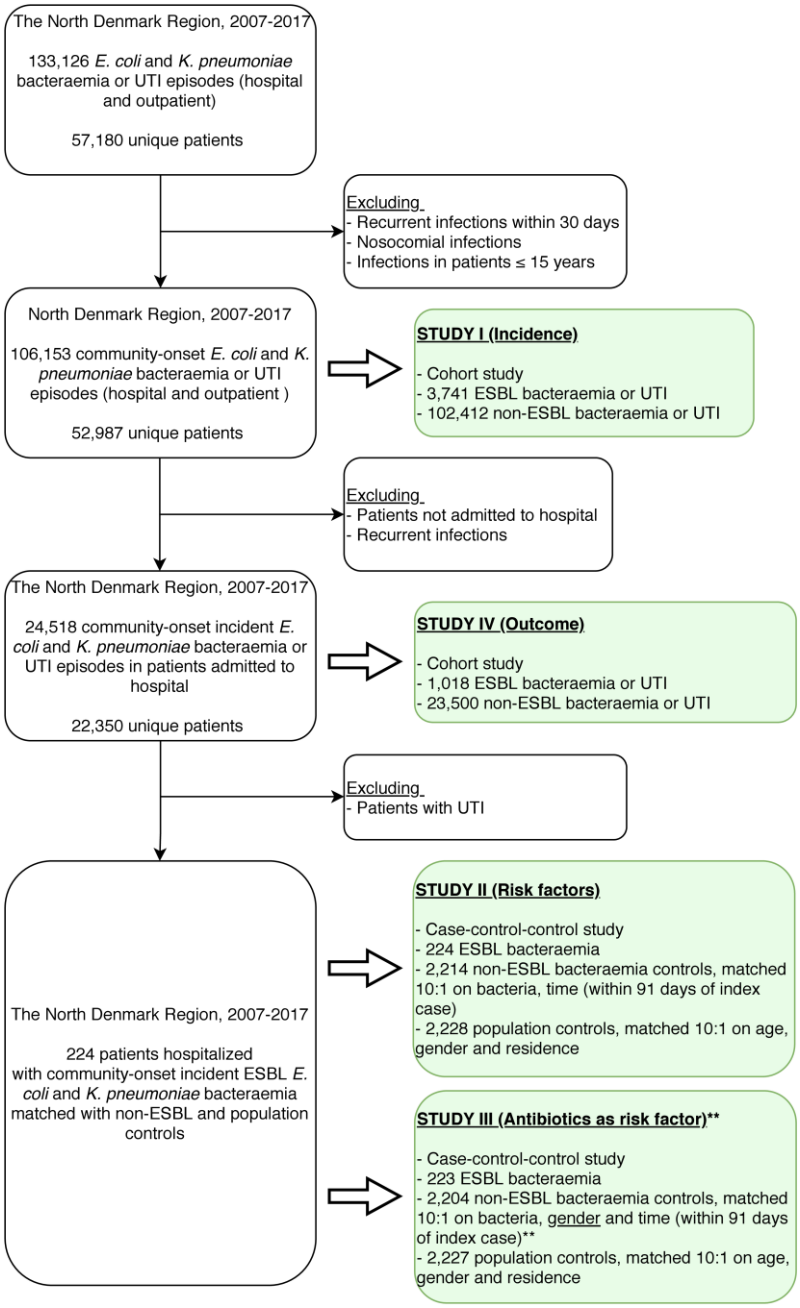
The study designs in this thesis included two historical population-based open cohort studies (Study I and IV) and two matched case-control-control studies (Study II and III). The study period was 1 January 2007 to 31 December 2017 for all four studies. All studies were approved by the Danish Patient Safety Authority (reference no.: 3-3013-2298/1). In Denmark, registry studies do not acquire approval by a science ethics committee.

3.5. STUDY POPULATIONS

In all studies, the population of interest was adult patients (>15 years) with community-onset *E. coli* or *K. pneumoniae* bacteraemia or urinary tract infection according to the definition of community-onset, ESBL, bacteraemia and urinary tract infection as described above. The study designs, including the number of cases and controls included in the four studies, are shown in Figure 3.

In **Study I**, the same patient could contribute with several episodes of both ESBL and non-ESBL-producing infections, and within different specimen groups (i.e. blood, urine collected from hospital and urine collected from primary care). However, episodes within the same group should be at least 30 days apart; otherwise, they were considered part of the same unresolved infection.[1] Studies II-IV were restricted to incident episodes, i.e. only the first community-onset infection per patient was included.

Figure 3. Flow diagram of the study population included in the four studies.



** Applying gender as an additional matching criterion slightly altered the study population between Study II and III.

In the risk factor studies (**Study II and III**), we restricted to include patients hospitalised with *E. coli* or *K. pneumoniae* bacteraemia. Cases included every patient with an ESBL-positive *E. coli* or *K. pneumoniae* bacteraemia. We used two different control groups; 1) non-ESBL controls, consisting of patients with a corresponding *E. coli* or *K. pneumoniae* bacteraemia but with a non-ESBL-producing isolate; and 2) population controls, consisting of patients randomly selected from the background population within the North Denmark Region. The cases and controls were matched in a 1:10 ratio using the risk set sampling technique, i.e. each control should be alive and at risk of an ESBL infection at the date of sampling. Non-ESBL controls were matched according to bacteria and time, i.e. the bacteraemia should be present within 91 days of the index date of the corresponding case. Population controls were matched according to age, gender and residency within the North Denmark Region, and they were assigned the same index date as the corresponding case.[2] Because male sex was found to be a risk factor of ESBL-producing bacteraemia in Study II, and because antibiotic treatment regimens differ between genders (e.g. treatment of urinary tract infection in males versus females), we decided also to match on gender in Study III as well.[3]

In the assessment of outcome (**Study IV**), we used a cohort design including all patients with *E. coli* or *K. pneumoniae* bacteraemia or urinary tract infection without performing any matching. Nor did we include population controls. Patients who had both a positive blood and a urinary sample collected within +/- 2 days were included only in the bacteraemia cohort (Figure 4).

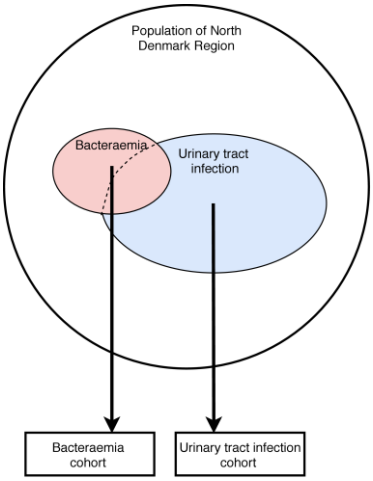


Figure 4. Base population of the study. Patients presenting with both bacteraemia and urinary tract infection were included only in the bacteraemia cohort (dark grey colour), while patients presenting solely with urinary tract infection constituted the urinary tract infection cohort (light grey colour).

3.6. OUTCOMES

The primary outcomes in the four studies were:

Study I: The incidence of community-onset ESBL-producing *E. coli* and *K. pneumoniae* bacteraemia and urinary tract infection (both in hospital and in primary care) in the North Denmark Region, 2007-2017.

Study II: Estimation of risk factors (expressed as ORs) of ESBL production in community-onset *E. coli* and *K. pneumoniae* bacteraemia. Temporal dynamics in selected risk factors between the time periods 2007-2011 and 2016-2017.

Study III: The influence of the chosen methodology on estimation of antibiotic exposure as a risk factor of ESBL production in community-onset *E. coli* and *K. pneumoniae* bacteraemia.

Study IV: Thirty-day and one-year mortality of community-onset ESBL-producing *E. coli* and *K. pneumoniae* bacteraemia or urinary tract infection in hospitalised patients compared with patients with non-ESBL-producing strains. LOS was considered a secondary outcome.

3.7. STATISTICAL ANALYSIS

Study I (Incidence):

Due to the dynamic open cohort design chosen, we measured incidence density as age- and gender-standardized incidence rates per 100,000 person years. ESBL-positive cases constituted the numerator data, while the catchment population of the North Denmark Region as per 1 July each year constituted the denominator data, i.e. the population at risk.[1] Information on population size and distribution is freely available at StatBank hosted by Statistics Denmark.[95] The base population of the North Denmark Region in 2007 was used in the direct standardization. The "overall" incidence rate was calculated by summarizing infections within each of the six groups, i.e. the same patient could contribute with several infections. We also restricted the analysis to incident infections, i.e. a patient could contribute with only one (the incident) infection in each group. The rationale behind this strategy is discussed in Study I.[1] Theoretically, in the analysis of incident infections where the "event" could *not* recur, the time contribution of an individual to the denominator of the incidence rate, i.e. the "time at risk", should end once the event occurred.[96] However, due to the relatively low number of ESBL cases compared with the general population, subtracting these cases from the total population at risk would only marginally influence the results and this approach was therefore ignored.[96] The study was solely descriptive, and no confidence intervals (CIs) were calculated.

Study II (Risk factors):

Due to the matched design chosen, we used conditional logistic regression to calculate crude and adjusted ORs with 95% CIs. To disentangle risk factors associated with bacteraemia from risk factors of "ESBL-producing bacteraemia", we included two separate control groups. When examining exposure to antibiotics as a risk factor of antimicrobial resistance (e.g. ESBL-producing bacteria), the traditional case-control design will generally tend to overestimate associated ORs.[97,98] In addition, other challenges exist that might influence the results when evaluating antibiotic exposure as a risk factor of antimicrobial resistance.[3] Thus inspired, we conducted an additional study (Study III) to explore in detail how the methodology might affect the results obtained.

Study III (Methodology):

We employed the same analytic approach as described in Study II. We graphically displayed ORs and CIs associated with the different criteria to allow for visual qualitative interpretation of the results.

Study IV (Outcome):

Mortality was assessed using time-to-event data. The index date was defined as the day of admission. The patients were followed until death, emigration or end of study (31 December 2017), whichever occurred first. To account for patients who died on the day of admission, these patients were arbitrarily assigned 0.5 days of survival. We graphically displayed 1-year mortality using the Kaplan-Meier methods. We estimated crude and adjusted mortality rate ratios (MRR) between patients with and without ESBL-producing isolates using Cox proportional hazard regression analyses. We *a priory* decided to adjust for well-known risk factors of mortality; age, gender and comorbidity. Sensitivity analyses with additional adjustments were performed. The proportional hazard assumption was assessed graphically with log-log plots and Schoenfeld residuals and found to be appropriate. Finally, risk factors of 30-day mortality were assessed by conventional logistic regression.[4]

CHAPTER 4. RESULTS

4.1. STUDY I (INCIDENCE)

During 2007 to 2017, we identified 106,153 community-onset *E. coli* and *K. pneumoniae* infectious episodes, whereof 3,741 (3.5%) were with an ESBL-producing strain.[1] The 106,153 infectious episodes occurred among 57,180 unique patients (2.0 episodes per patient). 32,094 (60.6%) of the patients had only one infectious episode; hence, 74,059 infectious episodes occurred among the remaining 25,086 patients (Figure 5). *E. coli* urinary tract infections (in-hospital and outpatient) accounted for 89,442 (84.3%) of the infectious episodes (Figure 6). During 2007 to 2017, we identified 3,741 community-onset ESBL *E. coli* or *K. pneumoniae* infectious episodes among 2,170 unique patients (1.7 episodes per patient). The number of ESBL-producing infectious episodes increased from <33 in 2007 to 553 in 2017, corresponding to an increase in the standardized incidence rate from <7.5 to 105 per 100,000 person years.[1] Obviously, this was not explained by an increasing trend of specimens tested, which is emphasized by the fact that the proportion of ESBL-producing isolates rose from 0.5% to 4.0% during the study period.[1] Even more important than the overall rate of ESBL are the rates within specific populations groups;[11] e.g., being elderly (>80 years) and having a community-acquired infection increased the incidence rate of ESBL-producing *E. coli* urinary tract infection in primary care in 2017 by a factor four compared with patients who were 60-79 years old and patients with a HCA infection, respectively.[1] Co-resistance among ESBL isolates was high; thus, in 2017 64.3% and 42.9% of community-onset ESBL-producing *E. coli* isolates from bloodstream infections were resistant to ciprofloxacin and gentamicin, respectively.[1]

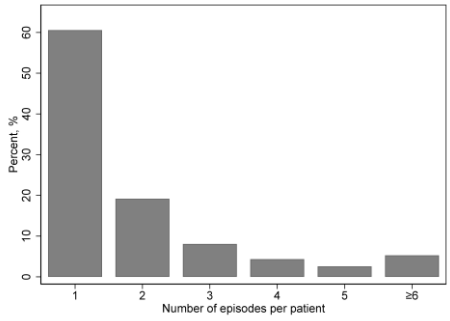


Figure 5. Proportion of the number of infectious episodes per patient.

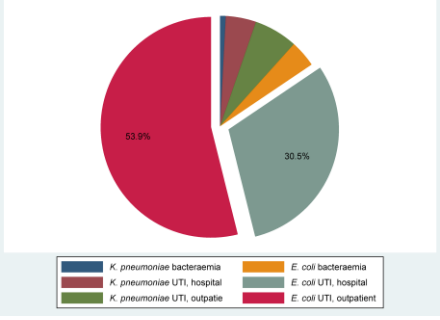


Figure 6. Proportion of infectious episodes according to bacteria and specimen. *E. coli* urinary tract infection accounted for the vast majority of infectious episodes.

4.2. STUDY II (RISK FACTORS)

We matched 224 ESBL cases to 2,214 non-ESBL controls and 2,228 population controls, respectively. Comparison with population controls revealed that most of the risk factors were associated with the risk of ESBL-producing bacteraemia. Compared with non-ESBL controls and following adjustment, the risk factors producing the highest ORs were in a decreasing order; exposure to fluoroquinolones (aOR 3.56 [95% CI; 2.52-5.05]), ≥3 antibiotic prescriptions within 15-365 days, ≥3 hospital admission within 1-365 days, male sex, hospital admission within 1-91 days and antibiotics within 15-91 days (aOR 1.82 [95% CI; 1.37-2.42]).[2] Only 14 (6.3%) of patients with ESBL-producing bacteraemia had all of the five most pronounced risk factors, while 49 (22.0%) had none of the risk factors (Figure 7). No specific comorbidity nor genitourinary surgery were statistically associated with ESBL production compared with non-ESBL controls; still, a high CCI score (≥3) was a risk factor (aOR 1.55 [95% CI; 1.08-2.24]).

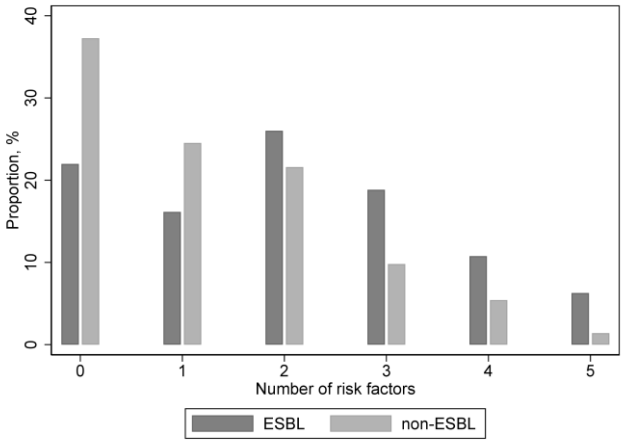


Figure 7. The proportion of the cumulative number of risk factors in patients with community-onset *E. coli/K. pneumoniae* bacteraemia with an ESBL-producing strain (dark grey) or non-ESBL-producing strain (light grey).

All antibiotics and antibiotic classes were associated with ESBL production compared with population controls; aORs ranging from 2.28 [95% CI; 1.57-3.31] for broad-spectrum penicillin to 9.96 [95% CI; 6.23-15.95] for fluoroquinolones. The aORs were reduced considerably compared with non-ESBL controls where the drug classes broad/narrow-spectrum antibiotics/penicillins remained associated with ESBL production, in addition to trimethoprim and fluoroquinolones, the latter conferring the

highest aOR of 3.56 [95% CI; 2.52-5.05]).[2] The association between the use of antibiotics and the risk of ESBL-producing infections is examined in dept in Study III.[3]

Neither proton-pump inhibitors (aOR 1.23 [95% CI; 0.92-1.62]) nor nitrofurantoin (aOR 1.32 [95% CI; 0.69-2.53]) was statistically significantly associated with ESBL production when compared with non-ESBL controls, though these have previously been shown to be associated with ESBL-producing *E. coli* urinary tract infection within our community of the North Denmark Region.[62]

4.3. STUDY III (METHODOLOGY)

Applying the different in/exclusion criteria notably reduced the overall amount of antibiotic exposure in the study population. Hence, when applying the least restrictive criteria (criteria 1) 74.7% of ESBL cases, 68.1% of non-ESBL controls and 33.1% of population controls had been exposed to antibiotics within the past year. This was reduced to 44.1%, 35.2% and 10.7% for ESBL cases, non-ESBL controls and population controls, respectively, when applying the most restrictive criteria (criteria 4). As expected, control group selection had a major impact of the estimated OR. Odds ratios of antibiotic exposure as a risk factor of ESBL-producing bacteraemia increased by almost a factor 4 or 5 compared with the population controls instead of non-ESBL controls, e.g. from an OR of 1.70 [95% CI; 1.29-2.25] for “any antibiotics” (criteria 4) compared with non-ESBL controls to an OR of 6.86 [95% CI; 4.80-9.82] compared with population controls . Additionally, excluding patients with a prior ESBL-positive urine culture considerably reduced ORs of non-β-lactam antibiotics, especially fluoroquinolones.[3]

4.4. STUDY IV (OUTCOME)

1,018 patients hospitalised with community-onset ESBL-producing infectious episodes (224 with bacteraemia and 749 with urinary tract infection) were accessible for follow-up and compared with 23,500 infectious episodes (4,341 with bacteraemia and 19,159 with urinary tract infection) in non-ESBL controls. The crude mortality appeared to be slightly higher among patients with ESBL-producing infections than among non-ESBL controls (Table 5). The exception was *K. pneumoniae* bacteraemia where none of 34 patients with ESBL-producing isolates experienced neither in-hospital or 30-day mortality. Of importance (and to our surprise), patients with community-onset ESBL-producing *K. pneumoniae* (n=34) bacteraemia differed on important predictors of mortality compared with the corresponding non-ESBL cohort (n=700); they were younger (median age 68 years [IQR; 59-84] versus 73 years [IQR, 63-82]), more often the focus of infection was the urinary tract (58.8% versus 42.6%),

and they less often had polybacteraemia (18.2% versus 26.3%).[4] Nonetheless, associations were attenuated following adjustment (Table 5). Neither ESBL production nor inappropriate empirical antibiotic treatment was associated with death. Independent predictors of death included polybacteraemia, non-urinary tract infection focus, high comorbidity and HCA infections.[4]

Table 5. Crude and adjusted 30-day mortality in patients hospitalised with first-time community-onset ESBL – and non-ESBL-producing *Escherichia coli* or *Klebsiella pneumoniae* bacteraemia and urinary tract infection (modified from Richelsen et al., J. Antimicrob. Chemother, 2020).[4]

	ESBL, % (95% CI)	Non-ESBL, % (95% CI)	cMRR[1] (95% CI)	aMRR[2] (95% CI)
E. coli				
Bacteraemia	15.8 (11.3.-21.8)	14.0 (12.9-15.2)	1.13 (0.78-1.63)	1.01 (0.70-1.45)
UTI	9.5 (7.5-12.1)	8.7 (8.3-9.2)	1.10 (0.85-1.42)	0.97 (0.75-1.26)
K. pneumoniae				
Bacteraemia	0 (0-10.2)[3]	17.2 (14.6-20.2)	-	-
UTI	13.8 (9.3-20.2)	10.7 (9.6-12.0)	1.33 (0.86-2.06)	1.13 (0.73-1.75)

Abbreviations: MRR, Mortality rate ratios; UTI, Urinary tract infection.
[1] Crude MRR: Estimated as hazard ratios using a Cox proportional hazards model (non-ESBL as reference).
[2] Adjusted MRR: Adjusted for age, sex and modified Charlson Comorbidity Index score.
[3] Estimated on basis of only 34 patients of whom no one experienced death within 30-days using the Wilson score.[99]

LOS appeared to be slightly longer among patients with community-onset ESBL-producing *E. coli* bacteremia, who had an adjusted hazard ratio of being discharged of 0.79 (95% CI; 0.68-0.93) compared with patients with non-ESBL-producing *E. coli* bacteraemia. The difference in LOS is visible from the cumulative incidence function (Figure 7). For example, after 10 days, only 51% of the patients hospitalised with ESBL-producing *E. coli* bacteraemia were discharged alive compared with 64% of patients with non-ESBL-producing isolates (Figure 7).

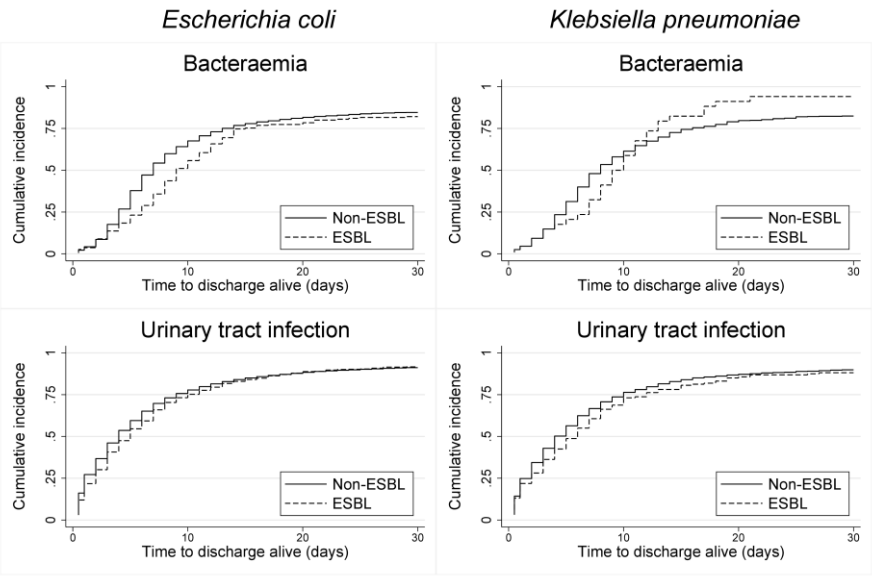


Figure 7. Cumulative incidence function of length of hospital stay (up to 30 days) following hospitalization with community-onset *Escherichia coli* and *Klebsiella pneumoniae* bacteraemia and urinary tract infection with ESBL – and non-ESBL-producing isolates. Death was considered a competing risk of being discharged alive.

CHAPTER 5. DISCUSSION

5.1. COMPARISON WITH OTHER STUDIES

Study I

The increasing incidence observed in our study were comparable with the incidence reported by Isendahl et al.[59] Thus, we reported an incidence of first-time community-onset ESBL *E. coli* and *K. pneumoniae* bacteraemias of 6.3 per 100,000 person years in 2017,[1] which was almost identical to the nationwide incidence of 6.0 per 100,000 person years in 2016-2017 in Sweden (i.e. Enterobacteriaceae bacteraemias whereof *E. coli* and *K. pneumoniae* accounted for the vast majority).[59] Few other studies have addressed temporal changes in the incidence rate of ESBL-producing bacteria in the community.[100,101] In Thailand, the incidence of community-onset ESBL-producing *E. coli* bacteraemia increased from 5.4 in 2008 to 12.8 per 100,000 person years in 2014,[100] twice the rate as in our population in 2017. In Minnesota (US), the incidence of extended-spectrum cephalosporin resistance *E. coli* bacteriuria increased from 2005-2009. As in our population, this increase was mainly observed in the elderly and in the community, e.g. the incidence of community-associated bacteriuria (excluding HCA) increased from 6 per 100,000 person years in 2005 to 14 per 100,000 person years in 2009.[101] The overall proportion of ESBL production in community-onset *E. coli* and *K. pneumoniae* infections was 4.9% in the North Denmark Region in 2017, which roughly corresponds to the results obtained from national Danish data,[44] and the increasing proportion is quite similar to that reported in studies from Norway[50] and the other Nordic countries as outlined in Table 1 and Table 2. In addition to reflecting the close proximity to these countries, the similarities probably also reflect similarities in healthcare systems, infection control and conservative antibiotic policies, i.e. antibiotics use requires a prescription. We were not able to report the genotypic characteristics of the ESBLs found in our study; however, national data from 2017 show that among 3rd-GCR *E coli* blood isolates, CTX-M-15 was the most prevalent (49%) enzyme followed by CTX-M-14 and CTX-M-27 (15% each).[44] These proportions are comparable with the distribution reported in Norway, whereas Sweden has a slightly higher proportion of CTX-M-15 (60%).[102]

Study II

We confirmed that many of the traditional risk factors of community-onset ESBL-producing infections (Table 3) were characteristic of our low-prevalence region, including recent and numerous healthcare contacts and use of antibiotics, especially fluoroquinolones.[2] Nonetheless, as illustrated in Figure 7, 22.0% of patients with ESBL-producing bacteraemia had none of the five most common risk factors, while almost two thirds of the patients with non-ESBL-producing bacteraemia had ≥1 risk factor; hence, identifying these patients by their risk factors might prove difficult. A

number of studies have aimed to predict the risk of ESBL-producing/3rd-GCR infections. Thus, prior resistant infection within recent years has repeatedly been found to be one of the strongest predictors.[67,69,72,103–108] A large international prospective study[109] recently validated a community-onset prediction rule ("the Utrecht score") of 3rd-GCR Enterobacterales bacteraemia including six variables developed by Rottier et al.[107] Model calibration was good and the prediction rule could improve empirical antibiotic use in community-onset infections.[109] Fröding et al.[69] applied the same prediction rule and compared it with their own "Stockholm score", which consisted of only three variables (prior ESBL-producing Enterobacterales infection, prior healthcare abroad, prior prostate biopsy) and demonstrated a comparable sensitivity but a higher specificity, emphasizing the need for using local data when implementing prediction models.[69] Likewise, the three variables; hospital care abroad, previous ESBL-positive urine and/or blood culture and ESBL-positive rectal swabs were found significant associated with 3rd-GCR Enterobacterales bacteraemia in another recent prediction study from Sweden.[51] In our study, previous genitourinary surgery was not associated with ESBL production compared with non-ESBL controls. Nor were prior ESBL-positive cultures nor healthcare abroad included as data on these variables was not readily available. A recent Danish study showed a 4.9% prevalence of ESBL colonization (rectum, nose or throat) in patients acutely admitted to the emergency department, and identified antibiotic consumption and travel activity to Asia/Oceania as main risk factors.[56] Prior studies should incorporate these variables.

This is the first study in Denmark to examine risk factors of community-onset ESBL-producing bacteraemia. A previous study from within our region investigated risk factors of EBSL-producing ESBL *E. coli* urinary tract infection in primary care.[62] Recent and numerous hospitalizations and exposure to antibiotics as well as male sex and a high Charlson Comorbidity Index score were common risk factors. However, use of nitrofurantoin, cancer, chronic pulmonary disease and renal disease were associated with ESBL-producing *E. coli* urinary tract infection in primary care, none of which were found to be risk factors in our study.[2] Also, using a triple-case-control design, Hertz et al. only identified hospital admission and fluoroquinolone use as risk factors of ESBL *E. coli* urinary tract infection in primary care in Denmark.[110] We inferred that many of the risk factors of ESBL-producing bacteraemia would probably be common to patients with urinary tract infections, as *E. coli* and *K. pneumoniae* are primarily uropathogens. However, this inference should be drawn with caution.

From a clinical perspective when deciding upon empirical antibiotic therapy, it is not possible to distinguish between an *E. coli* or *K. pneumoniae*. This should be reflected in the ascertained risk factors. Therefore, in line with several studies (Table 3), we decided to combine the two bacteria. However, we acknowledge that these two bacteria represents different epidemiological entities.[1,111–113] Thus, for infection control practices, tailoring of infection control measures to the specific bacteria should be considered.[112]

Study III

To our knowledge, this is the first study of community-onset ESBL bacteraemia specifically to examine the influence of different methodologies on the risk factor estimates. However, as mentioned above, several methodological studies have examined the association between antibiotic exposure and antimicrobial resistance. Harris et al. examined the importance of control group selection across three different bacteria, and found that, e.g., imipenem use was strongly associated with imipenem-resistant *Pseudomonas aeruginosa* compared with susceptible strains (OR 27.1 [95% CI; 13.9-52.9]); this resistance decreased compared with control patients in whom clinical cultures did not yield imipenem-resistant *P. aeruginosa* during their hospital stay (OR 6.3 [95% CI; 3.7-11.0]).[114] The crude OR of ESBL-producing bacteremia in patients exposed to fluoroquinolones was 17.25 [95% CI; 7.58-39.26] compared with population controls, when applying criteria 4. This was remarkably similar to the unadjusted OR of 15.95 [95% CI; 9.18-27.7] reported by Isendahl et al. when applying similar criteria (i.e. an antibiotic exposure duration of 8-91 days and excluding patients with a prior ESBL-positive culture).[59] This mutually strengthen our findings of fluoroquinolones as a risk factor of ESBL-producing bacteraemia. Nonetheless, when compared with non-ESBL controls the corresponding OR reduced to 2.89 [95% CI; 1.63-5.12], suggesting that much of this excess risk was merely mediated by the risk of bacteraemia rather than ESBL production. This, once again, highlights the impact of control group selection and the need of interpreting results carefully with respect to the selected control group(s). Isendahl et al. was one of only two studies[59,69] identified that excluded patients with prior ESBL-positive culture (supplementary material Table S1, Study III[3]) to avoid confounding by indication. However, this confounding is probably less pronounced in countries with a high ESBL prevalence and with a less restrictive use of fluoroquinolones. Also, only Isendahl et al.[59] examined and supported our finding that a shortening of the period of exposure to antibiotics increased the associated OR. However, this finding supports the proper choice of a 3-month exposure period utilized by almost all of the other studies in Table S1, Study III.[3] Nonetheless, in a recent Danish study previous use of antibiotics, especially fluoroquinolones, was associated with an increased risk of colonization for at least 2 years after treatment.[115]

Study IV

The 30-day mortality of 15.8% following community-onset ESBL *E. coli* bacteraemia found in our study falls within the range of 7.6-36% reported in other studies (Table 4), but it is higher than the 11.3% reported in the Swedish study by Isendahl et al.[59] Of notice, we included polybacteraemias, which might explain some of the excess mortality. Almost all studies reporting mortality following ESBL-producing infections focus on bacteraemia; yet in our cohort, ESBL *E. coli* urinary tract infection was associated with a 9.5% 30-day mortality. This is notably higher than the mortality of 1.33% (mortality period not specified) in patients with community-associated

(excluding HCA) ESBL-producing *E. coli* pyelonephritis.[82] While we cannot completely rule out that some of the patients in our cohort might actually have had a bacteremia that was never captured, the surprisingly high mortality probably mainly reflects the comorbid conditions of patients acquiring these infections.[4]

Our finding of an absence of excess mortality in the ESBL group is somewhat surprising as inappropriate empirical antibiotic treatment or/and delay in effective antibiotic treatment has repeatedly been shown to be associated with increased mortality;[116] a concept that fits well with our understanding of antibiotic treatment. In fact, why should we bother that much about community-onset ESBL-producing infections if they are no more dangerous than non-ESBL-producing infections? Nonetheless, our findings are far from unique, and several observational studies have been unable to demonstrate an excess mortality associated with ESBL-production (Table 4) or 3rd-GCR infections (most of which were ESBL producers).[117] Nonetheless, most of these studies adopted a statistical interpretation of results, while in the majority of studies ESBL production was associated with increased mortality, though not reaching significance. Thus, it is likely that many of the studies were underpowered to detect a "true" difference. Furthermore, in observational studies the outcome following ESBL-producing infections probably reflects a complex interaction of various variables that might be considered *both* intermediates and confounders, hereby complicating the interpretation of results even after adjustment (to be discussed below).[57,118]

Still, the results might likely be explained in other ways. Interestingly, a Swedish study demonstrated that community carriage of ESBL-producing *E. coli* was associated with low pathogenicity compared with ESBL-producing isolates causing invasive infections.[52] It might be speculated that invasive ESBL-producing infections too are associated with less virulent strains than non-ESBL-producing infections; a hypothesis that has been suggested in other studies.[119] Unfortunately, we were not able to support our findings with analysis of virulence genes. Another hypothesis is that empirical antibiotic treatment towards ESBL-producing infections may actually conserve some activity *in vivo*, though deemed "resistant" *in vitro.*[119] In our region, piperacillin/tazobactam with/without gentamicin is quite frequently used for empirical treatment of "severe sepsis of unknown origin". Piperacillin/tazobactam has been shown to retain some activity against ESBL infections, especially if used in high doses at concentrations above minimal inhibitory concentration for >55% of treatment time.[120] Our data did not allow us to account for the individual antibiotics dispensed in our study, but we assume that many of the patients with ESBL-producing bacteraemia would have been administered piperacillin/tazobactam upon admission. If reported as tested according to EUCAST clinical breakpoints piperacillin/tazobactam was susceptible in 85.3% of ESBL-producing *E. coli* blood isolates in 2017.[1] Furthermore, in observational studies comparing beta-lactam/beta-lactamase inhibitors (primarily piperacillin/tazobactam) with carbapenems in the treatment of ESBL-producing infections, no difference in

mortality was observed.[121] Despite these findings, in the MERINO trial, the only randomized controlled trial to date, piperacillin/tazobactam was found to be inferior to carbapenems in the definitive treatment of ceftriaxone-nonsusceptible *E. coli* and *Klebsiella* spp that tested susceptible to piperacillin/tazobactam.[122] Despite randomization, controversies about the MERINO trial exist, and it may be questioned whether these results are generalizable to our region.[123,124] A major issue with respect to the MERINO trial was that many of the isolates that tested susceptible to piperacillin/tazobactam at the participating sites were actually resistant by subsequent broth microdilution reference testing.[125] Lastly, despite all our efforts, we may speculate that our results as well as the results of other observational studies comparing mortality in patients with/-without ESBL-producing infections are hampered by some unmeasured variables or residual confounding as discussed below.

5.2. METHODOLOGICAL CONSIDERATIONS

"The purpose of clinical epidemiology is to develop and apply methods of clinical observations that will lead to a valid conclusion by avoiding being misled by systematic error and the play of chance", Robert H. Fletcher et al. [126]

Systematic and random error ("the play of chance") must be considered when evaluating the internal validity of observational studies. Systematic error is often categorized into selection bias, information bias or confounding, while random error refers to the statistical estimate of the precision. Random error, i.e. precision, will often be reduced if the sample size is increased, whereas systematic error will not.[96]

5.2.1. SELECTION BIAS

Selection bias is a systematic error arising from the procedures used to select study participants and from factors that influence participation into the study.[127] Consequently, the relation between exposure and outcome differs between those included (participants) and those excluded (non-participant) in the study, and the associations observed represents a mix of forces that determine participation and forces that determine disease occurrence.[127] The association between the exposure status and the outcome status in non-participants are rarely known; hence, selection bias must usually be inferred rather than observed.[96]

The population-based design and the setting of the Danish healthcare where unrestricted all members of the population receive tax-funded healthcare collectively ensured that we were able to capture most of the infectious episodes, hereby considerably limiting selection bias. Furthermore, the use of population-based (e.g.

NDRDB) and nationwide medical databases (e.g. the DNPR and DNPR*) with routinely collected data from the entire population considerably limits (to some extent exclude) selection bias.[128]

In **Study I,** we took account of the varying population at risk ("denominator") when estimating the incidence (Appendix A). As outlined in the supplementary material of Study I[1] and described above, the identification of ESBL ("the numerator") varied during the study period. Though a tremendous effort was put into correct identification of ESBL, differences in identification of ESBL are important when comparing with other studies as such differences may affect trends in incidence over time. Indeed, changes in culture methodology should also be considered when assessing time trends of bacteraemia.[129] Furthermore, the inclusion criteria by definition required that a blood or urine culture be performed. However, the thresholds and practices for blood and urine culturing might differ over time and across hospitals and specialities, among clinicians, and according to disease severity and comorbidity of the patient, etc. Especially with respect to urine specimens submitted by general practitioners, it is likely that samples from uncomplicated urinary tract infections are underrepresented; hence, a potential overestimation of resistance rates, e.g. ESBL-producing isolates, is possible.[130] In addition, even if a sample was collected, appropriate antibiotics administered before collection might prevent growth of susceptible bacteria and hence obscure microbiological detection. It should also be mentioned that some general practitioners do basic testing of specimen samples themselves, without involving the regional laboratory. Importantly, the definition of urinary tract infection was based solely on the presence of bacteria; and although we excluded urine samples with $<10^4$ colony forming units, we acknowledge that a proportion of these samples might represent colonization rather than infection. Therefore, the incidence of bacteraemia probably reflects the most precise incidence estimate, while the incidence rate of patients with urinary tract infections was less precise and probably *overestimated* due to inclusion of patients with asymptomatic bacteriuria.

In **Study II and III,** we utilized a case-control-control design which has previously been used in similar studies.[62,75,77] We chose a control group of population controls because our focus was on community-onset infections, and a control group with susceptible infections to disentangle risk factors from infection with risk factors that the strain was an ESBL-producing strain. As discussed in **Study III**[3]**,** it is notoriously difficult to ensure proper selection of control group(s) in these case-control "antimicrobial resistance studies" trying to isolate unique risk factors of ESBL production;[98,114] it is likely that using a control group with a susceptible strain has *overestimated* the associated risk of specific antibiotics as a risk factor as patients treated with an effective antibiotic are prevented from entering the non-ESBL control group.[97 98,114] Kaye et al.[131] developed the case-case-control study design, where two case groups (e.g. ESBL - and non-ESBL-producing *E. coli*) are compared with the same control group, and argue that this design is less flawed than the standard case-control design.[131] This design has subsequently been adopted and used in risk factor

studies of ESBL-producing infections, even in an extended version with a triple-case-control design.[110]

In **Study IV,** loss to follow-up might pose a problem of selection bias. However, the population-based design and the use of the CRS providing almost complete follow-up mitigate this problem in our cohort. Selection bias is of concern if the association between the exposure (ESBL-positive infections) and the outcome (mortality and LOS) differed between patients included and patients excluded in our study. The recommended treatment of patients with ESBL infections is carbapenems, which are only available at hospitals. Consequently, patients with ESBL-producing urinary tract infections might be admitted to the hospital for treatment, even though their clinical condition does not require hospitalization. This bias potentially reduced mortality in the ESBL group among patients with urinary tract infections. In addition, as mentioned above, patients with ESBL-producing *K. pneumoniae* bacteraemia experienced no 30-day mortality and differed on several important parameters from the non-ESBL cohort; thus, despite investigating and rethinking design meticulously, we speculate that some form of selection bias might have been introduced into this subgroup.

5.2.2. INFORMATION BIAS

Information bias might be introduced through collection of data if the exposed/diseased participants are classified as non-exposed/non-diseased participants. If measurement errors of exposure or outcome are randomly distributed among exposed and non-exposed participants, non-differential misclassification is introduced. This misclassification will most often (but not always) bias the estimate toward the null hypothesis. Differential misclassification occurs if a measurement error is unevenly distributed among exposed or unexposed participants, and this misclassification is directional, i.e. the misclassification might either increase or decrease any true association between exposure or outcome, and the direction might be difficult to predict.[127]

Study I. The selection bias described above in terms of "identification of ESBL" might also be considered a form of information bias, most of which would probably tend to be non-differential.

Studies II-IV. Misclassification of exposure, outcome and confounders might have been introduced in the register-based studies. However, all information on cases and controls was collected in the same way using nationwide registers with a high validity of data concerning admission and a high positive predictive value of discharged diagnoses (DNPR).[92] For hospitalised patients, we obtained data on comorbidity from one day prior to admission (and ten years back) to avoid including a diagnosis

recorded on the day of admission, which would not be available for the population controls, as this would potentially could lead to an overestimation of associated ORs. In Denmark, no antibiotics are available over the counter, and the data completeness in the DNPR* is generally considered to be very high. The DNPR*[93] measures *redeemed* prescriptions, which eliminates "primary non-compliance" (i.e. the prescription is not redeemed), and the natural motivation to get rid of infections makes us believe that redeemed antibiotics is a good surrogate marker for actual ingestion. A considerable limitation of antibiotic consumption in our study is the lack of data on antibiotics dispensed at the hospitals, resulting in a *reduced* exposure, which might hamper the results of the risk factor studies (Study II and III).[2] The virtually complete follow-up and recording of vital status on a daily basis in the CRS[91] ensures that misclassification of mortality in Study IV seems very unlikely. Finally, all data was collected irrespective of our study purposes, and data was retrieved in the same way for cases and controls; thus, any information bias is most likely non-differential leading to an underestimation of our results. However, some differential misclassification might have been introduced. For example, it is likely that physicians might be more attentive to patients with ESBL-producing infections. This might result in more "control" urinary samples and possibly the launch of a new treatment cause of antibiotics, or increased attention might result in more diagnoses being recorded among patients with exposure (ESBL-producing infections), i.e. surveillance bias.

5.2.3. CONFOUNDING

Confounding might be considered a distortion of effect, and arises if a variable associated with *both* exposure *and* outcome is imbalanced across exposure categories [96]. Confounding might be controlled for in several ways in both design (randomization, restriction, matching) and in analysis (stratification, adjustment, etc.). It is possible to adjust for measured confounders in the analyses. However, it is not possible to adjust for unmeasured and especially unknown confounders, i.e. residual confounding.[96] A variable on the causal pathway from exposure to outcome, i.e. an intermediate variable, is not a confounder. Consequently, intermediate variables should not be adjusted for.[127]

Study I was purely descriptive, with no exposure-outcome associations; hence, any confounding is highly unlikely.

In **Study II and III,** cases were matched by index date, age and gender to population controls; and by index date, specimen and bacteria (and gender – only **Study III**) to non-ESBL controls in order to eliminate confounding by these variables. Nonetheless, matching in case-control studies might introduce selection bias itself.[127] This selection bias behaves like a confounder; hence, of importance, the matching factor should be adjusted for in the analysis.[127] In **Study IV,** we *a priori* decided to adjust for age,

gender and comorbidity as these variables are well-established predictors of mortality. However, we were unable to adjust for unmeasured confounders, e.g. socioeconomic status[132] or history of travelling, which might be associated with both exposure and outcome. As discussed above, travelling to high-endemic ESBL regions is a risk factor of colonization. Yet, travelling generally "requires" a good health, and it is likely that patients who travels much are healthier than patients who do not travel, hereby reducing mortality in the travelling ESBL-exposed patients. Nonetheless, this *healthier-bias* effect might partly be "offset" by adjusting for comorbidities and age. A subtle question arises in outcome studies of antimicrobial resistance as discussed in Study IV;[4] how to properly take account of "inappropriate empirical antibiotic therapy" and "severity of infection" - intermediates variables or confounders?.[133–135] This issue is reflected and described in Appendix D. In Study IV, we provided sensitivity analyses by subsequently adjusting for focus of infection, inappropriate antibiotic therapy and polybacteraemia. We lacked data on severity of illness and were hence unable to include this variable into the sensitivity analysis.[4]

5.2.4. PRECISION

We expressed statistical precision by 95% CIs, thereby providing information of both the magnitude and the degree of precision (size of 95% CI) instead of using significance tests and associated p-values.[136] Moreover, we tried to avoid the common misconception of treating the CI as a significance test anyway, i.e. whether it contains the null value or not, and we aimed at reporting our results as associations rather than "true" casuals relationship as determined by a "significance test".[96,137] However, significant tests as well as CIs depends on denominator data and are meant to guard against sampling error and might perform poorly when the whole population is included.[138] In addition, a high precision cannot correct any systematic error introduced by study design.

5.3. MAIN CONCLUSIONS

Based on the results and the outlined methodological considerations, the main conclusions in the four studies were:

Study I

We demonstrated a marked increase in the incidence of community-onset ESBL-positive *E. coli* or *K. pneumoniae* infections during the study period. This increase was driven mainly by *E. coli* urinary tract infections. A shift in place of acquisition was observed throughout the study, with strictly community-associated infections

becoming more prevalent than HCA infections by the end of the study period. In 2017, 5.6% of *E. coli* and 13% of *K. pneumoniae* bloodstream isolates were ESBL producers, and co-resistance in ESBL-producing isolates was considerably.[1]

Study II

We confirmed established risk factors of ESBL-producing infections in our region where the prevalence of ESBL infections is traditionally low, and empirical antibiotic therapy covering ESBL-producing infections should be considered especially in patients with recent and numerous hospital admissions and antibiotic exposure, especially fluoroquinolones. A prior ESBL-positive culture and recent travel abroad to countries with a high prevalence of ESBL are well-established risk factors in other similar studies and should also be taken into account, though we did not address these exposures in Study II.

Study III

Control group selection highly influences the associated ORs; however, controversies as to which is the appropriate control group remains and this depends on the specific question addressed. Shortening the antibiotic exposure duration from 1 year to 3 months increases the associated ORs, and choosing an exposure duration of 3 months seems appropriate. Antimicrobial resistance studies are prone to confounding by indication, and sensitivity analysis in/excluding patients with a prior ESBL-positive culture may provide information as to if such confounding is present. Reverse causation, i.e. including antibiotics given in response to the present infection seemed to be of minor concern.

Study IV

Somewhat surprisingly, we found that patients with ESBL-producing isolates did not experience an increased mortality compared with patients with ESBL-negative isolates. The results were robust when adjusting for focus of infection, inappropriate empirical antibiotic therapy and polybacteraemia. Methodological challenges of observational outcome studies might influence our results. Nonetheless, the similarity of mortality proportions may be explained by a possible *in vivo* effect of piperacillin/tazobactam treatment in patients with ESBL-producing isolates, an immediate resuscitation of septic shock patients by supportive treatment rather than empirical antibiotics[139], and the fact that virtually all patients (if surviving initially) were treated definitively with appropriate antibiotics[140] (as carbapenem resistance in *E. coli* and *K. pneumoniae* is almost non-existent in our region). These results favour a conservative empirical antibiotic approach, i.e. piperacillin/tazobactam seems a reasonable choice in most circumstances. Nonetheless, critically ill patients, patients with high-inoculum infections, and patients with *a priori* high risk of ESBL-producing invasive infections should preferable be treated with carbapenems.[141]

CHAPTER 6. CLINICAL IMPLICATIONS AND PERSPECTIVES

This thesis extends our knowledge of the incidence, risk factors and prognosis of ESBL-producing infections within our region where the prevalence of antimicrobial resistance is low, and elaborates upon some of the methodological challenges in studies of antimicrobial resistance.

We confirmed an increasing incidence of community-onset *E. coli* and *K. pneumoniae* infections, consistent with the successful dissemination of ESBL-producing *E. coli* strains into the community since the millennium.

In the absence of a rapid point-of-care test of antimicrobial resistance, identification of patients at risk remains important when opting for empirical antibiotic therapy [69]. We confirmed the presence of common risk factors of ESBL-producing bacteraemia. These risk factors include exposure to antibiotics, especially fluoroquinolones, ≥3 antibiotics prescriptions within the past year, admission within 92 days, ≥3 admissions within past year, and male sex; these risk factors should raise awareness of the possible presence of ESBL-producing infections in these patients. Thus, when opting for empirical antibiotic therapy, clinicians should pay attention to these specific risk factors. However, many of these risk factors are common to patients admitted to hospital with suspected infection, and indiscriminate use of carbapenems would result in overuse. Prospective prediction studies including these risk factors and also data on prior ESBL-positive cultures (of any origin), prostate biopsy and recent travelling to high-endemic ESBL regions are warranted. However, these studies are very expensive and time consuming; in lack of such studies, guidance from this study as well as other studies with a similar prevalence, ESBL epidemiology and healthcare setting might prove useful.

We were not able to demonstrate any excess mortality in patients infected with an ESBL-producing strain. Lack of information on "severity of illness" and lack of information on specific empirical and definitive antibiotic treatment makes it difficult to draw firm conclusions. Still, from current evidence, it seems reasonable to conclude that empirical carbapenems should be restricted to severely ill patients or to patients with a high *a priori* risk of ESBL-infection, while awaiting further results of treatment recommendations in specific subpopulations.[142] A proportion of ESBL-producing *E. coli* or *K. pneumoniae* blood isolates around 6.0% in 2017 further justifies a restrictive use of broad-spectrum antibiotics. Nevertheless, data from the only randomized trial to date did not support the use of piperacillin/tazobactam compared with carbapenems in the treatment of ceftriaxone-resistant *E. coli* or *K. pneumoniae*.[122] Whether these results are applicable to our region remains questionable, and an RCT assessing this outcome in our population would be highly appreciated. However, this would be very

expensive; and in light of results of the previous RCT, it is unlikely to be conducted in the near future.

Nationwide and population-based healthcare registries provide researchers with a unique, inexpensive and time-sparing opportunity to conduct research studies. Nonetheless, a major drawback of such research is the lack of information that cannot be accessed through registries, e.g. information of important risk factors and "severity of illness" at admission. Also, "excessive" use of registries is prone to spurious findings with vague biological associations, and the databases should be used with caution.[128,138]

Finally, methodological heterogeneity among previous studies, e.g. the chosen design in risk factors studies, including the diversity of criteria for assessing antibiotic exposure and adjustment for confounders in outcome studies, might influence the estimated results and any conclusions drawn, emphasizing the challenges and difficulties in interpretation of these studies. In line with this, it would be interesting to conduct a case-case-control study of risk factors using the same base population as in our case-control-control risk factor study to reveal discrepancies in associated risk factors by these designs.

Whether we are approaching a post-hoc antibiotic era remains a question unanswered. Development of new effective antibiotics is indeed warranted; however, even new antibiotics will merely provide a much-needed breathing space, as almost 80 years of antibiotic use has demonstrated that so far resistance has developed towards all deployed antibiotics. Thus, restrictive use of antibiotics and proper infection control remain cornerstones in the fight against antibiotic resistance, and antibiotic stewardship should remain a focus of high priority.

REFERENCES

[1] Richelsen R, Smit J, Anru PL, Schønheyder HC, Nielsen H. Incidence of community-onset extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae infections: an 11-year population-based study in Denmark. Infect Dis (Auckl) 2020;52:547–56.

[2] Richelsen R, Smit J, Anru PL, Schønheyder HC, Nielsen H. Risk factors of community-onset extended-spectrum β-lactamase Escherichia coli and Klebsiella pneumoniae bacteraemia: an 11-year population-based case-control-control study in Denmark. Clin Microbiol Infect 2020:[Online ahead of print].

[3] Richelsen R, Smit J, Nielsen H. Impact of chosen methodology when evaluating antibiotic exposure as a risk factor of community-onset extended-spectrum β-lactamase-producing Escherichia coli or Klebsiella pneumoniae bacteraemia: A population-based Danish case-control-control study. Int J Antimicrob Agents 2020:[submitted].

[4] Richelsen R, Smit J, Schønheyder HC, Laxsen Anru P, Gutiérrez-Gutiérrez B, Rodríguez-Bãno J, et al. Outcome of community-onset ESBL-producing Escherichia coli and Klebsiella pneumoniae bacteraemia and urinary tract infection: a population-based cohort study in Denmark. J Antimicrob Chemother 2020:[Online ahead of print].

[5] Barber M. Staphylococcal Infection Due To Penicillin-Resistant Strains. Br Med J 1947;2:863–5.

[6] Fleming A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzae. Br J Exp Pathol 1929;10:226–36.

[7] Clatworthy AE, Pierson E, Hung DT. Targeting virulence: A new paradigm for antimicrobial therapy. Nat Chem Biol 2007;3:541–8.

[8] Tacconelli E, Carrara E, Savoldi A, Kattula D, Burkert F. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development og new antibiotics. World Heal Organ 2017.

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

[10] Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, et al. Prevalence and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect 2008;14:144–53.

[11] Livermore DM. Current Epidemiology and Growing Resistance of Gram-Negative Pathogens. Korean J Intern Med 2012;27:128–42.

[12] Echols RM, Tosiello RL, Haverstock DC, Tice AD. Demographic, Clinical, and Treatment Parameters Influencing the Outcome of Acute Cystitis. Clin Infect Dis 1999;29:113–9.

[13] Czaja CA, Scholes D, Hooton TM, Stamm WE. Population-Based Epidemiologic Analysis of Acute Pyelonephritis. Clin Infect Dis 2007;45:273–80.

[14] Laupland KB, Church DL. Population-Based Epidemiology and Microbiology of Community-Onset Bloodstream Infections. Clin Microbiol Rev 2014;27:647–64.

[15] Wilson J, Elgohari S, Livermore DM, Cookson B, Johnson A, Lamagni T, et al. Trends among pathogens reported as causing bacteraemia in England, 2004–2008. Clin Microbiol Infect 2010;17:451–8.

[16] Uslan DZ, Crane SJ, Steckelberg JM, Cockerill FR, Sauver JL St., Wilson WR, et al. Age- and Sex-Associated Trends in Bloodstream Infection. Arch Intern Med 2007;167:834–9.

[17] de Kraker M, Jarlier V, Monen J, Heuer O, 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;19:860–8.

[18] Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, et al. Health Care-Associated Bloodstream Infections in Adults: A Reason To Change the Accepted Definition of Community-Acquired infections. Ann Intern Med 2002;137:791–7.

[19] Kennedy KJ, Roberts JL, Collignon PJ. Escherichia coli bacteraemia in Canberra: Incidence and clinical features. Med J Aust 2008;188:209–13.

[20] Laupland KB, Gregson DB, Church DL, Ross T, Pitout JDD. Incidence, risk factors and outcomes of Escherichia coli bloodstream infections in a large Canadian region. Clin Microbiol Infect 2008;14:1041–7.

[21] Williamson DA, Lim A, Wiles S, Roberts SA, Freeman JT. Population-based incidence and comparative demographics of community-associated and

healthcare-associated Escherichia coli bloodstream infection in Auckland, New Zealand, 2005-2011. BMC Infect Dis 2013;13.

[22] Søgaard M, Nørgaard M, Dethlefsen C, Schønheyder HC. Temporal changes in the incidence and 30-day mortality associated with bacteremia in hospitalized patients from 1992 through 2006: A population-based cohort study. Clin Infect Dis 2011;52:61–9.

[23] Bush K. Past and Present Perspectives on β-Lactamases. Antimicrob Agents Chemother 2018;62:e01076-18.

[24] Abraham EP, Chain E. An Enzyme from Bacteria able to Destroy penicillin. Nature 1940;146:837.

[25] Paterson DL, Bonomo RA. Extended-Spectrum ß-Lactamases: a Clinical Update. Clin Microbiol Rev 2005;18:657–86.

[26] Giske CG, Sundsfjord AS, Kahlmeter G, Woodford N, Nordmann P, Paterson DL, et al. Redefining extended-spectrum β-lactamases: Balancing science and clinical need. J Antimicrob Chemother 2009;63:1–4.

[27] Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother 2010;54:969–76.

[28] Vasoo S, Barreto JN, Tosh PK. Emerging Issues in Gram-Negative Bacterial Resistance: An Update for the Practicing Clinician. Mayo Clin Proc 2015;90:295–403.

[29] Ambler RP, Coulson AF, Frère JM, Ghuysen JM, Joris B, Forsman M, et al. A standard numbering scheme for the class A β-lactamases. Biochem J 1991;276:269–72.

[30] Bush K, Jacoby GA, Medeiros AA. A Functional Classification Scheme for β-Lactamases and Its Correlation with Molecular Structure. Antimicrob Agents Chemother 1995;39:1211–33.

[31] Datta N, Kontomichalou P. Penicillinase Synthesis Controlled By Infectious R Factors In Enterobacteriaceae. Nature 1965;208:239–41.

[32] Sirot D, Sirot J, Labia R, Morand A, Courvalin P, Darfeuille-Michaud A, et al. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. J Antimicrob Chemother 1987;20:323–34.

[33] Philippon A, Labia R, Jacoby G. Extended-Spectrum β-Lactamases. Antimicrob Agents Chemother 1989;33:1131–6.

[34] Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 2007;59:165–74.

[35] Rogers BA, Sidjabat HE, Paterson DL. Escherichia coli O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother 2011;66:1–14.

[36] Rodríguez-Baño J, Navarro MD, Romero L, Muniain MA, Perea EJ, Pérez-Cano R, et al. Clinical and Molecular Epidemiology of Extended-Spectrum β-Lactamase-Producing Escherichia coli as a Cause of Nosocomial Infection or Colonization: Implications for Control. Clin Infect Dis 2006;42:37–45.

[37] Woerther P-L, Andremont A, Kantele A. Travel-acquired ESBL-producing Enterobacteriaceae: impact of colonization at individual and community level. J Travel Med 2017;24:S29–34.

[38] Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: Toward the globalization of CTX-M. Clin Microbiol Rev 2013;26:744–58.

[39] Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 2008;13:1–11.

[40] Doi Y, Iovleva A, Bonomo RA. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. J Travel Med 2017;24:S44–51.

[41] Teresa M. Coque, Ângela Novais, Alessandra Carattoli, Laurent Poirel, Johann Pitout, Luísa Peixe, et al. Dissemination of Clonally related Escherichia coli strains Expressing Extended-Spectrum β-lactamase CTX-M-15. Emerg Infect Dis 2008;14:195–200.

[42] Ne Nicolas-Chanoine M-H, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Caniça MM, et al. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother 2008;61:273–81.

[43] D'Andrea MM, Arena F, Pallecchi L, Rossolini GM. CTX-M-type β-lactamases: A successful story of antibiotic resistance. Int J Med Microbiol 2013;303:305–17.

[44] The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. DANMAP 2017 -Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark 2018:1–152.

[45] Hansen SK, Kaya H, Roer L, Hansen F, Skovgaard S, Justesen US, et al. Molecular characterization of Danish ESBL/AmpC-producing Klebsiella pneumoniae from bloodstream infections, 2018. J Glob Antimicrob Resist 2020;22:562–7.

[46] Vlieghe ER, Huang TD, Phe T, Bogaerts P, Berhin C, De Smet B, et al. Prevalence and distribution of beta-lactamase coding genes in third-generation cephalosporin-resistant Enterobacteriaceae from bloodstream infections in Cambodia. Eur J Clin Microbiol Infect Dis 2015;34:1223–9.

[47] Alagesan M, Gopalakrishnan R, Panchatcharam SN, Dorairajan S, Ananth TM, Venkatasubramanian R. A decade of change in susceptibility patterns of Gram-negative blood culture isolates: A single center study. Germs 2015;5:65–77.

[48] Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization with Extended-spectrum Beta-lactamase-Producing Enterobacteriaceae and Risk Factors among Healthy Individuals: A Systematic Review and Metaanalysis. Clin Infect Dis 2016;63:310–8.

[49] European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: 2017.

[50] Mylvaganam H, Kolstad H, Breistein RI, Lind G, Skutlaberg DH. Extended spectrum cephalosporin resistance among clinical isolates of Enterobacteriaceae in West Norway during 2006-2013; a prospective surveillance study. APMIS 2017;125:52–8.

[51] Holmgren A, Ljung A, Bremell D. An easy-to-use scoring system for predicting bacteraemia with third-generation cephalosporin-resistant Enterobacterales in a low-resistance setting. Infect Dis (Auckl) 2020;52:242–8.

[52] Ny S, Lö Fmark S, Bö Rjesson S, Englund S, Ringman M, Bergströ 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;72:582–8.

[53] Ulstad CR, Solheim M, Berg S, Lindbæk M, Dahle UR, Wester AL. Carriage of ESBL/AmpC-producing or ciprofloxacin non-susceptible Escherichia coli and Klebsiella spp. in healthy people in Norway. Antimicrob Resist Infect Control 2016;5.

[54] Strömdahl H, Tham J, Melander E, Walder M, Edquist PJ, Odenholt I.

Prevalence of faecal ESBL carriage in the community and in a hospital setting in a county of Southern Sweden. Eur J Clin Microbiol Infect Dis 2011;30:1159–62.

[55] Hammerum AM, Lester CH, Jakobsen L, Porsbo LJ. Faecal carriage of extended-spectrum β-lactamase-producing and AmpC β-lactamase-producing bacteria among Danish army recruits. Clin Microbiol Infect 2011;17:566–8.

[56] Skjøt-Arkil H, Mogensen CB, Lassen AT, Johansen IS, Chen M, Petersen P, et al. Carrier prevalence and risk factors for colonisation of multiresistant bacteria in Danish emergency departments: A cross-sectional survey. BMJ Open 2019;9:e029000.

[57] Trecarichi EM, Cauda R, Tumbarello M. Detecting risk and predicting patient mortality in patients with extended-spectrum β-lactamase-producing Enterobacteriaceae bloodstream infections. Future Microbiol 2012;7:1173–89.

[58] Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. BMC Med Inform Decis Mak 2007;7:1–6.

[59] Isendahl J, Ternhag A, Tegmark Wisell K, Giske CG, Nauclér P. Risk factors for community-onset bloodstream infection with extended-spectrum β-lactamase-producing Enterobacteriaceae: national population-based case–control study. Clin Microbiol Infect 2019;25:1408–14.

[60] Jansåker F, Boel JB, Thønnings S, Hertz FB, Hansen KH, Frimodt-Møller N, et al. Pivmecillinam compared to other antimicrobials for community-acquired urinary tract infections with escherichia coli, ESBL-producing or not – a retrospective cohort study. Infect Drug Resist 2019;12:1691–702.

[61] 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.

[62] Søgaard M, Heide-Jørgensen U, Vandenbroucke JP, Schønheyder HC, Vandenbroucke-Grauls CM. Risk factors for extended-spectrum β-lactamase-producing Escherichia coli urinary tract infection in the community in Denmark: a case-control study. Clin Microbiol Infect 2017;23:952–60.

[63] Helldal L, Karami N, Florén K, Welinder-Olsson C, Moore ERB, Åhrén C. Shift of CTX-M genotypes has determined the increased prevalence of extended-spectrum β-lactamase-producing Escherichia coli in south-western Sweden. Clin Microbiol Infect 2013;19:E87–90.

[64] Søraas A, Sundsfjord A, Sandven I, Brunborg C, Jenum PA. Risk Factors for Community-Acquired Urinary Tract Infections Caused by ESBL-Producing Enterobacteriaceae-A Case-Control Study in a Low Prevalence Country. PLoS One 2013;8:e69581.

[65] Schrøder Hansen D, Schumacher H, Hansen F, Stegger M, Boëtius Hertz F, Schønning K, et al. Extended-spectrum β-lactamase (ESBL) in Danish clinical isolates of Escherichia coli and Klebsiella pneumoniae: Prevalence, β-lactamase distribution, phylogroups, and co-resistance. Scand J Infect Dis 2012;44:174–81.

[66] Lindback H, Lindback J, Sylvan S, Melhus Å. Low frequency of antibiotic resistance among urine isolates of Escherichia coli in the community, despite a major hospital outbreak with Klebsiella pneumoniae producing CTX-M-15 in Uppsala County. Scand J Infect Dis 2010;42:243–8.

[67] Kim M, Song KH, Kim CJ, Choe PG, Park WB, Bang JH, et al. Clinical prediction score for community-onset bloodstream infections caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species. J Korean Med Sci 2019;34:e116.

[68] Lee Y, Kim YA, Kim D, Shin JH, Uh Y, Shin KS, et al. Risk factors of community-onset extended-spectrum β-lactamase-producing Klebsiella pneumoniae bacteraemia in South Korea using national health insurance claims data. Int J Antimicrob Agents 2019;54:723–7.

[69] Fröding I, Karlsson J, Bolinder L, Nauclér P, Giske CG. Prediction of bloodstream infection caused by extended-spectrum β-lactamase-producing Enterobacterales in patients with suspected community-onset sepsis. Int J Antimicrob Agents 2019;53:820–9.

[70] Gottesman BS, Shitrit P, Katzir M, Chowers M. Antibiotic exposure in the community and resistance patterns of Escherichia coli community-acquired bloodstream infection. Isr Med Assoc J 2018;20:382–4.

[71] Quan J, Zhao D, Liu L, Chen Y, Zhou J, Jiang Y, et al. High prevalence of ESBL-producing Escherichia coli and Klebsiella pneumoniae in community-onset bloodstream infections in China. J Antimicrob Chemother 2017;72:273–80.

[72] Lee CH, Chu FY, Hsieh CC, Hong MY, Chi CH, Ko WC, et al. A simple scoring algorithm predicting extended-spectrum β-lactamase producers in adults with community-onset monomicrobial Enterobacteriaceae bacteremia. Med (United States) 2017;96:e6648.

[73] Zahar J-R, Lesprit P, Ruckly S, Eden A, Hikombo H, Bernard L, et al.

Predominance of healthcare-associated cases among episodes of community-onset bacteraemia due to extended-spectrum β-lactamase-producing Enterobacteriaceae. Int J Antimicrob Agents 2017;49:67–73.

[74] Soo Park Y, Kwon Bae I, Kim J, Hoon Jeong S, Hwang S, Seo Y-H, et al. Risk Factors and Molecular Epidemiology of Community-Onset Extended-Spectrum β-Lactamase-Producing Escherichia coli Bacteremia. Yonsei Med J 2014;55:467–75.

[75] Park SH, Choi S-M, Lee D-G, Kim J, Choi J-H, Kim S-H, et al. Emergence of Extended-Spectrum β-Lactamase-Producing Escherichia coli as a Cause of Community-Onset Bacteremia in South Korea: Risk Factors and Clinical Outcomes. Microb Drug Resist 2011;14:537–44.

[76] Lee JA, Kang CI, Joo EJ, Ha YE, Kang SJ, Park SY, et al. Epidemiology and clinical features of community-onset bacteremia caused by extended-spectrum β-lactamase-producing Klebsiella pneumoniae. Microb Drug Resist 2011;17:267–73.

[77] Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Ruíz M, Peña C, et al. Community-Onset Bacteremia Due to Extended-Spectrum β-Lactamase-Producing Escherichia coli: Risk Factors and Prognosis. Clin Infect Dis 2010;50:40–8.

[78] Hsieh CJ, Shen YH, Hwang KP. Clinical Implications, Risk Factors and Mortality Following Community-onset Bacteremia Caused by Extended-spectrum β-lactamase (ESBL) and non-ESBL Producing Escherichia coli. J Microbiol Immunol Infect 2010;43:240–8.

[79] Kang C-I, Song J-H, Ryeon Chung D, Ran Peck K, Soo Ko K, Yeom J-S, et al. Risk factors and treatment outcomes of community-onset bacteraemia caused by extended-spectrum β-lactamase-producing Escherichia coli. Int J Antimicrob Agents 2010;36:284–7.

[80] Yang YS, Ku CH, Lin JC, Shang ST, Chiu CH, Yeh KM, et al. Impact of Extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae on the Outcome of Community-onset Bacteremic Urinary Tract Infections. J Microbiol Immunol Infect 2010;43:194–9.

[81] Lee C-C, Lee C-H, Hong M-Y, Hsieh C-C, Tang H-J, Ko W-C. Propensity-matched analysis of the impact of extended-spectrum b-lactamase production on adults with community-onset Escherichia coli, Klebsiella species, and Proteus mirabilis bacteremia. Immunol Infect 2018;51:519–26.

[82] Park SH, Choi SM, Lee DG, Cho SY, Lee HJ, Choi JK, et al. Impact of extended-spectrum β-lactamase production on treatment outcomes of acute

pyelonephritis caused by escherichia coli in patients without health care-associated risk factors. Antimicrob Agents Chemother 2015;59:1962–8.

[83] Jean SS, Ko WC, Xie Y, Pawar V, Zhang D, Prajapati G, et al. Clinical characteristics of patients with community-acquired complicated intra-abdominal infections: a prospective, multicentre, observational study. Int J Antimicrob Agents 2014;44:222–8.

[84] Kim B, Kim J, Seo M-R, Wie S-H, Cho YK, Lim S-K, et al. Clinical characteristics of community-acquired acute pyelonephritis caused by ESBL-producing pathogens in South Korea. Infection 2013;41:603–12.

[85] Marchaim D, Gottesman T, Schwartz O, Korem M, Maor Y, Rahav G, et al. National multicenter study of predictors and outcomes of bacteremia upon hospital admission caused by Enterobacteriaceae producing extended-spectrum β-lactamases. Antimicrob Agents Chemother 2010;54:5099–104.

[86] Apisarnthanarak A, Kiratisin P, Mundy LM. Predictors of Mortality From Community-Onset Bloodstream Infections Due to Extended-Spectrum β-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae. Infect Control Hosp Epidemiol 2008;29:671–4.

[87] Apisarnthanarak A, Kiratisin P, Saifon P, Kitphati R, Dejsirilert S, Mundy LM, et al. Clinical and molecular epidemiology of community-onset, extended-spectrum β-lactamase-producing Escherichia coli infections in Thailand: A case-case-control study. Am J Infect Control 2007;35:606–12.

[88] Borer A, Gilad J, Menashe G, Peled N, Riesenberg K, Schlaeffer F. Extended-spectrum beta-lactamase-producing Enterobacteriaceae strains in community-acquired bacteremia in Southern Israel. Med Sci Monit 2002;8:CR44–7.

[89] Laupland KB. Defining the epidemiology of bloodstream infections: the "gold standard" of population-based assessment. Epidemiol Infect 2013;141:2149–57.

[90] Schønheyder HC, Søgaard M. Existing data sources for clinical epidemiology: The North Denmark Bacteremia Research Database. Clin Epidemiol 2010;2:171–8.

[91] Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 2014;29:541–9.

[92] Schmidt M, Schmidt SAJ, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: A review of content, data quality, and research potential. Clin Epidemiol 2015;7:449–90.

[93] Kildemoes HW, Sorensen HT, Hallas J. The Danish National Prescription Registry. Scand J Public Heal 2011;39:38–41.

[94] Pottegård A, Schmidt SAJ, Wallach-Kildemoes H, Sørensen HT, Hallas J, Schmidt M. Data resource profile: The Danish national prescription registry. Int J Epidemiol 2017;46:798-798f.

[95] Statistics Denmark. StatBank Denmark. Population and elections, 2019 [in Danish] n.d.

[96] Rothman KJ. Epidemiology. An introduction. New York: Oxford University Press Inc.; 2002.

[97] Schechner V, Temkin E, Harbarth S, Carmeli Y, Schwaber MJ. Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. Clin Microbiol Rev 2013;26:289–307.

[98] Harris AD, Karchmer TB, Carmeli Y, Samore MH. Methodological Principles of Case-Control Studies That Analyzed Risk Factors for Antibiotic Resistance: A Systematic Review. Clin Infect Dis 2001;32:1055–61.

[99] Edwin B W. Probable Inference , the Law of Succession , and Statistical Inference. J Am Stat Assoc 1927;22:209–12.

[100] Sawatwong P, Sapchookul P, Whistler T, Gregory CJ, Sangwichian O, Makprasert S, et al. High Burden of Extended-Spectrum β-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae Bacteremia in Older Adults: A Seven-Year Study in Two Rural Thai Provinces. Am J Trop Med Hyg 2019;100:943–51.

[101] Swami SK, Liesinger JT, Shah N, Baddour LM, Banerjee R. Incidence of antibiotic-resistant Escherichia coli bacteriuria according to age and location of onset: A population-based study from Olmsted County, Minnesota. Mayo Clin Proc 2012;87:753–9.

[102] Brolund A. Overview of ESBL-producing Enterobacteriaceae from a Nordic perspective. Infect Ecol Epidemiol 2014;4:24555.

[103] Weinstein EJ, Han JH, Lautenbach E, Nachamkin I, Garrigan C, Bilker WB, et al. A clinical prediction tool for extended-spectrum cephalosporin resistance in community-onset enterobacterales urinary tract infection. Open Forum Infect Dis 2019;6:1–6.

[104] Tumbarello M, Trecarichi EM, Bassetti M, De Rosa FG, Spanu T, Di Meco E, et al. Identifying patients harboring extended-spectrum-β-lactamase-producing Enterobacteriaceae on hospital admission: Derivation and

validation of a scoring system. Antimicrob Agents Chemother 2011;55:3485–90.

[105] Johnson SW, Anderson DJ, Byron D, Drew RH. Utility of a Clinical Risk Factor Scoring Model in Predicting Infection with Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae on Hospital Admission. Infect Control Hosp Epidemiol 2013;34:385–92.

[106] Cano A, Gutiérrez-Gutiérrez B, Machuca I, Gracia-Ahufinger I, Pérez-Nadales E, Causse M, et al. Risks of Infection and Mortality Among Patients Colonized With Klebsiella pneumoniae Carbapenemase-Producing K. pneumoniae: Validation of Scores and Proposal for Management. Clin Infect Dis 2018;66:1204–10.

[107] Rottier WC, van Werkhoven CH, Bamberg YRP, Dorigo-Zetsma JW, van de Garde EM, van Hees BC, et al. Development of diagnostic prediction tools for bacteraemia caused by third-generation cephalosporin-resistant enterobacteria in suspected bacterial infections: a nested case–control study. Clin Microbiol Infect 2018;24:1315–21.

[108] Augustine MR, Testerman TL, Justo JA, Bookstaver PB, Kohn J, Albrecht H, et al. Clinical Risk Score for Prediction of Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae in Bloodstream Isolates. Infect Control Hosp Epidemiol 2017;38:266–72.

[109] Deelen JTWT, Rottier WC, Ortega JAG, Rodriguez-Baño J, Harbarth S, Tacconelli E, et al. An international prospective cohort study to validate two prediction rules for infections caused by 3rd-generation cephalosporin-resistant Enterobacterales. Clin Infect Dis 2020:[ePub ahead of print].

[110] Boëtius Hertz F, Schønning K, Christian Rasmussen S, Littauer P, Dahl Knudsen J, Løbner-Olesen A, et al. Epidemiological factors associated with ESBL-and non ESBL-producing E. coli causing urinary tract infection in general practice. Infect Dis (Auckl) 2016;48:241–5.

[111] Sakellariou C, Gürntke S, Steinmetz I, Kohler C, Pfeifer Y, Gastmeier P, et al. Sepsis Caused by Extended-Spectrum Beta-Lactamase (ESBL)-Positive K. pneumoniae and E. coli: Comparison of Severity of Sepsis, Delay of Anti-Infective Therapy and ESBL Genotype. PLoS One 2016;11:e0158039.

[112] Freeman JT, Rubin J, McAuliffe GN, Peirano G, Roberts SA, Drinković D, et al. Differences in risk-factor profiles between patients with ESBL-producing Escherichia coli and Klebsiella pneumoniae: a multicentre case-case comparison study. Antimicrob Resist Infect Control 2014;3.

[113] Scheuerman O, Schechner V, Carmeli Y, Gutiérrez-Gutiérrez B, Calbo E,

Almirante B, et al. Comparison of Predictors and Mortality Between Bloodstream Infections Caused by ESBL-Producing Escherichia coli and ESBL-Producing Klebsiella pneumoniae. Infect Control Hosp Epidemiol 2018;39:660–7.

[114] Harris AD, Samore MH, Lipsitch M, Kaye KS, Perencevich E, Carmeli Y, et al. Control-Group Selection Importance in Studies of Antimicrobial Resistance: Examples Applied to Pseudomonas aeruginosa, Enterococci, and Escherichia coli. Clin Infect Dis 2002;34:1558–63.

[115] Dualleh N, Chanchiri I, Skjøt-Arkil H, Pedersen AK, Rosenvinge FS, Johansen IS. Colonization with multiresistant bacteria in acute hospital care: the association of prior antibiotic consumption as a risk factor. J Antimicrob Chemother 2020:[Epub ahead of print].

[116] Raman G, Avendano E, Berger S, Menon V. Appropriate initial antibiotic therapy in hospitalized patients with gram-negative infections: systematic review and meta-analysis. BMC Infect Dis 2015;15.

[117] Rottier WC, Deelen JWT, Caruana G, Buiting AGM, Dorigo-Zetsma JW, Kluytmans JAJW, et al. Attributable mortality of antibiotic resistance in Gram-negative infections in the Netherlands: a parallel matched cohort study. Clin Microbiol Infect 2020:[Epub ahead of print].

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

[119] de Lastours V, Laouénan C, Royer G, Carbonnelle E, Lepeule R, Esposito-Farèse M, et al. Mortality in Escherichia coli bloodstream infections: antibiotic resistance still does not make it. J Antimicrob Chemother 2020;75:2334–44.

[120] Abodakpi H, Chang K-T, Song G, Sánchez-Díaz AM, Cantón R, Tam VH. Optimal Piperacillin-Tazobactam Dosing Strategies against Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae. Antimicrob Agents Chemother 2019;63:e01906-18.

[121] Sfeir MM, Askin G, Christos P. Beta-lactam/beta-lactamase inhibitors versus carbapenem for bloodstream infections due to extended-spectrum beta-lactamase-producing Enterobacteriaceae: systematic review and meta-analysis. Int J Antimicrob Agents 2018;52:554–70.

[122] Harris PNA, Tambyah PA, Lye DC, Mo Y, Lee TH, Yilmaz M, et al. Effect of piperacillin-tazobactam vs meropenem on 30-day mortality for patients

with e coli or Klebsiella pneumoniae bloodstream infection and ceftriaxone resistance. JAMA - J Am Med Assoc 2018;320:984–94.

[123] Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Kahlmeter G. Antibiotics for Ceftriaxone-Resistant Gram-Negative Bacterial Bloodstream Infections. JAMA - J Am Med Assoc 2019;321:612–3.

[124] Grant J, Afra K. Point–counterpoint: The MERINO trial and what it should imply for future treatment of ESBL bacteremia. J Assoc Med Microbiol Infect Dis Canada 2019;4:125–35.

[125] Henderson A, Humphries R. Building a better test for piperacillin-tazobactam susceptibility testing: Would that it were so simple (It's complicated). J Clin Microbiol 2020;58:1–4.

[126] Fletcher RH, Fletcher SW, Fletcher GS. Clinical Epidemiology: The Essentials. 5th ed. Baltimore: Lippincoot Williams & Wilkins; 2012.

[127] Rothman KJ, Greenland S, Lash TL. Validity in epidemiologic studies. Modern Epidemiology. 3rd ed. Philadelphia, US: Lippincoot Williams & Wilkins; 2008.

[128] Thygesen LC, Ersbøll AK. When the entire population is the sample: Strengths and limitations in register-based epidemiology. Eur J Epidemiol 2014;29:551–8.

[129] Søgaard M, Engebjerg MC, Lundbye-Christensen S, Schønheyder HC. Changes in blood culture methodology have an impact on time trends of bacteraemia: A 26-year regional study. Epidemiol Infect 2011;139:772–6.

[130] Hillier S, Bell J, Heginbothom M, Roberts Z, Dunstan F, Howard A, et al. When do general practitioners request urine specimens for microbiology analysis? The applicability of antibiotic resistance surveillance based on routinely collected data. J Antimicrob Chemother 2006;58:1303–6.

[131] Kaye KS, Harris AD, Samore M, Carmeli Y. The case-case-control study design: addressing the limitations of risk factor studies for antimicrobial resistance. Infect Control Hosp Epidemiol 2005;26:346–51.

[132] Winkleby MA, Jatulis DE, Frank E, Fortmann SP. Socioeconomic status and health: How education, income, and occupation contribute to risk factors for cardiovascular disease. Am J Public Health 1992;82:816–20.

[133] Schwaber MJ, Carmeli Y. Antimicrobial resistance and patient outcomes: the hazards of adjustment. Crit Care 2006;10:164.

[134] Marcos M, Soriano A, Martinez JA, Mensa J. Septic Shock Should Be Included in Multivariable Models Analyzing the Effect of Empirical Antibiotic Therapy on Mortality. Clin Infect Dis 2007;45:1401.

[135] McGregor JC, Rich SE, Harris AD, Perencevich EN, Osih R, Lodise TP, et al. A Systematic Review of the Methods Used to Assess the Association between Appropriate Antibiotic Therapy and Mortality in Bacteremic Patients. Clin Infect Dis 2007;45:329–37.

[136] Amrhein V, Greenland S, McShane B. Retire statistical significance. Nature 2019;567:305–7.

[137] Rothman KJ. Six persistent research misconceptions. J Gen Intern Med 2014;29:1060–4.

[138] Leibovici L, Turjeman A, Paul M. The temptation of large numbers. Clin Microbiol Infect 2018;24:931–2.

[139] Lim CL, Spelman D. Mortality impact of empirical antimicrobial therapy in ESBL- and AmpC-producing Enterobacteriaceae bacteremia in an Australian tertiary hospital. Infect Dis Heal 2019;24:124–33.

[140] Joo EJ, Park DA, Lee NR, Moon SY, Choi JK, Ko JH, et al. Impact of appropriateness of empiric therapy on outcomes in community-onset bacteremia by extended-spectrum-β-lactamase producing Escherichia coli and Klebisella pneumoniae definitively treated with carbapenems. Eur J Clin Microbiol Infect Dis 2017;36:2093–100.

[141] Tamma PD, Rodriguez-Baño J. The use of noncarbapenem β-lactams for the treatment of extended-spectrum β-lactamase infections. Clin Infect Dis 2017;64:972–80.

[142] Gutiérrez-Gutiérrez B, Rodríguez-Baño J. Current options for the treatment of infections due to extended-spectrum beta-lactamase-producing Enterobacteriaceae in different groups of patients. Clin Microbiol Infect 2019;25:932–42.

[143] Kang C-I, Mi Wi Y, Young Lee M, Soo Ko K, Ryeon Chung D, Ran Peck K, et al. Epidemiology and Risk Factors of Community Onset Infections Caused by Extended-Spectrum-Lactamase-Producing Escherichia coli Strains. J Clin Microbiol 2012;50:312–7.

APPENDICES

The appendices contain the

Appendix A: Catchment population of the North Denmark Region from 2007 to 2017.

A reform of local government in 2006 merged two former counties into one health region. Consequently, in a transition period up to end 2009, the Department of Clinical Microbiology, Aalborg University Hospital, did not serve as referral laboratory for the former Thisted and Morsø county, as evidenced by the low number of urine and blood cultures handed in to the Department of Microbiology from these to former counties in 2007 to 2009 (Table A1). Therefore, the catchment population increased during the study period (Table A2 - data freely available from Statistics Denmark). The reference population was used as denominator when calculating the annual incidence rates. From 2010 and onwards, the reference population corresponded to the North Denmark Region (Table A2).

Table A1. Number of patients from Thisted and Morsø county with urine and blood cultures delivered to the Department of Clinical Microbiology, Aalborg University Hospital in the transitional period.

Specimen	2007	2008	2009	2010
Urine	<10	<10	~150	1765
Blood	0	0	171	1859

Table A2. Catchment population >15 years during the study period 2007-2017. The reference population is used as denominator when calculating incidence rates.

Year	The North Denmark Region	Thisted county	Morsø county	Reference population
2007	465317	36314	17882	411121
2008	467965	36444	17916	413605
2009	469503	36648	17951	414904
2010	470539	-	-	470539
2011	472570	-	-	472570
2012	473936	-	-	473936
2013	476116	-	-	476116
2014	479044	-	-	479044
2015	482252	-	-	482252
2016	485098	-	-	485098
2017	487148	-	-	487148

Appendix B. Definitions of communiy-onset and healthcare-associated infection.

Table A3. Studies reporting on risk factors of community-onset ESBL-producing bacteraemia and their applied definitions of community-onset and healthcare-associated infections.

Author Year	Definition: Community onset (CO) Healthcare-associated (HCA)
Lee Y., et al. [68] 2019	- CO: Bacteraemia confirmed within 2 days of admission. - HCA: Not specified.
Isendahl J., et al. [59] 2019	- CO: Blood cultures performed ≤2 days of admission and >3 days after discharge. - HCA: Not specified.
Kim M., et al. [67] 2019	- CO: Presence of BSI established within 48 hours of admission. - HCA: not addressed.
Fröding I., et al. [69] 2019	- CO: *"Patients attending the ED… had a blood culture drawn, were admitted and had antibiotic treatment (with a Gram-negative agent) started ≤24 h".* - HCA: Long-term care facility centre, any intravenous administration, prostate biopsy or wound care ≤30 days, haemodialysis and >48 h hospitalization during the past 90 days.
Lee CH., et al. [72] 2017	- CO: Blood cultures drawn in the emergency department within 48 hours of admission. - HCA: Not assessed.
Quan J., et al. [71] 2017	- CO: Positive blood culture obtained within 48 hours of admission. - HCA: >48 h of hospital admission within 3 months, invasive catheters (including urinary catheter, peritoneal drainage tube, mechanical ventilation, etc.) within 3 months, receipt of haemodialysis or peritoneal dialysis or intravenous medication in the previous 30 days, residence in a nursing home or long-term care facility.
Park YS., et al. [74] 2014	- CO: Bacteraemia that was present in the outpatient department or within 48 hours of admission. - HCA: Intravenous therapy, wound care, or nursing care received at home within 30 days; attendance at a hospital or haemodialysis clinic or receipt of intravenous chemotherapy within 30; >48-hour hospital admission or performance of invasive procedures such as urinary catheter, endoscopy, and nasogastric tube within 90 days; or residence at a nursing home or long-term care facility.
Park SH., et al. [75] 2011	- CO: Positive blood culture obtained at time of admission or <48 hours after hospitalization. Patients hospitalised within 2 weeks before admission were defined as having nosocomial infections. - HCA: Intravenous therapy, wound care, or specialized nursing care at home or in a day hospital within 30 days, including the performance of urinary or digestive tract endoscopy or other invasive procedures. (2) attending a hospital or haemodialysis clinic within 30 days before bacteraemia. (3) hospitalization for > 2 days in an acute care hospital or the patient resided in a nursing home or long-term care facility the past year.
Lee JA., et al. [76] 2011	- CO: Infection diagnosed within the first 48 hours of admission, and blood cultures drawn in the ED within 48 hours of admission. - HCA: history of >48-hour hospital admission in the previous 90 days, haemodialysis, intravenous medication, home wound care in the previous 30 days, or residence in a nursing home or long-term care facility.

Hsieh CJ., et al. [78] **2010**	- **CO: Positive blood cultures taken on, or within, 48 hours, of admission. Patients who had been hospitalised in the month prior to admission were defined as having a nosocomial infection.** - **HCA: Not addressed specifically.**
Kang CI., et al. [79] **2010**	- **CO: Infection diagnosed within the first 72 hours of admission.** - **HCA: A history of >48h hospital admission in the previous 90 days; receipt of haemodialysis, receipt of intravenous medication, home wound care in the previous 30 days; or residence in a nursing home or long-term care facility.**
Rodríguez Banós, J. et al. [77] **2010**	- **CO: Infection occurring in non-hospitalised patients or <48 h after hospitalization.** - **HCA: Intravenous therapy, wound care, or specialized nursing care at home or in a day hospital within 30 days (including the performance of urinary or digestive tract endoscopy or other invasive procedures); attending a hospital or haemodialysis clinic, or had been hospitalised for ≥2 days in an acute care hospital or resided in a nursing home or long-term care facility during the past year.**
Kang C-I., et al. [143] **2011**	- **CO: Positive blood cultures drawn in the ED or an outpatient clinic, and infection diagnosed within the first 48 hours of hospitalization.** - **HCA: A history of a >48-h hospital admission in the previous 90 days, haemodialysis, intravenous medication, home wound care in the previous 30 days, or residence in a nursing home or long-term care facility.**
Zahar JR., et al. [73] **2017**	- **CO: Any positive blood culture drawn within the first 48 h of admission in patients coming directly from home or from a non-medical nursing home. Patients admitted from long-term care facilities or rehabilitation centres or with a recent (<7 days) of acute care hospital admission were considered hospital-acquired and excluded.** - **HCA: Patients receiving home intravenous (i.v.) therapy or home care, having a recent hospital stay (≥24 h) in the past 90 days, receiving haemodialysis, or having received i.v. chemotherapy within 30 days before occurrence of the bacteraemia episode; patients admitted from a nursing home were also included in this group.**

Abbreviations: CO, community-onset; HCA, Healthcare-associated

Appendix C.

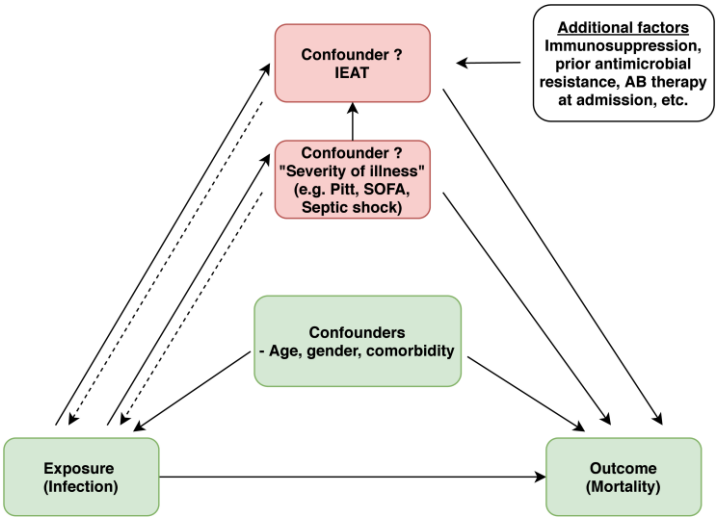
Table A4. Embase and Pubmed search strategy details.

Studies	Query	Hits	Included**
Base search*	Pubmed (("Community-Acquired Infections"[Mesh]) OR (community*[Text Word])) AND (((((("beta-Lactam Resistance"[Mesh:NoExp]) OR "Cephalosporin Resistance"[Mesh]) OR "Penicillin Resistance"[Mesh]) OR "Ampicillin Resistance"[Mesh]) OR ((ESBL[Text Word]) OR (extended spectrum*[Text Word]))) Embase ('extended spectrum beta lactamase'/exp OR 'extended spectrum*':ti,ab,kw OR esbl:ti,ab,kw) OR ('community acquired infection'/exp OR community*:ti,ab,kw)	3,987 2,436	
Study I (Nordic prevalence)	Pubmed "Base search" AND (Scandinavian and Nordic Countries [Mesh]) OR (danish*[Text word] OR swedish*[Text word] OR norwegian*[Text word] OR finnish*[Text word] OR icelandic*[Text word]) Embase "Base search" AND ('scandinavia'/exp OR 'danish'/exp OR 'swedish'/exp OR 'norwegian'/exp OR 'finnish' OR 'icelandic')	83 49	 8
Study II/III (Risk factors/Methodology)	Pubmed "Base search" AND ("Bacteremia"[Mesh]) OR ((((Bacteremia*[Text Word]) OR (bacteraemia*[Text Word])) OR (sepsis[Text Word])) OR (bloodstream infection*[Text Word])) Embase "Base search" AND (('bacteremia'/exp OR 'bloodstream infection'/exp OR bacteremia*:ti,ab,kw OR bacteraemia*:ti,ab,kw OR sepsis:ti,ab,kw OR 'bloodstream infection*':ti,ab,kw) AND ('risk factor'/exp OR risk*:ti,ab,kw OR predictor*:ti,ab,kw OR exposure*:ti,ab,kw))	239 205	 15
Study IV (Outcome)	Pubmed "Base search" AND ((("Treatment Outcome"[Mesh]) OR (("Mortality"[Mesh] OR "mortality" [Subheading]) OR "Length of Stay"[Mesh])) OR ((((outcome*[Text Word]) OR (mortalit*[Text Word])) OR ("length of hospital stay"[Text Word])) OR ("length of stay"[Text Word]))) Embase "Base search" AND (('prognosis'/exp OR 'treatment outcome'/exp OR 'mortality'/exp OR 'length of stay'/exp) OR (prognosis*:ti,ab,kw OR mortalit*:ti,ab,kw OR outcome*:ti,ab,kw) OR (length NEAR/2 stay):ti,ab,kw))	790 652	 17

*** The "Base search" was used in all four studies to identify strictly community-onset ESBL-producing infections. Next, the identified records were combined with the individual search strategies for the different studies.**

**** Following removal of duplicate studies and inclusion of additional papers identified from reference lists of selected papers.**

Appendix D. A subtle question. Inappropriate empirical antibiotic therapy and "severity of illness" - confounder or intermediate variable?



Abbreviations: IEAT, Inappropriate empirical antibiotic therapy.

Control for confounding is crucial in outcome studies. Solid arrows represent correctly identified pathways, while dotted arrows represent falsely identified pathways. Age, gender and comorbidity are classic confounders associated with both exposure and outcome and precede infection onset (green boxes); hence, these variables should be adjusted for in the analysis. "Severity of illness" and IEAT are both associated with the outcome (red boxes); however, they are both *caused* by the exposure (solid arrows), i.e. they are intermediate variables that should not be adjusted for. Accounting for "Severity of illness" or/and IEAT as confounders (dotted lines) might provide false results. This is particularly the case when including only one of the intermediate variables; e.g., adjusting for IEAT without adjusting for "Severity of illness" might falsely lead to the conclusion that appropriate empirical antibiotic treatment is associated with increased mortality. However, this is itself, confounded by the fact that severely ill patients are more likely to receive *broad*-spectrum antibiotics. In addition, several additional factors might influence the choice of empirical antibiotics, further complicating this analysis (white box). Nonetheless, including "Severity of illness" and IEAT in sensitivity analyses might provide additional information in order to accurately determine true independent predictors of outcome [118,133].

Appendix E. Full versions of studies I-IV including supplementary material.

ISSN (online): 2246-1302
ISBN (online): 978-87-7210-813-1

AALBORG UNIVERSITY PRESS