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BURDEN OF DISEASE AND IDENTIFICATION OF INTERVENTION TARGETS IN EXTENDED-SPECTRUM ß-LACTAMASE-ASSOCIATED INFECTIONS

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BURDEN OF DISEASE AND IDENTIFICATION OF INTERVENTION TARGETS IN EXTENDED-SPECTRUM ß-LACTAMASE-ASSOCIATED INFECTIONS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my Swedish and American families

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The front-page illustration depicts a group of extended spectrum β-lactamase-producing *Escherichia coli*. It is a three-dimensional computer-based recreation based on scanning electron microscopic imagery. Courtesy of James Archer and the United States' Centers for Disease Control and Prevention.

The illustration "Common Good Market" in the foreword was drawn by Frits Ahlefeldt and reproduced with his permission.

Foreword

An unmanaged commons in a world of limited material wealth and unlimited desires inevitably ends in ruin.

Garret Hardin (1915-2003), American ecologist and philosopher

The "tragedy of the commons" is a term that was coined by the economist William Forster Lloyd in 1833. Lloyd described how utilization of a common, finite resource by individual actors in their own self-interest can be detrimental to the longer-term interest of the community. The incentive for each actor is to maximize use of the resource, while over time such usage can deplete the resource altogether. Popularized by Garret Hardin in 1968 to point out the perils of global population growth, the term has since been applied to describe how over-exploitation of natural resources lead to environmental depletion.

Without proper regulation, individual incentives for production and consumption of antibiotics and other important "common goods" dominate the market. This eventually exhausts the resource.

The development of bacterial resistance to our most important antibiotic drugs can be described as an example of a tragedy of the commons. Due to insufficient public management of the antibiotic resource, mass production and consumption occurs to satisfy individual demand. This compromises drug effectiveness, since Darwinian mechanisms reward bacteria that prosper in antibiotic-laden environments. To overcome the tide of antibiotic resistance of our own making, we resort to using drugs with ever-wider bacterial spectra. This, in turn, propels further development of antibiotic resistance.

Antibiotic drugs are indispensable to us by virtue of their potential for absolution of severe invasive bacterial infections. Their *commercial* success, however, hinges on large-scale utilization for common, often self-limiting ailments such as upper respiratory tract infections, skin infections and cystitis. For doctors to accurately identify patients that are in real need of antibiotic treatment, trustworthy treatment guidelines are needed that are based on sound epidemiological research. The prospect of contributing something to that end has been a guiding light in my work with this thesis.

Antibiotic overuse, however, is just one denominator of the antibiotic resistance problem. Examples of others are environmental contamination during production, lack of microbiological diagnostics to guide correct treatment and antibiotic use in food production. I believe Mr. Hardin would join me in saying that meticulous management of the common good of antibiotic drugs is the only way in which we can steer clear of its collapse.

ABSTRACT

If I am to speak ten minutes, I need a week for preparation; if fifteen minutes, three days; if half an hour, two days; if an hour, I am ready now.

Woodrow Wilson (1856 – 1924), 28th president of the USA

Extended-spectrum β-lactamase-producing Enterobacteriaceae (EPE) have surged globally since the early 2000s, partly due to dissemination of CTX-M-15 producing *Escherichia coli* (*E. coli*) sequence type 131 and its sub-lineages *H*30-R and *H*30-Rx. The overarching aim of the thesis was to increase the knowledge of the disease burden in and risk factors for bloodstream infection (BSI) with EPE.

For **papers I** and **II**, 408 children were enrolled in a cross-sectional study upon seeking emergency medical care at a referral hospital in Bissau with signs of systemic infection (fever or tachycardia). In **paper I**, we reported that the proportion of these children that were intestinally colonized with *E. coli* or *Klebsiella pneumoniae* (*K. pneumoniae*) that produced extended-spectrum β-lactamases (ESBLs) was 33% and that co-resistance to other antibiotic drugs was common. There was considerable genetic heterogeneity among the isolates, suggesting wide community dissemination. This descriptive knowledge of EPE dispersion in Guinea-Bissau can help inform local antibiotic treatment guidelines for infections potentially caused by Gram-negative bacteria.

In **Paper II**, we assessed the etiology of severe infections in the same population. Pathogenic bacteria were identified from the bloodstream of 12% of the children. *Staphylococcus aureus* was the commonest species (26 findings), followed by non-Typhoidal *Salmonella* (5) and *Streptococcus pneumoniae* (4). Bloodstream infection (BSI) was common among non-febrile children. Malaria was clinically diagnosed in 64% of the subjects, but could only be laboratory-verified in 5%. The findings can be used to inform treatment in patients with signs of systemic infection and support previous studies indicating fever as an insufficient predictor of BSI.

In **Paper III**, the risk of EPE BSI was studied in a cohort of Swedes with a previous finding of EPE in urine (urine cohort) or feces (feces cohort) from 2007-12 and compared to the risk in the Swedish population. The incidence rate in the urine cohort peaked at 22.4 events per 1000 person-years 31-90 days after the initial finding and fell to 1.8 events after 2-6 years. The cumulative 6-year incidence was 3.8% and 1.6% for the urine and feces cohorts, respectively. The relative risk of incident EPE BSI in the respective cohorts compared to the Swedish population was 62- and 32-fold. Urological disorders and other underlying morbidities were associated with increased risk. **Paper IV** was a nation-wide case-control study of disease burden and risk factors for community-onset EPE BSI. The incidence rate of EPE BSI in Sweden was 1.7 events per 100 000 person-years during 2007-12. Male sex, high age and urological morbidity were strong risk factors. The 30-day mortality after EPE BSI was 11.7%. Fluoroquinolone consumption 8-91 days before the outcome was associated with 5.5-fold odds, which in a causal interpretation yields a population attributable fraction of 14%. The findings from **papers III** and **IV** can be used to identify high-risk populations that are suitable targets for interventions such as eradication therapy and antibiotic stewardship programs.

In conclusion, the thesis contributes to the knowledge of BSI and EPE dissemination in Guinea-Bissau and can be used to inform treatment guidelines and public health policy. It also improves our understanding of the natural history of EPE colonization in terms of subsequent risk of EPE BSI and the attribution of morbidity, antibiotic consumption and other risk factors to that outcome.

LIST OF SCIENTIFIC PAPERS

I. Isendahl J, Turlej-Rogacka A, Manjuba C, Rodrigues A, Giske CG and Nauclér P. Fecal carriage of ESBL-producing *E. coli* and *K. pneumoniae* in children in Guinea-Bissau: a hospital-based cross-sectional study. PLoS ONE 2012;7(12):e51981

II. Isendahl J, Manjuba C, Rodrigues A, Xu W, Henriques-Normark B, Giske CG and Nauclér P. Prevalence of community-acquired bacteraemia in Guinea-Bissau: an observational study. BMC Infect Dis 2014;14:3859

III. Isendahl J, Giske CG, Hammar U, Sparén P, Tegmark Wisell K, Ternhag A and Nauclér P. Temporal Dynamics and Risk Factors for Bloodstream Infection With Extended-spectrum β-Lactamase–producing Bacteria in Previously-colonized Individuals: National Population-based Cohort Study. Prepublished online 2018. Clin Infect Dis; ciy539. DOI: 10.1093/cid/ciy539

IV. Isendahl J, Giske CG, Sparén P, Tegmark Wisell K, Ternhag A and Nauclér P. Risk Factors for Community-Onset Bloodstream Infection with Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae: National Population-Based Case-Control Study. Manuscript.

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LIST OF ABBREVIATIONS

aCSHR	Adjusted cause-specific hazard ratio
AmpC	Ampicillinase C
aOR	Adjusted odds ratio
bla	β-lactamase gene
BSI	Bloodstream infection
CI	95% confidence interval
CTX	Cefotaximase
CTX-M	Cefotaximase-München
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
ECDC	European Center for Disease Prevention and Control
EPE	ESBL-producing Enterobacteriaceae
ESBL	Extended spectrum β-lactamase
HR	Hazard ratio
ICD	International Classification of Diseases
K. pneumoniae	*Klebsiella pneumoniae*
KPC	*Klebsiella pneumoniae* carbapenemase
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
NDM	New Delhi metallo-β-lactamase (β-lactamase of ESBL$_{CARBA}$ type)
NTS	Non-Typhoidal *Salmonella*
OXA	Oxacillinase (β-lactamase of ESBL$_{M}$ and ESBL$_{CARBA}$ type)
pAmpC	Plasmid-mediated AmpC (β-lactamase of ESBL$_{M}$ type)
PCR	Polymerase chain reaction
PPV	Positive predictive value
S. aureus	*Staphylococcus aureus*
SHV	Sulfahydryl variable (including β-lactamases of ESBL$_{A}$ type)
ST	Sequence type
S. pneumoniae	*Streptococcus pneumoniae*
TEM	Temoneira (including β-lactamases of ESBL$_{A}$ type)
UTI	Urinary tract infection
WHO	World Health Organization

1 BACKGROUND

1.1 BACTERIA

If you don't like bacteria, you're on the wrong planet

Stewart Brand (born 1938), American author

Bacteria are the most prevalent living organisms and roughly constitute half of the world's living biomass. They are extremely adaptable and can inhabit almost any environment. Bacterial interaction with humans is vastly synergetic, meaning that it benefits both the microbe and its human host. Colonizing, *commensal*, bacteria frequently live on outward facing body surfaces such as the skin, the respiratory tract and the gastrointestinal tract. In 1884, Danish bacteriologist Hans Christian Gram developed a basic classification of bacteria based on their ability to retain a crystal violet dye. Bacteria with a thick peptidoglycan layer in their cell wall retain the violet-colored stain after alcohol treatment and are therefore classified as *Gram-positive*. *Gram-negative* bacteria, on the other hand, are equipped only with a thin peptidoglycan layer which dissolves in the presence of alcohol and hence lose their stain (1). Bacteria are further categorized into *cocci* (spherical-shaped), *bacilli* (rod-shaped) and *spiral bacteria* based on their morphology in a light microscope.

In bacteriology, a *clone* refers to a cell line with similar genotype due to common ancestry (2). Commensal clones have pathogenic potential chiefly after translocation to sterile loci or wounds. Other clones possess virulence mechanisms that enable them to circumvent the body's defenses and cause disease. In clinical medicine, the most important species that colonize the gut are members of the Enterobacteriaceae family, which most prominent members are the *Escherichia*, *Klebsiella*, *Salmonella*, *Enterobacter*, *Citrobacter* and *Proteus* genera. Enterobacteriaceae are Gram-negative bacilli that are facultative anaerobes and that do not sporulate. Biochemically they are known to reduce nitrate to nitrite. *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) cause a vast majority of Enterobacteriaceae infections in humans and are the focus of this thesis.

1.1.1 Escherichia Coli

Discovered by Theodor Escherich in the late 1800s, *E. coli* was early established as a common colonizer of the human intestine. Commensal strains of the species synthesize Vitamin K and live symbiotically in numbers around 7.8 log colony-forming units per gram feces (3, 4). Simultaneously, pathogenic strains of the same species cause disease, ranging from trivial infections such as common tourist diarrhea to potentially life-threatening intestinal, meningeal and urinary tract infections. *E. coli* cause more than 80% of community-acquired urinary tract infections (UTIs) (5) and the majority of bloodstream infections (BSIs) in high-income countries (6).

1.1.2 Klebsiella Pneumoniae

Also identified by a German microbiologist, Edvin Klebs, *Klebsiella* is frequent in soil and water. In humans, *K. pneumoniae* is commonly isolated in infections in the urinary and respiratory tracts, wounds and BSIs and is also a commensal species in the intestines (7). To a larger extent than *E. coli*,

invasive infections with *K. pneumoniae* are related to comorbidity, healthcare contacts, urinary and central catheterization and immunosuppression (8, 9).

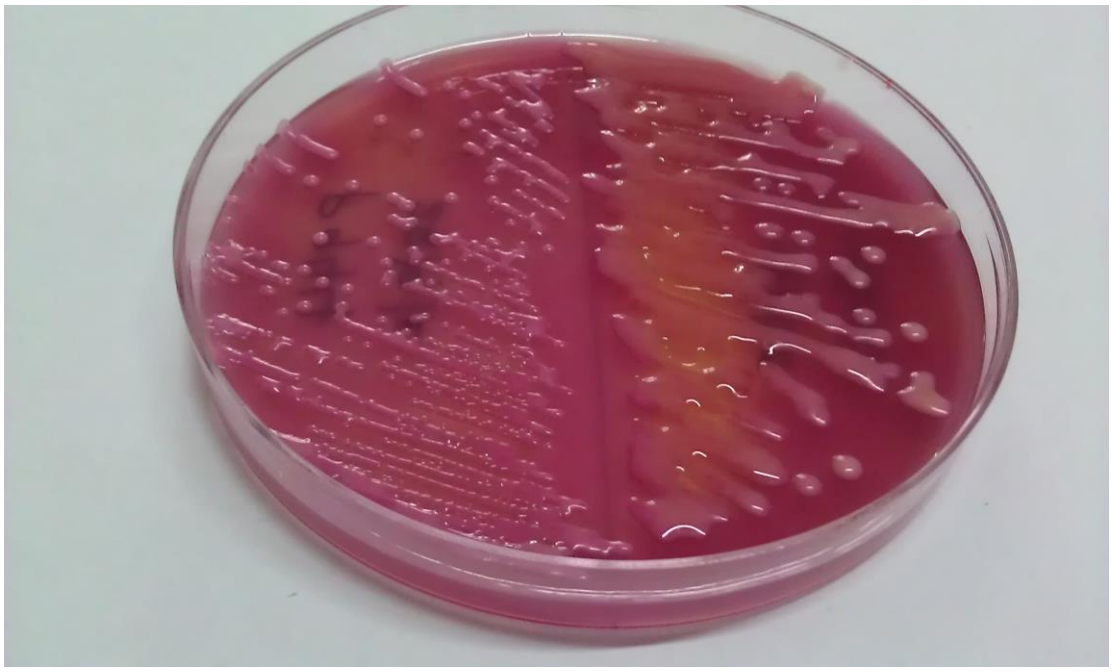

Figure 1. Photograph of *Escherichia coli* (left) and *Klebsiella pneumoniae* (right) colonies on a MacConkey's agar plate. The former yields dry growth, the latter characteristically mucoid, confluent colonies. Photograph by Macorao, reproduced under a Creative Commons 4.0 license.

1.2 BACTERIAL INFECTIONS

All disease begins in the gut

Hippocrates (c. 460 – c. 370 BC.), Greek physician

1.2.1 Intestinal Colonization

The relationship between intestinal colonization and infection is central to this thesis. Research conducted over 100 years ago established that colonization of the colon by commensal bacteria is a natural process that starts immediately after birth (10). *E. coli* is quintessential to the healthy intestine, yet there are two principled scenarios in which the species can cause disease. First, bacteria may translocate *passively* from the intestinal mucosa to the peritoneal cavity and cause invasive infection. This can occur when the host's intestinal mucosa or the immune system is impaired due to a traumatic or ischemic gut injury, an intense inflammatory response or another underlying malady (11-13) and it is the most common scenario behind *E. coli* infections (14). Sometimes under such circumstances, even low-pathogenic or commensal clones may cause life-threatening disease. Second, pathogenic clones can *actively* invade the urinary tract or damage the intestinal lining using a myriad of *virulence factors* (15, 16). Eventually they reach the bloodstream and the triggering of massive immune responses cause fulminant disease. A bacterium possessing uropathogenic virulence mechanisms can be a harmonious colonizer of the intestine while it can go on to cause infection upon invading the urinary tract.

1.2.2 Urinary Tract Infection

UTIs are among the commonest infections in humans and are more frequent in women than in men. Every other woman in the United States is estimated to experience a lower UTI during her life (17), while the lifetime prevalence in men is 14% (18). Lower UTI (cystitis) is frequently uncomplicated and self-limiting, however bacteria ascending the ureters can cause infection in the renal pelvis (pyelonephritis) which in turn can lead to bloodstream infection (BSI). Treatment for lower UTI is advised by most guidelines although not immediately necessary. The modelled mechanism behind infection is bacterial relocation from the gut to the urinary tract (19), why the composition of the gut flora in terms of prevalent species and resistance elements is important to treatment.

1.2.3 Bloodstream Infection

"As regards prognosis, it is evident that a negative [blood] culture does not give much assistance, while a positive result gives a very unfavorable prognosis in the majority of cases"

Franklin W. White in a 1899 research article in Journal of Experimental Medicine (20)

BSI is a medical condition marked by the presence of viable microorganisms in the bloodstream and simultaneous signs of infection (21). This is generally operationalized as presence of one or more positive blood cultures in conjunction with alterations of clinical, laboratory and hemodynamic parameters (21). The term *sepsis* is akin to BSI and defined as a "life-threatening organ dysfunction caused by a dysregulated host response to infection" (22). Severe sepsis is an exacerbation of the septic condition in which "the circulatory and cellular/metabolic abnormalities are profound enough to increase mortality substantially" (22). The incidence rate of severe sepsis was estimated to 300 events per 100 000 person-years in the USA in 2001 with a mortality of 28.6% (23). Incidence appears to be increasing (24), possibly attributable to ageing and more comorbid populations. Septic shock is the most severe form of the condition, characterized by a need of vasopressor agents to maintain blood pressure (22). The outcome measurement in this thesis is mainly based on positive blood cultures, which we found better described by the term BSI.

1.3 ANTIBIOTICS

Antibiotics are drugs used to treat bacterial infections. Analogue to chemotherapeutic agents for cancer treatment, they target key survival or replication functions of the foreign cell, with no or limited interference with host cells. *Bactericidal* antibiotics immediately kill off the bacterium while *bacteriostatic* drugs impede its replication, each representing one main mode of antibiotic action. However, certain antibiotic compounds can be bacteriostatic in lower concentrations and bactericidal in higher. In the name of conciseness and as this thesis focuses on β-lactam antibiotics, other antibiotic drugs and their respective mechanism of action will not be presented in further detail.

1.3.1 β-Lactams

For the discovery of what remains the most important antibiotic group to date, the β-lactams, Alexander Fleming was awarded the 1945 Nobel Prize in Medicine and Physiology. β-lactams are cyclic amides that bind irreversibly to and thereby inactivate *penicillin-binding proteins*, which serve to crosslink the peptidoglycan layer that fortifies the bacterial cell wall.

Initially limited to the narrow-spectrum compound penicillin G, the β-lactam family now encompasses penicillins, cephalosporins, carbapenems and monobactams. β-lactam-β-lactamase inhibitor combinations constitute an additional group, which exploit synergy between the β-lactam and an auxiliary drug that inhibit β-lactamases but that possesses little innate antibiotic effect. Within each of these groups, myriads of structurally more complex compounds have been developed in the endeavor to optimize bacterial spectrum and circumvent antibiotic resistance.

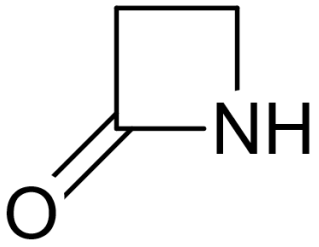

Figure 2. Molecular structure of the β-lactam ring.

1.4 ANTIBIOTIC RESISTANCE

The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to infection with the penicillin-resistant organism

Alexander Fleming (1881-1955), discoverer of penicillin

Bacteria can counter antibiotic drugs in a variety of ways. Production of enzymes that inactivate antibiotics, such as β-lactamases, is one strategy. Decreasing the permeability of the cell wall, activating drug-excreting pumps and modifying the drug target within the cell machinery are others (**Figure 3**).

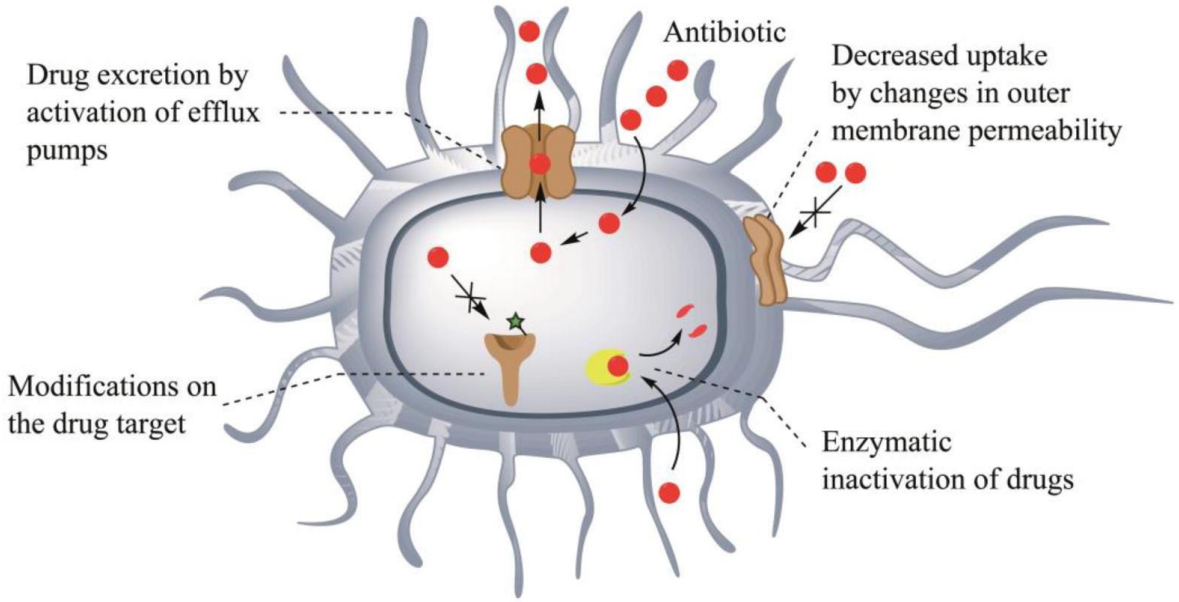

Figure 3. Principled mechanisms of bacterial resistance to antibiotics. Reprinted from González-Bello (25) under a CreativeCommons 4.0 license.

1.4.1 β-Lactamases

β-lactamases are enzymes excreted by bacteria to neutralize β-lactam antibiotics. Elements that code for them are referred to as *bla* genes and these can either be incorporated into the bacterial chromosome or be carried on *plasmids*, genetic elements that float freely in the bacterial cytoplasm (see 1.4.3). The first described plasmid-borne β-lactamase is TEM-1, named after the Greek patient Temoneira from whom it was first isolated in 1965 (26). TEMs were initially limited to hydrolyzing semi-synthetic penicillins (including ampicillin) but minor structural changes soon enabled them to break down first generation cephalosporins. In combination with plasmid-mediated aminoglycoside resistance, emerging in the late 1970s, this development resulted in increased fluoroquinolone and cephalosporin use, which in turn paved the way for widespread resistance to these drugs in the form of extended spectrum β-lactamases (ESBLs) (27).

1.4.2 Extended Spectrum β-Lactamases

ESBLs are β-lactamase enzymes that degrade third generation cephalosporins. The first ESBL enzyme was reported by Knothe in *K. pneumoniae* and *Serratia marescens* in Germany in 1983 (28). The term ESBL was however not coined until 1988, when Phillipon (29) sought to distinguish the new, plasmid-mediated β-lactamases that conferred resistance to third generation cephalosporins from previously recognized β-lactamases.

ESBLs are mainly shared within the Enterobacteriaceae family, but have also been reported from *Pseudomonas aeruginosa* (30). ESBL enzymes are defined by their capacity to hydrolyze extended-spectrum cephalosporins and monobactams in conjuncture with being inhibited by clavulanic acid. They are frequently found on plasmids, which frequently also carry genetic elements conferring resistance to other antibiotic groups. In a much-used functional classification of β-lactamases by Bush, Jacoby and Medeiros classification from 1995 (31), ESBLs are categorized as class 2be enzymes (**table 1**). The Ambler system is another frequently used classification, which denotes ESBLs as class A enzymes based on their molecular structure (32). These schemes exclude enzymes that neutralize clavulanic acid, most prominently AmpC.

Propelled by a need for better communicating the problem of rapidly increasing third generation cephalosporin resistance with clinicians, research funding bodies and policy makers, Giske *et al.* proposed a new classification in 2009 (33). This classification widened the ESBL definition, dividing ESBLs into three groups: $ESBL_A$ corresponds to molecular class A functional class 2be (**Table 1**) and is characterized by *in vitro* growth inhibition by clavulanic acid. $ESBL_M$, where M stands for miscellaneous, include plasmid-encoded, acquired AmpC and OXA enzymes that are inhibited by cloxacillin and/or boronic acid. They roughly correspond to Ambler class C and Bush-Jacoby-Medeiros class 1. $ESBL_{CARBA}$ enzymes, as the name suggests, are also capable of hydrolyzing carbapenems. The Giske classification is the system currently used in Sweden and Norway and also in this thesis, which focuses on $ESBL_A$ and $ESBL_M$.

Table 1. Classifications of β-lactamase enzymes

Ambler classification	
Class A	Serine based penicillinases
Class B	Zink based metallo-β-lactamases
Class C	Serine based cephalosporinases
Class D	Serine based oxacillinases

Bush-Jacoby-Medeiros classification	
Group 1	Cephalosporinases not inhibited by clavulanic acid
Group 2	
Group 2a	Penicillinase
Group 2b	Extended-spectrum penicillinase
Group 2be	ESBLs
Group 2br	Penicillinases not inhibited by clavulanic acid
Group 2c	Hydrolyzing carbenicillin
Group 2d	Hydrolyzing cloxacillin
Group 2e	Extended spectrum cephalosporinases
Group 2f	Carbapenemases
Group 3	Metallo-β-lactamases inhibited by EDTA and DPA
Group 4	Penicillinases not inhibited by clavulanic acid

Giske classification	
$ESBL_A$	Classical ESBLs inhibited by clavulanic acid
$ESBL_M$	Miscellaneous enzymes including plasmidic AmpC and OXA ESBLs
$ESBL_{CARBA}$	Carbapenemases
$ESBL_{CARBA-A}$	Carbapenemases such as KPC, NMC and SME
$ESBL_{CARBA-B}$	Metallo-β-lactamases
$ESBL_{CARBA-D}$	OXA carbapenemases

Adopted from Brolund **(34)** with permission.

Early $ESBL_A$ enzymes belonged either to the TEM or SHV groups and commonly surfaced as single nucleotide mutants from plasmid-borne (extended-spectrum) penicillinases. In contrast, the later-pandemic CTX-M enzymes (see 1.4.2.1) were initially mobilized from the chromosome of the species *Kluvyera* (35). TEM and SHV were the most common ESBL groups globally in the 1980s and 1990s but their relative proportions have since receded and together made up just over 10% of clinical isolates in the USA in 2016 (36). The receding proportion is not explained by declining TEM and SHV prevalence but rather by the massive spread of CTX-M genes, which has become the most important enzyme group globally.

1.4.2.1 CTX-Ms

CTX refers to cefotaximase, highlighting the preference of CTX-Ms to hydrolyze cefotaxime over ceftazidime (both are clinically important third generation cephalosporin antibiotic compounds). The M stands for Munich, Germany, where Bauernfeind *et al.* first identified a CTX-M type enzyme in a clinical isolate in 1989 (37). However, CTX-Ms reached prominence only in the early 2000s, when rapid global dissemination was reported (see chapter 1.5.3 for a presentation of their spatial epidemiology) (38). Its enzymes are divided into six phylogenetic groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25 and KLUC, each differing ≥10% in their amino acid sequence (39, 40). The CTX-M-1 group includes the widely dispersed enzyme CTX-M-15, while the CTX-M-9 group

includes CTX-M-14. The number of reported CTX-M alleles was 164 as of July 1, 2015 and continues to grow (41).

1.4.2.2 Carbapenemases

Carbapenemases, denoted $ESBL_{CARBA}$ in the Giske classification, are enzymes that hydrolyze carbapenem antibiotics. Displaying activity against most Gram-positives, Gram-negatives and anaerobes, including species that are notoriously difficult to treat such as *Acinetobacter* and *Pseudomonas*, carbapenems are among the most valuable broad-spectrum antibiotic groups. For instance, carbapenems are used as first-line agents for nosocomial aspiration pneumonia and other severe hospital-acquired infections. Depending on regional variations in resistance to third-generation cephalosporins, they also constitute an important treatment option for BSIs and other severe infections of unknown origin(42). (*Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus* and *Stenotrophomonas maltophilia* are noteworthy exceptions to their impressive spectrum.) Carbapenems are generally acclaimed within the medical community for their effectiveness, tolerability and safety. In regions with high ESBL prevalence, carbapenems may also be the first-line drug for septic patients with community-acquired infections with a suspected focus in the intestines or the urinary tract. As is the case with $ESBL_A$, carbapenemase-producing bacteria frequently carry simultaneous co-resistance to other antibiotics (43).

1.4.3 Mechanisms of Dissemination

Transmission of antibiotic resistance genes between bacteria can occur either through *vertical* or *horizontal* dissemination. Both these mechanisms are considered to spur in antibiotic-laden environments (44, 45). Vertical dissemination occurs when bacteria divide: the chromosome is replicated in the offspring cell, and any antibiotic resistance genes in the mother chromosome is passed on to the offspring.

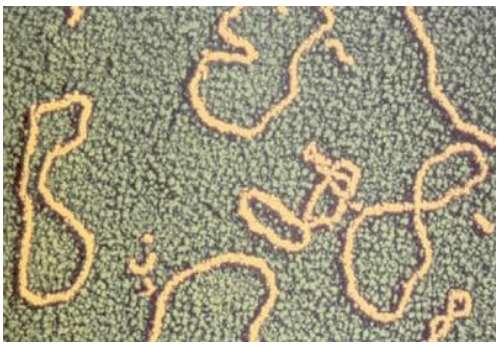

Figure 4. Small bacterial plasmid captured with scanning electron microscopic imagery. Reprinted from Bennet (15) with permission.

In the case of horizontal dissemination, same-generation bacteria exchange resistance amongst themselves by direct sharing of genetic material (46). Horizontal gene transfer is mediated by *conjugation*, *transformation* or *transduction*. The most important mechanisms in the exchange of ESBLs, conjugation, is popularly referred to as "bacterial sex". A temporary, pili-like structure is erected between the bacterial cells, allowing for exchange of genetic material through vectors, most commonly *plasmids*. Plasmids are extrachromosomal elements that replicate independently of their host and can be regarded as "auxiliary chromosomes" (44). They consist of circular units of double-string DNA, which carry genes conferring resistance (47). Frequently, plasmids with ESBL-encoding genes simultaneously carry other genes that confer co-resistance to fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole and other drugs that constitute important treatment alternatives in clinical medicine (44).

The *replicon* is a highly conserved part of the plasmid DNA string which contains genes essential for replication initiation and control. Each plasmid contains only one replicon, why replicon typing

can be used to classify plasmids into so-called *incompatibility groups*. Aside from the stable genetic core, plasmids can incorporate new genes from the bacterial chromosome or surroundings (44). In her doctoral dissertation, Brolund (34) describes a patient that over a study course of one year was colonized with different strains of *E. coli* and *K. pneumoniae*, all carrying an identical resistance plasmid. This illustrates the propensity of plasmids to disseminate across strains and species.

Transformation occurs when a bacterium incorporates genes from the external environment, for instance dead bacteria. Transduction is the infection of a bacterium by phages, viruses tropic for bacteria, that insert viral DNA into the bacterial cell (47).

1.5 THE ESBL EPIDEMIC

An epidemic is not confined to the one from whom it originates

Wolof Proverb

1.5.1 Gene Families

The number of reported β-lactamase enzymes types has risen sharply since the 1980s (48) and ESBLs constitute the bulk of this increase (49). In the 1990s, ESBLs were mainly considered a nosocomial problem limited to *K. pneumoniae* producing SHV and TEM enzymes. Such outbreaks of ESBL-producing *K. pneumoniae* are still an important problem (50), especially in high prevalence countries such as Italy and Bulgaria where 56% and 73% of reported *K. pneumoniae* isolates, respectively, were ESBL-producing in 2016 (51). However, since the turn of the 21st century, CTX-M enzymes in community-acquired ESBL-producing *E. coli* have been the main propagators of ESBLs globally. CTX-M group 1 is the most prevalent enzyme group today due to the widely dispersed enzyme CTX-M-15 (52-58), which was first reported from New Delhi, India in 1999 (59). CTX-M-15 has been particularly successful in causing infections in the community (60). It largely accounts for what has been referred to as a CTX-M β-lactamase pandemic with spread to all continents (52), including Antarctica (61).

$ESBL_{CARBA}$ comprises a separate set of enzyme groups with its own clonal, spatial and temporal distribution. This new treat is presented under heading 1.5.4.

1.5.2 Clones

In a population-based study of ESBL colonization, Ny *et al.* (62) observed greater pathogenicity in blood isolates of ESBL-producing *E. coli* than in fecal isolates of asymptomatic individuals. This indicates that invasive infection may be modest in many community carriers and thereby nuances the discussion of the relationship between ESBL production and virulence. In particular, ST131 including its subclone *H*30-Rx was common among the invasive isolates. Since it surfaced in 2008, it has expanded rapidly worldwide (63-66). The clone is virulent, causes both nosocomial and community-acquired infections, frequently produces CTX-M-15 and is resistant to fluoroquinolones (67). It is associated with UTI and BSI (63) and has been described to predict treatment failure in UTIs (68). Its prevalence varies widely (67), however several studies performed in the mid-2010s reported the clone to represent 20-40% of clinical findings of ESBL-producing *E. coli* (67-71). Constituting a large

proportion of EPE, the success of this clone has strongly contributed to the spread of CTX-M-type ESBLs. Examples of other *E. coli* clones that are associated with ESBL dissemination are ST38, ST393 and ST405 (72). In *K. pneumoniae*, the role of strain ST258 in the spread of carbapenemase-producing KPC enzymes has been debated (73).

The virulence of EPE compared to non-ESBL-producing Enterobacteriaceae is insufficiently studied. *In vitro*, Demirel *et al.* (74) found that polymorphonuclear cell response was higher to ESBL-producing *E. coli* than to that of susceptible strains and that this coincided with increased bacterial growth after six hours. Additionally, the response of interleukins 6 and 8 was lower when exposed to the ESBL-producing strains compared to the sensitive strains. Similarly, Bristianou *et al.* (75) identified that ESBL-producing *E. coli* stimulated production of pro-inflammatory cytokines from monocytes to a higher degree than their susceptible dittos and that this was beneficial for bacterial urinary tract tissue invasion.

1.5.3 Global Distribution and Burden of Disease

The overall prevalence of ESBLs is increasing in the community worldwide this has been presented to be particularly true for developing countries (76). Specifically, beginning in the early 2000s, the spread of CTX-M ESBLs has reached pandemic proportions (52). When reading this section, it is important to consider that studies differ in their choice of target population, inclusion strategies, source of included specimens and laboratory protocols. These differences limit the direct comparability between studies, *e.g.* in terms of prevalence figures and the magnitude of associations.

1.5.3.1 Sweden and Europe

From a global perspective, the prevalence of ESBLs in Sweden is low. However, since many years there is a relentless upward trend and the proportion of clinical *E. coli* isolates that are ESBL-producing tripled from 2.6% to 8.3% between 2010 and 2016, according to data reported to the European Antimicrobial Resistance Surveillance System Network (51, 77).

All clinical findings of EPE became notifiable to SmiNet (see 3.4.1.1) on February 1, 2007 according to the Swedish Communicable Disease Act. Since then, the number of findings increased from 2300 findings February - December that year to 8902 in 2014 (78). These figures include rectal samples from risk groups and may therefore vary with regional and temporal variations in health-care providers' screening policies (see 3.4.2 and **Table 2**). The proportion of invasive isolates in SmiNet was stable at around 5% of the total, increasing from 101 in 2007 to 594 in 2017 (78). CTX-M phylogroup 1 enzymes were found in 67% of the isolates while CTX-M-9 enzymes accounted for 27%, although these figures may be subject to bias since reporting of molecular data was not required in the reporting procedure to the register (79). According to a recently published study of attributable disease burden of antibiotic resistance in Europe in 2015 published by the European Center for Disease Prevention and Control (ECDC), the number of cases attributable to third generation cephalosporin resistance in *E. coli* and *K. pneumoniae* in Sweden in 2015 was 3601 and 167, respectively (80). The corresponding figures for mortality was 110 and 9 for the two species, respectively.

Broadening the perspective to Europe, EPE have disseminated widely in the community since the early 2000s (54). The region has experienced regional convergence in the molecular epidemiology of ESBL enzymes with increasing predominance of CTX-M-15 (52), the most notable exception being

CTX-M-14 endemicity in Spain (81). There is a geographical north-south gradient in ESBL prevalence. In 2013, the Nordic countries (and the Netherlands) reported that the ESBL-producing proportions of the *E. coli* population from clinical samples was around 5%. The corresponding proportions in Poland, Ireland, Germany, France and Austria were around 10%. Continuing south, the Iberian Peninsula, United Kingdom and Greece reported figures around 15% while Hungary, Romania, Italy and Slovakia had figures between 20% and 30% (77). According to the previously mentioned study from ECDC, the number of infections caused by resistance to third generation cephalosporins in Europe in 2015 was 297 416 for *E. coli* and 68 588 for *K. pneumoniae* (80). The figures exclude carbapenemase resistance.

1.5.3.2 Guinea-Bissau and Africa

There is very scarce data on antibiotic resistance epidemiology in Guinea-Bissau. In **Paper I** in this thesis (82) we reported that 32.6% of 408 children seeking care with signs of systemic infection at a pediatric emergency department in urban Bissau carried ESBL-producing *E. coli* and *K. pneumoniae* upon presentation. Previous studies have reported varying prevalence figures. Woerther *et al.* (56) reported that the prevalence of intestinal carriage of EPE among severely malnourished children in Niger was 31%. In healthy children at three health centers in different socio-economic settings in Antananarivo, Madagascar (83), 10% were colonized with EPE. In 2009, Ruppé (58) reported findings of CTX-M-15-producing *E. coli* from an allegedly antibiotic-naive population in Kagnoube in eastern Senegal. In a systematic review (84) from 2014 of colonization or infection with EPE in Africa, 16 out of 26 studies were reported to have a total proportion of ESBL-producing isolates of <15%.

Regarding data on BSI with EPE from the same study, the prevalence varied widely between 0.7% in Malawi and 75.8% in Egypt. These differences are likely to reflect both local epidemiology, calendar year when the study was performed and differences in the selection and enrolment of study subjects. A recently published surveillance study of blood cultures from patients with fever or suspected sepsis in Blantyre, Malawi, exemplified the common scenario where ESBL prevalence increases over time (85). The reported proportion of ESBL-producing *E. coli* in blood cultures out of the total number of *E. coli* isolates rose from 0.7% in 2003 to 30.3% in 2016. A 2005 hospital-based study in Dar-Es-Salaam, Tanzania found that 35% of *E. coli* and 17% of *K. pneumoniae* isolates among children with bacteremia were EPE and these children suffered nearly double mortality (86).

1.5.3.3 Asia

The Indian subcontinent is the epicenter of the ESBL epidemic and as noted previously this is where the CTX-M-15 genotype was first identified (59). The prevalence of EPE colonization in feces is among the highest in the world (87) and a recent study of pregnant woman with bacteriuria in Hyderabad, India identified 48% of the isolates as ESBL-producers (88). Furthermore, reports of $ESBL_{CARBA}$ worldwide have frequently had links to the region (89), which is discussed under heading 1.5.4. Ensor *et al.* (90) reported the ESBL-producing proportion of *E. coli* and *K. pneumoniae* in diverse clinical isolates in three distant Indian referral hospitals to be 73%, all producing CTX-M-15.

CTX-M-14 remains the predominant enzyme type in China, Japan, South Korea and parts of South-East Asia, although the proportion of CTX-M-15 has increased (57, 76, 91-93). In a 2017 multicenter report of consecutively enrolled subjects with community-onset BSI in China, 56% of *E.*

coli and 17% of K. pneumoniae findings produced ESBLs and CTX-M-14 was the most commonly isolated enzyme. In the SENTRY study from 1998-2002 (92), the proportion of ESBL-producing *E. coli* isolates in China was reported to be between 13-35%, with corresponding figures of 60% for *K. pneumoniae*. All isolates produced CTX-M-14. In a more recent study from Fuzhou, China (94), 51% of healthy adults attending a primary care clinic carried EPE. The share of isolates producing CTX-M-9 group enzymes was 75%, while 30% produced CTX-M-1 group enzymes.

1.5.3.4 Latin America

CTX-M-2 enzymes secured a substantive foothold in Latin America in the 1990s and during that decade, some of the highest prevalence figures in the world were reported from the region (95). For instance, during the first years of the SENTRY Antimicrobial Surveillance Program, it was reported that 38% of *K. pneumoniae* and 8% of *E. coli* isolates from UTIs across Latin America in 1997-99 produced ESBLs (96). The Tigecycline Evaluation and Surveillance Trial (TEST) (97) reported similar figures from 33 Latin American hospitals in 2004-2007, although a shift from *K. pneumoniae* toward *E. coli* as the foremost threat in clinical infections had started with resistance detected in 21% of those isolates. In 2013, prevalence in 49 healthy volunteers was compared to that of Franklin's gulls in central Chile (98). Surprisingly, while 12% of the volunteers were colonized with EPE, as many as 30% of the gulls were. CTX-M-1-production was predominant. The SENTRY Antimicrobial Surveillance Program's composite resistance figures towards third generation cephalosporins from 2011-2014 in diverse clinical specimens were 38% in *E. coli* and 57% in *K. pneumoniae*, with the highest proportions out of the larger countries found in Mexico (70% and 54 %, respectively) and the lowest in Brazil (15% and 56%, respectively) (99). Confirmatory testing to identify an ESBL phenotype was not reported which might lead to overreporting of ESBLs *e.g.* due to AmpC production. AmpC production, in turn, is reported to be common in food production animals (100) which is a sizable industry in Latin America. The relative frequency of CTX-M-2 has declined as CTX-M-15 has become pandemic. Indicative of this, a 2018 study of community-acquired UTIs from Southern Brazil (which reported the proportion of EPE to a modest 7%), CTX-M-1 group enzymes were detected in a majority of isolates and followed in prevalence by CTX-M-8/25 (101).

1.5.3.5 North America

The United States is traditionally a low-prevalence country in terms of EPE. For example, a 2012 study from New York City reported that less than 2% of a group of outbound travelers were fecally colonized with EPE (102). Recent nation-wide studies show higher figures, however, and the prevalence is increasing (103). Hoffman-Roberts and colleagues reported that 6.8% of *E. coli*, *K. pneumoniae* and *P. mirabilis* from 346 nation-wide hospitals were ESBL-producing in 2015 (104). The proportion of ESBL-producing varied only slightly between the Midwest, (5.7%), the Northeast (7.9%), the South (7.3%) and the West (6.6%). *Castanheira et al.* (105) reported in 2012 that the proportion of ESBL-producing isolates was 16% for *K. pneumoniae* and 12% in *E. coli*. The study measured the proportion of EPE in nine U.S. census regions. In line with the previously mentioned study, the highest proportions of ESBL-producing bacteria were found in the Eastern regions (5.8%-34.7%) and the lowest in the Central regions (1.7%-6.5%). CTX-M-1 group enzymes (including CTX-M-15) were identified in nearly half of the isolates with findings distributed across the country. CTX-M-9-group

enzymes, on the other hand, were identified in 10% of the isolates. More common than in Europe, one quarter of the isolates produced SHV-type enzymes, consistent with previous studies from the Eastern USA (106) and Canada (107) (where also production of CTX-M-14 was common).

1.5.4 $ESBL_{CARBA}$

In parallel with widespread CTX-M dissemination in the early 2000s, enzymes of the Verona integron-encoded metallo-β-lactamase (VIM) and *K. pneumoniae* carbapenemase (KPC) types emerged in Greece and the United States, respectively (108, 109). ST258 has been quintessential to the spread of KPCs (110). Dissemination of KPCs was mostly limited to *K. pneumoniae*, effectively limiting community spread. In short sequence, however, a new enzyme group named New-Delhi metallo-β-lactamases (NDM), which together with VIM enzymes are grouped as enzymes of the metallo-β-lactamase (MBL) type, was discovered in 2009 in Sweden in a patient returning from India (111). There were soon-to-follow findings of the enzyme group in a multi-country study from India, Pakistan, UK and Bangladesh (112). NDMs have since disseminated to *E. coli* (113) and the community setting on all continents (89, 114). Oxacillinase-48 (OXA-48) carbapenemases have since arisen as a fourth $ESBL_{CARBA}$ enzyme group with dispersion similar to that of NDM-type enzymes (89).

The international surge of $ESBL_{CARBA}$ and the increased mortality in bacteremia with $ESBL_{CARBA}$-producing bacteria is a cause for great concern (108, 114-117). NDM genes are the main drivers in the current global increase in $ESBL_{CARBA}$ prevalence although local and regional resistance patterns vary (108). There is already considerable disease burden that is attributable to carbapenemase production in Europe. According to figures from 2015 from ECDC, carbapenem and/or colistin resistance on the continent caused 9775 and 23 397 infections in *E. coli* and *K. pneumoniae*, respectively (80). Dissemination is however focused to a few endemic centers beyond which spread to the community is still limited (109). For instance, Sweden, a low-prevalence country, contributed with a mere 41 cases to the above-mentioned European figures (80).

Molecular data of carbapenem-resistant Enterobacteriaceae in an American multi-center study revealed wide genomic background of the isolates (118). This indicates undetected transmission chains within medical care, including asymptomatic carriage and transmission. Wide community spread is thus an imminent threat, which to a certain extent has already materialized in the Middle East and India (89). A species shift from *K. pneumoniae* to *E. coli* like that seen for CTX-M enzymes is a cause for particular concern, since wide dissemination to the community could ensue. This could in turn result in community-acquired UTIs for which there are very limited treatment options (109).

1.5.5 Duration of Colonization

Duration and dynamics of carriage is complex to study, since resistance genes move between bacteria and bacterial species in the intestines. Furthermore, repeated findings can represent recolonization and fecal cultures to identify EPE have limited sensitivity (119-121).

Gut colonization with ESBL-producing commensals is a problem *per se*, since the intestine functions as a reservoir for ESBL genes. Invasive infection can then ensue if the host becomes susceptible, or the genes can translocate horizontally to invasive strains and cause BSI (122). In a low-prevalence setting in Stockholm, Sweden, Titelman *et al.* (121) studied the association between

antibiotic consumption and other exposures and continuous carriage of EPE among 61 EPE carriers after detection of carriage. After one year 43% of the cohort still carried EPE and CTX-M-9 phylogroup B2 was associated with prolonged carriage. In a study from Southern Sweden, Tham *et al.* (123) investigated returning travelers with diarrhea for EPE carriage. After 3-8 months, 24% (10/41) of the original carriers were still colonized, four of them with different, simultaneous *E. coli* strains. After three years 10% (4/41) were carriers, one of them with new strains. However, in a larger study of travelers from Paris, France, half of the study subjects (51%, 292/574) that were not colonized by EPE before the trip had EPE-positive cultures upon returning to France (124). Out of the 515 of these travelers that performed a follow-up culture after 3 and 6 months, only 24 (5%) were still colonized with EPE and the proportion shrunk further after 6 (2%) and 12 (1%) months. Travel to Asia was associated with increased risk of acquisition when compared to Latin America and Africa. These findings suggest that the mainstay of travelers are acquitted of their EPE carriage within 3 months of returning from their trip, an interpretation that has been subject to debate (125).

1.5.6 Risk Factors

… risk factors predict disease but do not necessarily cause disease or predict benefit from an intervention: low income is associated with more illness, but health may not be improved by winning a large sum of money on the football pools.

Geoffrey Rose (1926-1993), Professor of epidemiology

The increasing prevalence of EPE stresses the importance of identifying mechanisms that underlie dissemination. Developing a clinical prediction rule to identify patients at risk of EPE colonization and infection has hitherto proven difficult (126, 127). As CTX-M-producing ST131 and other virulent clones emerge and the foremost setting of EPE dissemination shifts from hospitals to the community, the relative importance of different risk factors may change. A recent prospective study from five major American cities by Doi *et al.* (128) reported that 37% of EPE infections (of which 82% were UTIs) were community-acquired, without presence of any of the investigated risk factors. The corresponding figure from an earlier Israeli study was 20% (129).

1.5.6.1 Antibiotic Consumption

A statement accepted as true as the basis for argument or inference

Definition of "axiom" in Marriam-Webster's Dictionary of the English Language

That antibiotic consumption drives antibiotic resistance is an axiom within the medical community. Much effort has therefore been put into reducing its use. Establishing causality and the magnitude of effect for different subgroups of antibiotics *ex vitro* is however notoriously difficult (130-132).

Numerous studies (127, 132-142) have identified antibiotic consumption as a risk factor for colonization or infection with EPE. In a case-control study in a French referral hospital, Cassier *et al.* (134) found that exposure to third or fourth generation cephalosporins and fluoroquinolones was associated with a six-fold increase in the risk of developing EPE bacteremia. Similarly, Rodriguez-Baño *et al.* (135) found a several-fold risk increase for various antibiotic classes, with an emphasis on ß-lactams and fluoroquinolones. In hospitalized patients in a medical center in Boston, USA, Wener *et*

al. (143) reported that fluoroquinolones (OR 2.86, CI 1.37-5.97) and β-lactam-β-lactamase inhibitor combinations (OR 10.17, CI 1.19-86.92) were main risk factors for *K. pneumoniae* isolation, rather than broad-spectrum cephalosporins which were associated with increased risk only among patients that previously had not received fluoroquinolone treatment. One pathway that that has been reported to mediate the effect of antibiotic consumption on the risk of EPE BSI is through induction of *Clostridium difficile* infection, which in turn has been reported to increase the risk of EPE BSI (144).

The large methodological heterogeneity in the field regarding data collection, included pathogens, selection of cases and controls makes direct comparisons difficult. Specifically, the validity of many studies on the attribution of antibiotic consumption to antibiotic resistance may be low (133). Controls selected from a cohort of patients with sensitive Enterobacteriaceae infections can be expected to be less likely to have consumed antibiotics that select for ESBL than the general population (the study base), since such consumption would have prevented them from succumbing to an antibiotic-sensitive infection (145). This method of control selection violates the basic principle that the exposure distribution among the controls should represent that of the population that gave rise to the cases (146). In a review of risk factors for EPE bacteremia performed by Trecarichi *et al.* (133), 28/30 case-control studies used such non-ESBL-Enterobacteriaceae control groups. Furthermore, measures of effect size are limited in many studies (127, 138, 139, 147) due to limits in study size or self-reporting of exposure. Prospective, population-based studies that quantify the attribution of specific antibiotic classes to the disease burden in EPE bacteremia are needed to optimize antibiotic treatment policies in order to minimize resistance development.

1.5.6.2 Previous Colonization with EPE

In a case-control study (148) of 70 patients with ESBL-producing *E. coli* bacteremia and 140 controls with BSI with non-ESBL-producing *E. coli* in Scania, Sweden, Van Aken *et al.* found that previously documented EPE carriage was the only independent risk factor for EPE BSI (OR=87, CI 11-697). Freeman *et al.* (137) reported from New Zeeland that EPE colonization antedated EPE BSI in 36% (16/44) of the study subjects, compared to just 2% (1/44) of the control group of subjects with BSI with non-ESBL-producing Enterobacteriaceae. A majority of these cases had recently travelled to the Indian sub-continent. A study by Platteel *et al.* (126) identified a higher incidence (45.5/10 000) of nosocomial EPE infection in subjects with documented previous EPE colonization compared to previously non-EPE-colonized (2.1/10 000). Rottier *et al.* (149) reported a PPV of 7.4% for bacteremia with third generation cephalosporin-resistant bacteria among patients with suspected sepsis, previously colonized with such bacteria.

1.5.6.3 Travel to High-Prevalence Regions

According to a study by Tängden *et al.*, one in four Swedes that travel outside of Europe acquires EPE in the intestinal flora (150). For travelers to India, the EPE acquisition frequency was 70-90%, compared to 30-45% in South East Asia and the Middle East. Tham *et al.* (151) reported from Malmö, Sweden, that 36% of patients seeking medical care for diarrhea after travelling outside of Europe were colonized with EPE, while the corresponding proportion for within-Europe travelers with diarrhea was 3%. Similarly, from Stockholm, Sweden, Vading *et al.* reported that 32% of previously EPE-negative travellers were colonized with EPE upon returning from four high-prevalence regions (152).

Few virulence factors were detected in molecular analysis and no clinical infections with EPE were reported during 26 months of follow-up. This may indicate that the risk of infection subsequent to colonization in healthy travelers is low. In line with other studies (150, 153, 154), an increased risk of EPE acquisition was seen in subjects that reported diarrhea or antibiotic consumption during the trip. In the Netherlands (155), EPE carriage prevalence increased from 9% before international travel to 34% after and travel to India and South East Asia were overrepresented among the colonized. From Canada (156), a five-fold risk increase for carriage was reported in travelers compared to that of non-travelers, once again with a marked risk increase for travelers to India and Africa.

1.5.6.4 Hospitalization

During the 1980s and 1990s, most reported ESBL-associated infections were caused by *K. pneumoniae* in hospitals and long-term care units. A study in New Zeeland from 2012 (157) found that 5.4% of room surfaces of patients colonized with ESBL-producing *K. pneumoniae* at hospital admission were contaminated with that species, compared to 0.4% of rooms of patients colonized with ESBL-producing *E. coli* ($p<0.0001$). This propensity of *K. pneumoniae* to survive in the environment may explain its propensity to cause nosocomial outbreaks (53, 158).

Since the early 2000s, a shift toward a high community prevalence of CTX-M-15-producing *E. coli* has occurred (62, 136, 159, 160) and one study observed that the transmission of *E. coli* is more likely to occur between members of a household with a colonized individual than in hospitals (161).

Several studies have reported hospitalization as a risk factor for colonization by or infection with EPE (133). Previous hospitalization also predicts EPE carriage in studies from the Netherlands (126) and France (127), however the PPVs were poor. Intensive care unit admission (143, 162) and mechanical ventilation treatment are further risk factors for EPE bacteremia (133, 144). A multi-hospital Canadian intervention study (163) reported that the implementation of an EPE screening program for risk patients at admission in itself reduced the burden of hospital-acquired EPE infections by as much as 50 percent.

1.5.6.5 Socio-Economic Status

Social rank and other social determinants of health are known to influence most health outcomes, which has been increasingly recognized (164) since the Whitehall I Study in which a strong gradual association was found between employment grade and the risk of cardiovascular disease (165). In the field of antibiotic resistance, Nomamiukor *et al.* reported from England in 2015 that resistance to all tested antibiotics was higher in *E. coli* isolates from the most socially deprived population compared to the most privileged. In urban Hyderabad, India, a recent study identified that pregnant women at the lower half of the income spectrum had twice the odds of ESBL-producing compared to a non-ESBL-producing organism in their clinical urinary tract specimens (88). Using a different approach, a new global ecological study matching antibiotic consumption figures with reported resistance figures in different regions point in the same direction (166). In a previously mentioned multi-center study from urban Antananarivo, Madagascar, poverty was identified as a risk factor for EPE colonization (83). It has been pointed out that there is a need to identify the mechanisms that underlie these findings (167).

1.5.7 Ecological Determinants

Identifying risk factors for EPE carriage and infection in individuals is not sufficient to explain the rapid dissemination of EPE globally. A full source attribution model includes health system-related factors such as drug availability and drug dispensation policies. Furthermore, it encompasses ecological determinants such as sanitation, consumption in livestock and a life-cycle analysis of antibiotic production, consumption and degradation. A recent, above-mentioned reported figures of antibiotic resistance around the globe matched with factors such as antibiotic consumption and macro indices such as Gross Domestic Product, education and infrastructure (166). Multivariate analysis found evidence for an association between good infrastructure, good governance and lower antibiotic resistance. Interestingly, no evidence was found for antibiotic consumption.

The World Health Organization (WHO) considers improved sanitation and access to clean drinking water as important means to reduce antimicrobial resistance (168). Indeed, improved sanitation contributed to a rapid decline of infectious diseases and increase in life expectancy in the industrializing countries during the 19th century (169). In developing countries, lacking sanitation is still a major detriment to public health. One quarter of the global population lacks basal infrastructure for safe handling of urine and feces (170). It is estimated that 650 million Indians lack access to flush toilets, the New Delhi sewage system is only dimensioned for half of its citizens and 18% of the tap water in the city is fecally contaminated (170). $bla_{\text{NDM-1}}$ genes were found in 4% (2/50) of drinking water samples and 30% (51/171) of seepage samples from that city (114) and such circumstances may facilitate fecal-oral dissemination of EPE (27). In studies of river water downstream of 90 drug manufacturing factories near Hyderabad, India, Kristiansson *et al.* (171) and Fick *et al.* (172) measured ciprofloxacin concentrations of 1 g/L, surpassing therapeutic *in vivo* concentration in humans. The concentration corresponds to an outlet of 44 kg of ciprofloxacin in the river daily, by far surpassing the daily consumption in Sweden.

Drug dispensing practices differ between countries. In the industrialized world, markets are regulated so that a prescription is necessary to buy an antibiotic drug. In Guinea-Bissau and other low-income countries, antibiotics are sold over the counter in pharmacies or market stalls. At such open-air city markets, tablets may be sun-exposed for hours and are sold individually to anyone with stomach pain, viral nasopharyngitis or, possibly, even an aching toe.

Antibiotic use in livestock, poultry and fish farming exceeds that of human use (173) and is projected to increase by 67% until 2030 (174). The increase is particularly driven by increased use in emerging markets with large populations such as Brazil, Russia, India and China. Animal husbandry and domestic animals are vehicles for dissemination of antibiotic resistance (168, 175-177) and domestic animals have been suggested as possible reservoirs of resistance genes in the community (178).

E. coli ST131 sub-lineage *H*22 is a human uropathogen that is common in poultry worldwide (179). It is capable of carrying *mcr* mobile gene elements that confer resistance to colistin (180). A recent study from Flagstaff, USA, compared the prevalence of *E. coli* ST131 *H*22 in meat and clinical infections and presented compelling evidence that human infections results from transmission from the poultry population through contaminated meat (179), which is in line with previous research (177). Other studies have however disputed this hypothesis due to the limited resemblance between animal and human ESBL genotypes found in some studies (178, 181). In light of the projected increase

in global consumption of antibiotics in animal husbandry, its role for EPE propagation in humans remains an important field for future studies.

1.5.8 Treatment Options

If a patient is cold, if a patient is feverish, if a patient is faint, if he is sick after taking food, if he has a bed-sore, it is generally the fault not of the disease, but of the nursing.

Florence Nightingale (1820-1910), Statistician and founder of modern nursing

Carbapenems are the treatment option of choice for infections with $ESBL_A$-producing bacteria, particularly in case of severe sepsis or septic shock (136, 182). The ß-lactam-ß-lactamase-inhibitor combinations piperacillin-tazobactam and amoxicillin-clavulanic acid are useful carbapenem-sparing alternatives (183). However, co-production of OXA-1 enzymes, mediating resistance to this drug class, is common among CTX-M-15-producing ST131 *E. coli*. This limits its use in empiric treatment of severe infections (72).

Other treatment alternatives include fluoroquinolones, cephamycins, trimethoprim-sulfamethoxazole and aminoglycosides (136, 182). Treatment success depends on co-resistance patterns and the utility of these agents is strongly compromised due to high prevalence of co-resistance to these drugs among EPE (69, 96, 136, 184, 185). Furthermore, the effectiveness of these drugs may be better *in vivo* than *in vitro* (184). For UTI, pivmecillinam (186), fosfomycin (187, 188) and nitrofurantoin (188) have been reported as viable treatment alternatives.

The glycylcyclines are an antibiotic class that make up a potential treatment alternative for infections with $ESBL_{CARBA}$-producing bacteria. Clinical evidence of their use is limited and a meta-analysis (189) has indicated increased mortality with treatment of invasive infections with tigecycline, a glycylcycline compound, although this may partly be explained by its usage on erroneous indications such as *Pseudomonas* infections. Colistin is another treatment option, however its effectiveness at killing off bacteria as a monotherapeutic agent is insufficient and pan-resistant isolates have been reported (108). Another treatment group which is of current interest is cephalosporin-β-lactamase-inhibitor combinations (190). One such compound, ceftolozane-tazobactam, is viable for treatment of bacteria that produce $ESBL_A$ and $ESBL_M$ (191), while ceftazidime-avibactam is another, which is also reported to cover *e.g.* KPC-producing $ESBL_{CARBA}$-associated infections (192). Meropenem-vaborbactam and imipenem-relebactam are carbapenem-β-lactamase-inhibitor combinations that are putative treatment option for $ESBL_{CARBA}$-associated infections and of which the former recently fared well in a phase III trial with complicated UTIs (193). Along with imipenem-relebactam, cefiderocol (194) and plazomicin (195) are two other exciting treatment options for $ESBL_{CARBA}$-associated infections that are in the active pipeline.

1.6 CONSEQUENCES

Nature knows no pause in progress and development and attaches her curse on all inaction

Johann Wolfgang von Goethe (1749 – 1832), German author and statesman

Several studies have reported increased mortality in BSI with EPE (122, 196-200) compared to BSI with non-ESBL-producing Enterobacteriaceae. Schwaber and Carmeli concluded in a review of 16 studies in 2007 that the relative risk of mortality between the mentioned groups was 1.85 (95% confidence interval [CI] 1.39-2.47) (197). Delayed initiation of effective therapy predicted mortality in that study (RR 5.36, CI 2.73-10.53) and others (201, 202) and was indicated in a meta-analysis (203) as a mediator on the pathway between EPE bacteremia and mortality. Similarly, a previously mentioned study by Blomberg *et al.* (86) of children with signs of systemic infection at a pediatric emergency department in Dar Es Salaam, Tanzania found that the mortality in BSI with EPE was 63%, compared to 40% in BSI with non-ESBL-producing Enterobacteriaceae. De Kraker *et al.* (204) estimated the number of deaths attributable to ESBL production in Europe in 2007 to 27 000, while the Centers for Disease Control and Prevention estimated a more modest 1700 attributable deaths per year in the USA in 2013 (205). A fresh publication by Cassini *et al.* reports that 9066 deaths were attributable to resistance to third generation cephalosporins in *E. coli* in Europe in 2015 (80). The corresponding figure for *K. pneumoniae* was 3686 deaths. ESBL production resulted in 120 000 excess days of hospital stay in 2007, corresponding to a cost of 18 million Euro (204). The Public Health Agency of Sweden put the direct costs of ESBL production in Sweden in 2014 at 90 million Swedish kronor, corresponding to 9.2 million Euro (206).

Infections with $ESBL_{CARBA}$-producing Enterobacteriaceae are an expected consequence of increasing $ESBL_A$ and $ESBL_M$ prevalence. *Cassini et al.* report that 763 and 3753 deaths in Europe in 2015 were attributable to $ESBL_{CARBA}$-producing *E. coli* and *K. pneumoniae*, respectively(80). About half of these isolates were also colistin-resistant. BSI with $ESBL_{CARBA}$-producing bacteria has been reported to have 40% mortality, which is higher than for BSI with carbapenem-sensitive bacteria (207, 208). Falagas *et al.* estimated the attributable excess mortality of $ESBL_{CARBA}$ at 26-44 percent (209).

2 AIMS

Only one who attempts the absurd is capable of achieving the impossible.

Miguel de Unamuno (1864-1936), Spanish novelist and philosopher

The overarching objective of this thesis was twofold. First, we aimed to contribute knowledge regarding the prevalence and disease burden of colonization and invasive infection with EPE in Guinea-Bissau and Sweden. Second, we sought to identify modifiable risk factors that are suitable intervention targets for health policy designed to inhibit dissemination of antibiotic resistance.

Specifically, the aim of **paper I** was to determine the prevalence of community-acquired fecal colonization with EPE among children presenting with signs of systemic infection to a referral hospital in Guinea-Bissau. The aim of **Paper II** was to determine the prevalence and distribution of invasive pathogens and molecular pathogenic traits in the same population. We also sought to identify clinical signs and symptoms that could predict a positive blood culture. In **Paper III**, we sought to provide absolute and relative measures of EPE BSI risk among Swedes with a previous EPE finding in feces or from the urinary tract. Furthermore, the aim was to identify risk factors for EPE BSI in that cohort. The aim of **paper IV** was to investigate the impact of underlying morbidity, education and antibiotic consumption on the risk of BSI with EPE.

3 MATERIALS AND METHODS

The way you ask a question chooses its answer

João Ubaldo Ribeiro (1941 – 2014), Brazilian Author and Camões Prize Laurate

This section aims to introduce the reader to the study populations and the epidemiological, statistical and laboratory methods that were used in the preparation of the papers in this thesis. For an exhaustive presentation of the methodological protocols, please see the methods sections of the respective papers.

3.1 EPIDEMIOLOGY

Epidemiology is a scientific field that deals with the distribution and determinants of disease in populations. The epidemiologist may wish either to simply *describe* the distribution of a disease in a population or to *analyze* the determinants underlying its occurrence. In practice, the data which is at the epidemiologist's disposal is a limiting factor that influences the choice of study design and analytical approach.

There are two types of analytical study designs. *Experimental* studies are characterized by random allocation of study subjects to an intervention group, where the researcher assigns the exposure, and a control group. The *randomized controlled trial*, a type of experimental design, is often seen as the superior epidemiological study design. Since the main exposure under study is assigned randomly to the study participants at the start of the study, the risk of confounding decreases. However, ethical considerations render randomized controlled trials unsuitable to pursue many coveted research areas. In *observational* studies, the researcher merely collects information on exposures that the study subjects have subjected themselves to, and it becomes the researcher's job is to evaluate how other factors interact with the exposure under study to cause disease. This is typically done by *restriction* of the study population to certain subgroups or by performing *adjustments* using statistical methods. **Papers I-IV** in this thesis are all examples of observational studies.

A few concepts are central to the venture of establishing causality and investigating the magnitude of effect in epidemiological studies. *Confounding* occurs when a factor that is associated with both the exposure and the outcome fully or in part explain an observed association between exposure and outcome (**Figure 5**). *Mediation* occurs when a factor lies on the causal pathway between the exposure and the outcome (**Figure 6**). If only one mediator is present on the causal pathway and it is adjusted for in the statistical analysis, the exposure-outcome association under study will be nullified. *Effect modification* refers to the alteration of effect exerted by a factor that is on the causal pathway between the main exposure under study and the outcome. When many slices make up the etiological pie, correctly identifying these factors and accessing reliable data is a major challenge for the researcher.

In register-based research, the purpose of data collection is frequently unrelated to the study question and the dataset may therefore lack data on important factors. The researcher than may have to do without, which will result in *residual confounding*. Alternatively, he or she may seek to prevent such confounding by identifying available data which distribution in the study population overlaps

with the original factor of interest and create a so-called *proxy variable*. Residual confounding may still persist, however, if the chosen proxy variable unsatisfactorily mirrors the confounder.

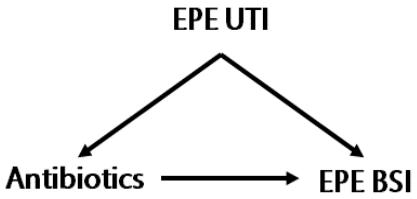

Figure 5. Confounding. In this scenario, EPE UTI is a confounder on the pathway between the exposure, antibiotic consumption, and the outcome, EPE BSI

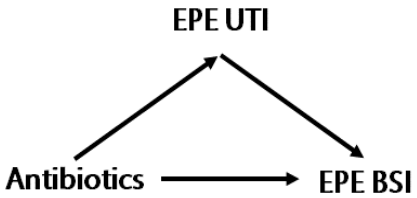

Figure 6. Mediation. In this scenario, EPE UTI is a *mediator* for the effect of the exposure, antibiotic consumption, on the outcome, EPE BSI

3.2 STUDY DESIGN

3.2.1 Cross-Sectional Design

Cross-sectional studies are simple and intuitive in their design. As the name implies, the desired information about the participants is collected at *one specific point in time*. Dividing the number of diseased by the total number of participants gives the *prevalence* of the disease in question. Similarly, biological samples and other relevant data on the study individuals can be collected and used to assess the distribution of other exposures of interest in the study population. The major drawback of the cross-sectional designs is that the temporal provenance of the factors under study cannot be ordained, which precludes causal inference based on the findings.

3.2.2 Cohort Design

Cohort studies are *longitudinal*, which means that study participants are recruited *before* the occurrence of the outcome event and then observed over time to detect its occurrence (and, possibly, exposure to time-varying covariates during follow-up). The number of outcome events can then be related to the total number of individuals at risk to calculate the *rate* of disease occurrence during any specified period of time. *Linear* or *logistic* regression models can be used to measure disease occurrence depending on whether the outcome variable is *continuous* or *ordinal*.

Survival analysis is a commonly used strategy in cohort studies. For instance, in *register-based* or *repeated-measurement* cohort studies, data on death, migration or loss-to-follow-up is collected at various time points during follow-up. Subjects contribute *risk-time* either until experiencing one of these events or until developing the outcome. The rate at which the outcome occurs is then calculated using the collective time at risk in the denominator, rather than the number of participants itself. In survival analysis, the rate itself is referred to as the *hazard rate*, which is defined as the risk of developing the outcome at time t, conditional on still being at risk at time $t - 1$. Using Poisson or Cox regression, the *hazard ratio* can be modelled by comparing the rate between exposed and unexposed

subjects. An assumption of both regression models is *proportional hazards*, meaning that the ratio between exposed and unexposed does not change over the analyzed time period.

To increase power and/or prevent confounding, a *matched cohort study* can be performed, in which the selection of the study population is performed to increase the number of exposed individuals (146, 210). Cohort designs are often viewed as superior to the case-control design. Specifically, they are said to be less prone to *selection bias* since study participants are enrolled irrespective of whether they will later experience the outcome or not. This is not necessarily true; if controls in a case-control study are selected so that they represent the *study base* (*i.e.* the population from which the cases arose) in terms of the studied exposures, also that design can yield valid results (146).

3.2.3 Case-Control Design

The benefit of a case-control design compared to the equivalent cohort study is that it reduces the required study size. When the disease under study is rare, first identifying the cases and then selecting representative controls focuses data collection efforts to subjects whose experience is scarce in the analysis, *i.e.* the cases. With the wake of large-scale registers and modern database management capability, however, case-control studies are rendered less useful for many study questions since data can be retrieved from databases with comparatively minor efforts. Since the researcher determines the number of controls, the ratio between cases and controls only provides the *odds* of disease occurrence rather than the incidence rate, since the rate presupposes an intact quantitative relationship between exposed and unexposed time. An exception to this is when the case-control study is nested within an existing cohort. In a nation-wide register setting such as Sweden's, the entire population can be defined as the cohort. Using *incidence density sampling*, the controls are then matched to the case on follow-up time. In this way, the person-time at risk can be used and the incidence rate ratio estimated.

The methodology used **for papers III** and **IV** are a good illustration of the benefits of the case-control vis-à-vis the cohort study in a large-scale register data setting. Most data for the **paper III** was collected from such registers, why a cohort design could be used in spite of the outcome being rare. However, information on inpatient antibiotic consumption was not retrieved, since that would require manually accessing medical charts of thousands of controls. In **paper IV**, we used a case-control design to limit the study population to two controls per case. This decreased the number of medical charts that we needed to review to an acceptable number. Since controls were selected using incidence density sampling, we were still able to calculate the incidence rate ratio of the outcome event.

3.3 STUDIES IN GUINEA-BISSAU

Always bear in mind that the people are not fighting for ideas … They are fighting to win material benefits, to live better and in peace … to guarantee the future of their children.

Amilcar Cabral (1924 – 1973), independence movement leader for Guinea-Bissau and Cape Verde

3.3.1 Setting

Papers I and **II** are mostly based on the same participants. An inclusion site was set up at the pediatric emergency department at Hospital Nacional Simão Mendes in Bissau, the national reference hospital in Guinea-Bissau. In spite of its status as national referral hospital for pediatrics, 90% of the study participants reported that they resided in or near Bissau. Inclusions were carried out at the hospital between 8 June and 22 September of 2010 during regular office hours. All children ≤ 5 years of age that presented to the department with tachycardia (≤ 1year ≥ 160 and 1-5 years ≥ 120 beats per minute and/or fever ≥ 38°C) were eligible for inclusion.

Bissau city had an estimated 423000 inhabitants in 2010. It is the capital of Guinea-Bissau, a country of 1.6 million inhabitants which is consistently indexed in the tier of the world's least developed countries; the human development index in 2010 was 0.426, 176 out of 187 nations. The rain season lasts from May-November, followed by a 6-month draught. Malaria, HIV-1 and HIV-2 are (low-) endemic in the region with prevalence figures of 3-5 percent in diverse populations in the years preceding the study (211-213). A 70-90% coverage of BCG, polio, diphtheria and tetanus vaccination was estimated while yellow fever, hepatitis B and *Haemophilus influenzae* type b vaccinations started in 2008-09 (214), recently before the study was performed. Malaria slide reading was available at the clinic at an extra patient fee, while blood cultures were unavailable altogether.

3.3.2 Analytical Approach

The purpose underlying the choice of inclusion criteria in **papers I** and **II** was to identify the pathogens and antibiotic resistance distribution among children with signs of invasive infections and to evaluate the prevalence of BSI in a non-febrile population but with signs of organ dysfunction, *i.e.* tachycardia.

Paper I is a hospital-based cross-sectional study with the aims of identifying the proportion of participants colonized with ESBL-producing *E. coli* or *K. pneumoniae*. Since the children were sampled shortly after presenting at the hospital, EPE colonization was unlikely to be related to the ongoing hospital visit. The underlying assumption in the choice of study design was therefore that the proportion of colonized individuals and their molecular profile would reflect that of the community.

Paper II is an investigation into clinical and microbiological features of community-acquired bacteremia in the mentioned pediatric population. By including non-febrile children with tachycardia, we aimed to investigate the sensitivity of fever as an inclusion criterion into studies of bloodstream infection, and the appropriateness of WHO treatment guidelines that limits its indication for antibiotic treatment to febrile children with signs of systemic infection.

3.3.3 Microbiology

As any doctor can tell you, the most crucial step toward healing is having the right diagnosis. If the diagnosis is precisely identified, a good resolution is far more likely. Conversely, a bad diagnosis means a bad outcome, no matter how skilled the physician

Andrew Weil (born 1942), American physician

A diverse array of microbiological analyses was performed based on the collected specimens. Rectal swabs were performed on all 408 participants in **paper I** and venous blood samples, malaria slides and a filter paper with a droplet of blood for malaria PCR assays were collected for the 370 subjects in **paper II.**

3.3.3.1 On-Site Sampling and Culturing

Commercially available screening kits (Copan Italia S.p.A, Brescia, Italy) were used for the collection of fecal screening samples for **paper I**. Cultures were performed at the National Public Health Laboratory in Bissau on in-house cysteine, lactose and electrolyte deficient agars and commercial ChromID ESBL media, which is ESBL-selective based on chromogenic properties (bioMérieux, Marcy l`Etoile, France). Morphologically unique colonies were frozen in an in-house medium for sensitive bacteria and transported to Sweden on dry ice. Semi-automated species identification was performed using VITEK2 (bioMérieux) while antibiotic resistance patterns were obtained using VITEK2 and antibiotic disk diffusion (Oxoid, Basingstoke, UK). European Committee on Antimicrobial Susceptibility Testing breakpoints for antibiotic susceptibility classification were used.

Analogue to the procedure used in **paper I**, blood samples for **paper II** were collected in BactALERT Paediatric-fan blood culture bottles (bioMérieux). After 24 to 48 hours of incubation, cultures were performed on in-house blood and chocolate agars and on a cysteine, lactose and electrolyte deficient agar. Unique colony morphologies were then frozen and transported to Sweden for species confirmation, antibiotic susceptibility testing and molecular analyses.

Malaria slides were performed on all children included in **paper II**. Slide reading came at an extra cost to the patient, but was provided for free for study participants. Since the hospital experienced frequent and long power outages, however, the availability of a microscope for slide reading was erratic. To prepare for molecular analysis of malaria parasites in Sweden, a droplet of blood was collected on a filter paper and sealed in a plastic bag.

3.3.3.2 Biochemical and Phenotypical Analyses

Re-culturing of frozen preliminary findings was performed at the Microbiological Laboratory at Karolinska University Hospital. ESBL-producing strains were once again cultured on ESBL-selective ChromID media (bioMérieux). Biochemical species determination was performed using the semi-automated VITEK2 (bioMérieux) for all species. Antibiotic susceptibility testing was performed using VITEK2 and/or disk diffusion methods in accordance with local Karolinska University Laboratory guidelines. Antibiotic susceptibilities were determined using MICs and breakpoints advised by the European Committee on Antibiotic Susceptibility Testing (215).

3.3.3.3 Polymerase Chain Reaction Based Analyses

Findings of EPE in the fecal cultures in **paper I** (and the EPE findings reported in **paper II**) were subjected to multiplex TaqMan polymerase chain reaction (PCR) to identify the presence and phylogenetic subgroup of CTX-M-producing isolates (39). CTX-M-negative isolates were analyzed with the Check-MDR CT101 assay to identify production of carbapenemase, AmpC, ESBL TEM and SHV enzymes (216).

DiversiLab (bioMérieux), a semi-automated repetitive-sequence PCR based typing system was used to determine the genetic relatedness of the *E. coli* and *K. pneumoniae* isolates, respectively. By incorporating isolates with important multi-locus sequence types (MLSTs) into the DiversiLab analytical software, relatedness to those MLSTs could be determined (217).

For **paper II**, DNA was extracted from blood specimens collected from the patients on filter paper using the Chelex boiling method (218). The sample was then classified with regard to *Plasmodium* species using a nested PCR targeting the *cytb* gene followed by a restriction fragment length polymorphism assay. Parasite density was determined using an 18S-qPCR assay.

3.3.3.4 Other Molecular Assays

For species identification of four *Acinetobacter* isolates in **paper II**, a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) assay was used. *Streptococcus pneumoniae* (*S. pneumoniae*) serotypes were determined using gel diffusion or capsular reaction testing. Protein A (*spa*) types and presence of Panton-Valentine leukocidin (PVL) cytotoxin production was determined for *Staphylococcus aureus* isolates using in-house methods at the Public Health Agency of Sweden.

3.4 STUDIES IN SWEDEN

Papers III and **IV** are both population-based, nation-wide register studies. **Paper III** is a matched cohort study while **paper IV** is a matched case-control study.

3.4.1 Setting

Sweden, accompanied by the other Nordic countries, has an impressive set of national registers of renown quality (219). Most health and population registers are kept at the National Board of Health and Welfare and Statistics Sweden, both government agencies. Study participants and exposure data for **papers III** and **IV** were all obtained from these and other pre-existing registers (**Figure 7**). The vast majority of data on antibiotic consumption is owned by the 21 counties, which are the main health-care providers in Sweden. A minority of the data is owned by private hospitals but is mostly accessible through the counties' databases.

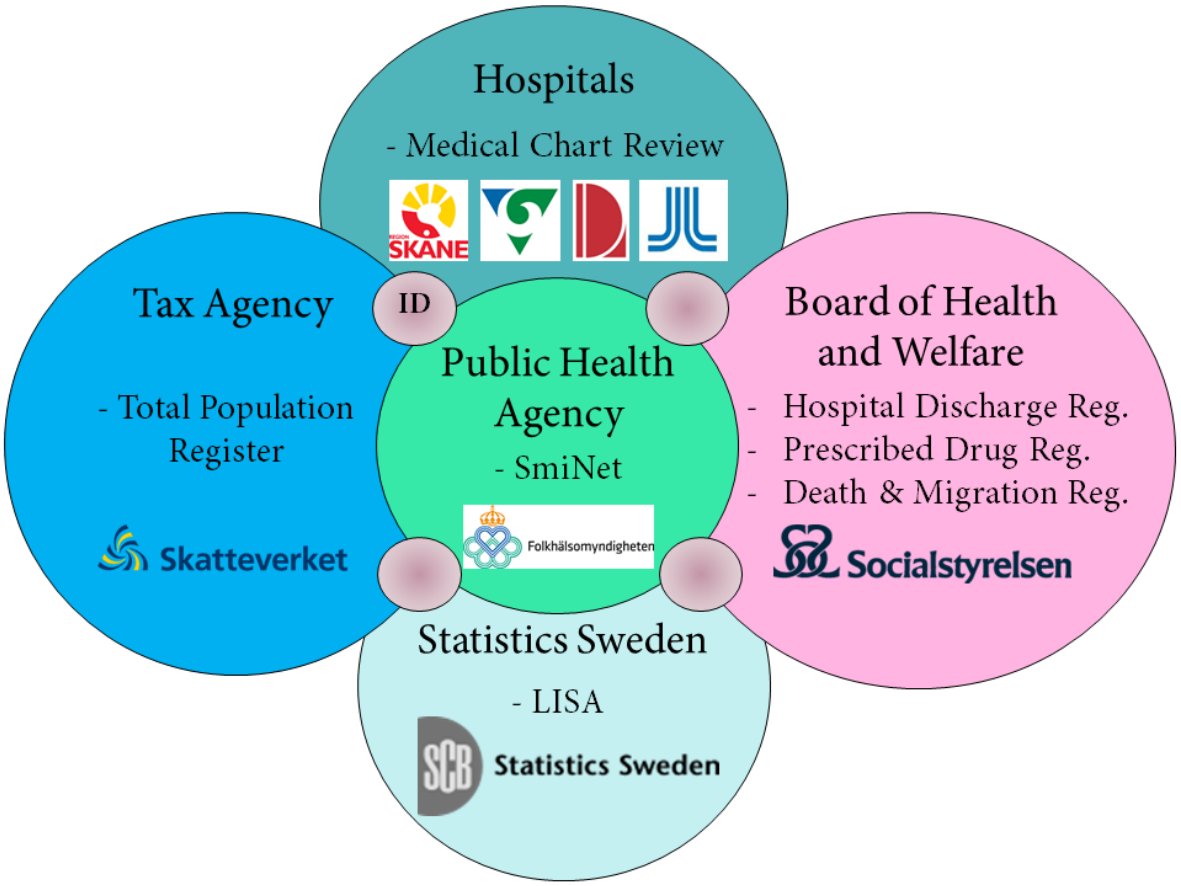

Figure 7. Illustration of the register infrastructure used for papers III and IV. Cases were obtained from SmiNet. Population-based comparison persons (**paper III**) and controls (**paper IV**) were randomly selected from the Total Population Register. Exposure data was obtained using population-based registers at the Board of Health and Welfare, Statistics Sweden and Swedish hospitals ID refers to the personal identity number which was used to link the register data.

3.4.1.1 SmiNet

Clinical findings of ESBLs became notifiable to the national electronic system for communicable disease surveillance, SmiNet, on February 1, 2007. The register is maintained by Public Health Agency of Sweden. Findings were reported from all of the counties' clinical microbiological laboratories and culturing was performed in accordance with each county's routine. Although the Public Health Agency has a sizable proportion of the reported isolates in-house, further analysis of these are beyond the scope of this thesis. During the first decade of reporting, the number of isolates increased each year, starting at 2099 from February-December 2007, reaching 7225 in 2012 and 10659 in 2016 (220). In 2017, the first decrease was seen as the total number of reports dropped to 10084 (221). Urine was the most common source of findings, followed by feces and blood. The proportion of invasive isolates was stable at around 5% of the total, increasing from 101 in 2007 to 594 in 2017. *E. coli* is the commonest species in SmiNet, constituting roughly 85% of the findings, while the corresponding figure for *K. pneumoniae* was around 8% (221).

3.4.1.2 The Total Population Register

This register is kept by Statistics Sweden and contains data on all residents in Sweden. Death is captured to 100% while 95% of immigrations and 91% of emigrations is captured within 30 days (219).

This underrepresentation of emigrations in the registers leads to a slight over-coverage of the register, which has been estimated to 0.5% (222). Controls for register-based research can be obtained at random from the register, matched on basic characteristics such as age, sex and county of residence.

3.4.1.3 The Hospital Discharge Register

Also called the National Inpatient Register, this register collects dates of admission and discharge and all International Classification of Diseases (ICD) diagnosis codes from in- and outpatient care at Swedish hospitals. Comprehensive national registration of inpatient care was achieved in 1987 and nearly 100% of provided inpatient care in Sweden was covered during the study period (223). The register is kept by Sweden's National Board of Health and Welfare.

3.4.1.4 The Prescribed Drug Register

Anatomical Therapeutic Chemical (ATC) Classification System codes, along with prescription and dispensation dates and other variables related to drug prescription and dispensation are collected in this register. All Swedish pharmacies report to the register. Inpatient drug administration is not included. Just like the Hospital Discharge Register, it is maintained by the National Board of Health and Welfare.

3.4.1.5 LISA

The name derives from the acronym for the Swedish name of the Longitudinal Integration Database for Insurance and Labour Market Studies. Data on the occupational status of all Swedish residents is registered here, along with variables such as highest attained educational level, income and family size.

3.4.1.6 Medical Charts

Medical care in Sweden is provided and administered at county level, each of the 21 county councils being a health service provider. Each clinic within each hospital owns its patients' medical charts, however in some counties the permission for researchers to access medical chart data has been delegated to the county's Chief Medical Officer. A minority of medical care is provided by privately owned hospitals (most of their revenue however comes from the public system), each of which own their own medical chart data.

3.4.2 Study Population

3.4.2.1 Inclusion Criteria, Control and Comparison Groups and Outcome Definition

The study subjects in **Papers III** and **IV** are nested within the Swedish population. For **paper III**, the first finding of EPE in urine (n = 17189) or feces (n = 5513) in an individual warranted inclusion in the EPE-exposed cohort. Individuals with a preceding finding in blood were excluded. A population-based comparison group (n = 45161) was selected at random with a ratio of two reference subjects per EPE-exposed subject, individually matched on age, sex and county of residence. The idea behind the comparison group was to calculate the risk of EPE BSI among those with previous "trivial" findings of EPE and compare it to the risk of EPE BSI in the community. To limit data collection on covariates, matching was performed on age in years, sex and residence county with a ratio of two individuals per

case. All individuals were followed in time until the occurrence of a censoring event or the outcome. Criteria for censoring were death, emigration from Sweden or surviving to the end of the study period (December 31, 2012). The outcome was defined as the first EPE-positive blood culture reported to SmiNet during the follow-up.

For **paper IV**, all individuals in Sweden with a first EPE-positive blood culture reported to SmiNet were initially included as cases. Since SmiNet is a passive surveillance system, individuals with a previous finding of EPE in urine or feces were followed in the Hospital Discharge Register for up to 6 years. If a diagnosis code that possibly indicates Gram-negative sepsis (A415, A419 and N109) was identified, the clinical microbiological laboratory was contacted to confirm whether the subject had a corresponding EPE-positive blood culture. This process captured an additional 70 EPE BSI episodes.

Occurrences of BSI events that occurred ≥ 3 days after admission to hospital or within 3 days of discharge were considered nosocomial and hence excluded. As opposed to fecal and urine cultures, blood cultures are routinely performed on patients with signs of sepsis, why the identified proportion of outcomes is likely to be a good reflection of the total number of events.

A control group was randomized from the general population matched on sex, age and county of residence at a ratio of 10 controls per case. The underlying rationale for choosing population-based controls was that a majority of previously available research has used controls with non-EPE BSIs. Such controls are likely to have an exposure distribution that is lower than that of the study base (133, 145, 224), which may lead to overestimation of the risk that is attributable to antibiotic consumption.

Finally, in the analysis of in- and outpatient antibiotic consumption in relation to the outcome, we excluded patients with a previous finding reported to SmiNet. This restriction was performed in order to limit *confounding by indication*, *i.e.* the physician prescribing a certain drug because of a previously known EPE exposure resulting in a spurious association between the drug and outcome. Furthermore, to limit manual chart review, two out of the ten controls in each risk set were randomly selected.

3.4.2.2 Definition of Previous Exposure to EPE

Sampling from both feces and urine are generally performed as result of a medical condition *per se* or because of seeking health care within some defined time after receiving care abroad. The screening and sampling practices that gave rise to the exposed cohort in **paper III** are important since differences in the distribution of other covariates in the population, notably comorbidity, needs be properly adjusted for in the analyses. The exact indications for sampling are established by each health-care provider, however the Public Health Agency has published a guiding document (225) of which a condensed version is presented in **Table 2**.

In 2007 and 2012, 13% and 24% of reported findings respectively originated from fecal samples. Possibly, this reflects more liberal screening policies or practices. As the number of total findings decreased slightly in 2017, the number of urine findings continued to increase, for which possible explanations are alterations in the counties' indications for fecal cultures or decreased physician motivation. Alternatively, the spike in refugees, predominantly from Syria, during fall 2015 resulted in an increased number of findings 2015-16 (226). In this interpretation, the decline in 2017 can be regarded as a "normalization" in the number of reported findings.

Table 2. Patients implicated in most Swedish health care providers' screening programs

Group	ESBL	$ESBL_{CARBA}$	MRSA†	VRE‡
Patient hospitalized or received advanced policlinic treatment outside of the Nordic countries during the last 6 months	x	x	x	x
Patient hospitalized in a Nordic country in a unit with ongoing transmission	x	x	x	x
Patient resided ≥2 months in high endemic region* during the last 6 months	x	x	x	
Patient with colonized household members		x	x	
Patient with wounds or abscesses that developed in a highly endemic region*			x	
Patient that worked with animal stock with known MRSA in Sweden or abroad			x	

* Africa, Asia, Central or South America, the Middle East
† Methicillin-resistant *Staphylococcus aureus*
‡ Vancomycin-resistant *Enterococci*

3.5 STATISTICAL ANALYSES

To understand God's thoughts one must study statistics, the measure of his purpose

Florence Nightingale (1820-1910), Statistician and nurse

The confidence level of all reported CIs in the thesis is 95%. All analyses were performed using Stata version 12.

For the analysis of risk factors in **papers I** and **II**, Fischer's exact test and the Pearson $\chi 2$ test were used for categorical data while Student's t-test was used for continuous data. Two-sided p-values ≤0.05 were considered significant.

In **paper III**, the cumulative incidence of EPE BSI in the respective cohorts was determined using the cumulative incidence function, with death treated as a competing risk. The temporal development of risk for EPE BSI among previously EPE-positive subjects was compared to that of the reference population using an exponential Poisson regression model. Cox regression was used to model the relative risks of host-related exposures such as species in baseline sample, underlying morbidity status, education and pharmacy-dispensation of antibiotic drugs among those with a previously documented EPE finding in feces or urine compared to those without.

In **paper IV**, total population figures for Sweden during the study years were collected from Statistics Sweden and used in the calculation of incidence rates. Conditional logistic regression was used to determine the odds of EPE BSI depending on education, morbidity and antibiotic consumption compared to population-based controls. Multivariate logistic regression was used to identify risk factors for 30-day mortality after the EPE BSI episode.

3.6 ETHICAL CONSIDERATIONS

Data collection and analysis was approved by the Regional Ethical Review Board in Stockholm. The diary number of the permit regarding analyses in Sweden for **papers I** and **II** is 2011/64-31/1. The diary numbers for the permission for **papers III** and **IV** is 2012/2104-31/2 and 2013/704-32. The research carried out in Guinea-Bissau was approved by Comité Nacional de Ética na Saúde, Guinea-Bissau's national ethical committee for health-care, on April 5, 2010. The diary number of the permission is 15/CNES/2010.

For **papers I** and **II**, where enrolment was carried out in Guinea-Bissau, a signature (or in case of illiteracy a fingerprint) from the child's guardian served as proof of consent. For papers **III** and **IV** no patient consent was collected for three reasons. First, data was analyzed and presented on aggregate level with minimal intrusion of privacy. Second, the total number of study subjects was close to 80 000, rendering consent-seeking difficult. Third, the mortality in the study cohorts was high. All personal numbers were replaced with a personal key after government register data was added at Statistics Sweden and the National Board of Health and Welfare. The researchers did not have access to this key during the analysis of the data, with the exception of subjects who were re-identified with the purposes of 1) identifying missing cases at the country's clinical microbiological laboratories or 2) performing individual medical chart review to identify inpatient antibiotic consumption. This procedure was limited to subjects that had one or more hospitalization episodes registered in the Hospital Discharge Register less than one year prior to index date.

4 RESULTS AND DISCUSSION

However beautiful the strategy, you should occasionally look at the results

Unknown

4.1 EPIDEMIOLOGY OF BACTERIAL FINDINGS IN CHILDREN IN GUINEA-BISSAU

4.1.1 Fecal Carriage of ESBL-producing *E. coli* and *K. pneumoniae*

A total of 408 children were enrolled, since 9 children had been excluded since their fecal cultures had not been frozen according to protocol. Nearly one third (133/408, 32.6%) of the fecal cultures were positive for ESBL-producing *E. coli* or *K. pneumoniae* and 37 children (9.1%) had ≥2 ESBL-producing strains in the same sample. Out of the 174 samples, 83 (47.7%) were *E. coli* and 91 (52.3%) were *K. pneumoniae*. An overwhelming majority (81.9%) of the *E. coli* findings and nearly half of the *K. pneumoniae* findings (48.4%) were resistant to fluoroquinolones. The inverse of the proportion of resistant isolates was true for gentamicin (43.4% of *E. coli* and 93.4% of *K. pneumoniae*) while the prevalence of resistance to trimethoprim-sulfamethoxazole was near uniform in both species (94.0 and 91.2%, respectively). Simultaneous resistance to these three agents was found in 38.5% of the isolates, which all remained susceptible to carbapenems.

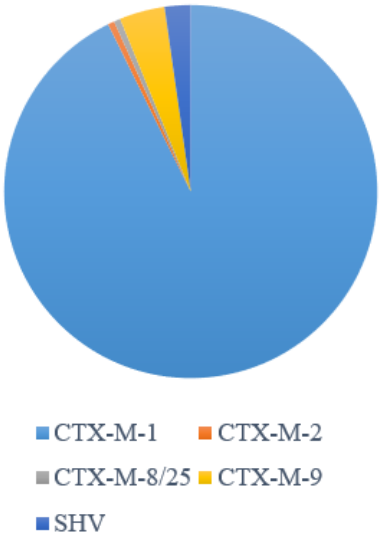

Figure 8. CTX-M enzymes produced by E. coli and K. pneumoniae in ESBL-producing fecal isolates from children in Guinea-Bissau.

Nearly all isolates (165/174, 94.8%) carried $bla_{\text{CTX-M-1}}$ phylogroup genes which includes CTX-M-15, while bla_{SHV} genes were detected in four isolates (2.3%) (**Figure 8**).

A total of 30 DiversiLab types were identified (**Figure 9**) of which 14 were *E. coli* and 16 *K. pneumoniae*. Only 7 of the DiversiLab types contained ≥5 isolates while 63 types were unique to the isolate. When external isolates known to belong to epidemic STs were added to the DiversiLab analysis software, only 3/83 (3.6%) of the *E. coli* isolates clustered with the added ST 131 isolates. Similarly, none of the *K. pneumoniae* isolates clustered with STs 11, 14, 15 or 258.

Weak evidence was found of an association between bed sharing with another child <5 years of age (p=0.04) and fecal colonization. To the contrary, no association was detected between differences in age, sex, weight, ongoing breastfeeding, number of children in the household, mid-upper arm circumference, reported ongoing antibiotic treatment or antibiotic consumption in the month preceding inclusion.

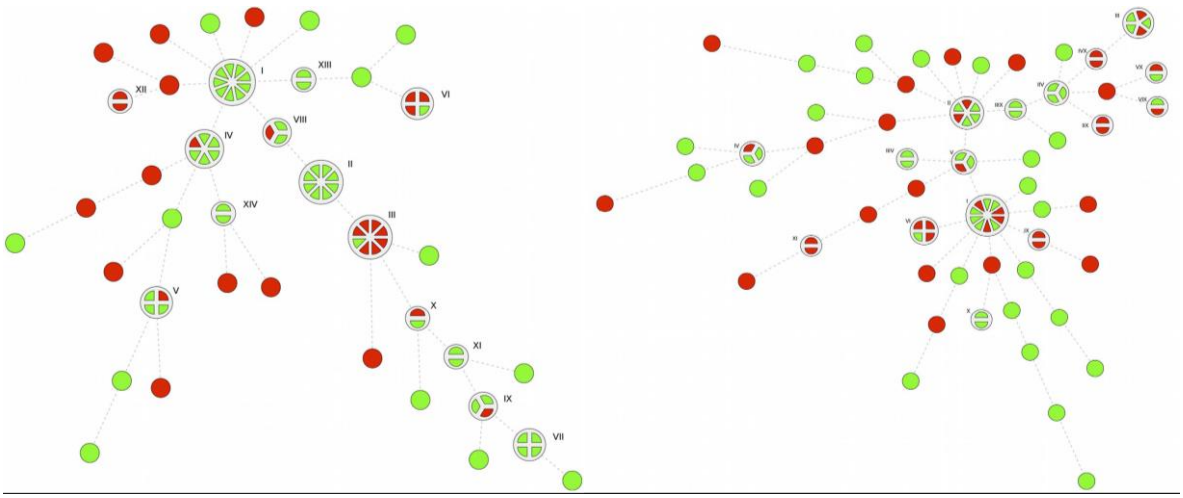

Figure 9. Minimal spanning trees depicting the clonal relatedness of *E. coli* (left) and *K. pneumoniae* (right) in fecal isolates from children in Guinea-Bissau. Isolates showing ≥95% similarity in DiversiLab analysis were considered a cluster and represented as a pie, where each slice represents one isolate. Green isolates were susceptible to either gentamicin, ciprofloxacin or trimethoprim-sulfamethoxazole, which at the time of study were the three easily available antibiotics for treatment of Gram-negative bacterial infections in Guinea-Bissau.

4.1.2 Clinical and Epidemiological Aspects of Bloodstream Infection

A total of 372 consecutively enrolled children were included in the analysis, after the exclusion of 42 children whose samples could not be properly conserved. The number of children seeking care at the department that fulfilled the inclusion criteria but declined participation is not known, however the study nurses declared that they were very few. In total 46% of the children (172/372) presented with both tachycardia and fever while 45% (167/372) had only tachycardia and 9% (33/372) had only fever). About half (48%, 180/372) of the participating children were hospitalized due to their ailment. The mean age was 1.7 years and 44% (163/372) were female.

4.1.2.1 Prevalence, Distribution of Bacterial Findings and Mortality

The prevalence of bacteremia was 12% (46/372) of which *S. aureus* was the most common pathogen (54%, 26/48) followed by non-Typhoidal *Salmonella* (10%, 5/48), *S. pneumoniae* (8%, 4/48) and *Salmonella* Typhi (6%, 3/48). Among children under 60 days of age, Enterobacteriaceae was cultured from 3 out of 5 samples. Out of the 6 children who were under the age of 1 year, 55% (6/11) of positive cultures grew Enterobacteriaceae, compared to 24% (9/37) between the ages of 1 and 5. A total of 5% (17/320) of the children had either slide-verified (6%, 13/227) or PCR-verified (5%, 14/311) malaria, while 64% (237/372) received a clinical malaria diagnosis. There was no difference in the proportion of positive isolates between those admitted to hospital and those who were discharged after the consultation ($p = 0.40$). Mortality was captured for inpatients during hospitalization. Two out of 25 (8%) hospitalized children with positive blood cultures died, both neonates with an Enterobacteriaceae finding. Suspected contaminants were isolated from 31% (117/372) of the cultures, of which coagulase-negative *Staphylococci* ($n = 87$) were the most prevalent. MALDI-TOF was used to identify possible *Acinetobacter baumannii* findings, however all four *Acinetobacter* isolates belonged to other species which were presumed contaminants.

4.1.2.2 Predictors

No difference in prevalence of BSI was seen based on age (p = 0.54) or sex (p = 0.19). Nearly all children with a positive blood culture (96%, 44/46) were included due to presence of tachycardia. Nevertheless, the PPV of tachycardia for BSI was only 13%. If inclusion had been based solely on the presence of fever, 21 BSI episodes (46%) would have been missed. The specificity of both the inclusion criteria were poor, along with all other investigated clinical signs and parameters, *inter alia* oxygen saturation, diarrhea, reduced consciousness, lung crepitations and mid-upper arm circumference. In a subgroup analysis of risk factors for positive blood cultures that were not *S. aureus*, there was a weak indication that fever of 39°C (p = 0.08) and a leukocyte particle concentration of $\geq 20 \times 10^9$/l (p = 0.07) was associated with increased BSI risk.

4.1.2.3 Antibiotic Resistance and Clinical Molecular Biology of Isolates

All *S. aureus* findings remained sensitive to methicillin and both *E. faecalis* findings were susceptible to both ampicillin and vancomycin. Three out of 4 *S. pneumoniae* were penicillin-susceptible, while 1 isolate showed decreased susceptibility at 0.5 mg/l. Notably, among *K. pneumoniae*, two isolates were ESBL-producing, one due to TEM and the other due to SHV enzyme production. These two isolates in conjunction with one *E. cloacae* isolate were resistant to the entire antibiotic testing panel except imipenem.

Frequent *spa* types in *S. aureus* were t084 (n = 7), t355 (n = 5), t127 (n = 2), t1476 (n = 2) and t4690 (n = 2). The *spa* types t008, t024, t314, t491, t571, t760, t939 and t1458 were found in one isolate, each. In total, 38% (10/26) produced PVL. The four *S. pneumoniae* isolates belonged to serotypes 6B (n = 2), 5 (n = 1) and 23F (n = 1).

4.1.2.4 Clinical Management

Treatment with adequate coverage of the causative pathogen was administered to 61% (28/46) of the children with BSI. Ampicillin with a single dose of gentamicin was the most common treatment, yet 22% (10/46) of the children with BSI did not receive any antibiotic. No differences in BSI prevalence were seen based on the attending physician's clinical diagnosis. Although there was no evidence of BSI being less common among children with diagnosed malaria (p = 0.67), the likelihood of receiving antibiotic treatment decreased after a such diagnosis (p = 0.002).

4.1.3 Discussion

Data allow your political judgments to be based on fact, to the extent that numbers describe realities.

Hans Rosling (1948-2017), Professor of global health

4.1.3.1 Principal Findings

In **papers I** and **II**, we systematically enrolled approximately 400 children seeking care at the pediatric emergency department at the national hospital in Guinea-Bissau. A proportion of 33% of the study population was colonized with EPE. There was considerable molecular heterogeneity among the samples, however 95% produced CTX-M-15 or another phylogroup 1 enzyme. Blood cultures from 12% of the children grew pathogenic bacteria, of which *S. aureus* was the most prevalent finding.

Malaria was diagnosed in 64% of the children based on clinical criteria but found only in 5% of the samples. A large proportion (46%) of the children did not present with fever, nor did we identify other clinical parameters that could detect BSI with acceptable sensitivity and specificity.

4.1.3.2 Fecal EPE Colonization

It is important to distinguish between studies of EPE prevalence in hospitalized patients on the one hand and studies of the community prevalence on the other, since the findings reflect nosocomial dynamics of dissemination in the first case and community dynamics in the second. For that reason, prevalence studies in hospitalized populations is unlikely to reflect the community colonization prevalence. Furthermore, in health care systems and hospitals lacking adequate human, institutional and economic capital for preventing spread, nosocomial dissemination of bacteria is widespread. For instance, in a Nigerien study of hospitalized, severely malnourished children in Maradi (56), the acquisition rate among those that were not colonized by EPE at admission was a staggering 94%. Although our study was formally hospital-based, the fecal sampling was carried out soon after the children presented to the department, virtually excluding EPE acquisition during that hospital visit.

A study of the point prevalence of EPE in the community in Madagascar reported that 10.1% of 484 subjects seeking care at three medical health centers were colonized (83). In the above-mentioned study from Niger, 30.9% of the children were carriers (56). A Madagascan study (227) of inpatients carried out a year before the previously mentioned study from the same country reported an EPE colonization prevalence of 21.2%, a significantly higher figure. As in the mentioned studies, the prevalence of co-resistance was high in our study. As many as 38.5% of the fecal EPE isolates were co-resistant to ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole. This is of great concern, since these were the only agents that were readily available to treat infections with Gram-negative bacteria in the Guinea-Bissau at the time of the study.

A review from 2014 reported that the proportion of Enterobacteriaceae isolates from various clinical specimens that produced ESBLs in Africa was similar to figures in Europe (84). In the light of that fact, it is important to underscore that an equal proportion of isolates being ESBL-producing translates into higher absolute mortality and morbidity in countries such as Guinea-Bissau, where the overall burden of bacterial infections is larger and treatment with broad-spectrum antibiotics such as carbapenems rarely are available.

4.1.3.3 Blood Culture Findings

S. aureus is known both as a commensal residing on the skin and a pathogen that can cause fulminant BSIs. In line with our study and published in the same journal, the species was also the predominant finding in two studies from Nigeria (228, 229) and one from Malawi (230). Nevertheless, often-cited previous studies of pediatric bacteremia in Sub-Saharan Africa identified *S. pneumoniae* (231, 232) and non-Typhoidal *Salmonella* species (86, 233, 234) as the most prevalent causes of BSI. These were the second and third most common findings in our paper. Children with *S. aureus* findings were less likely to have fever, implying that at least some findings represent contamination. Using two or more blood culture bottles could have increased our ability to exclude possible contaminants. Simultaneously, such a procedure would possibly have increased the sensitivity of the culturing process for fastidious organisms such as *S. pneumoniae*.

Although the veracity of the *S. aureus* findings cannot be established *post hoc*, their exclusion from the analysis did not alter the important observation that fever was insufficiently predictive of BSI. Resembling a previous study (231), only 68% of the children presented with fever. This is noteworthy in light of many previous studies of BSI that are limited to febrile subjects (228, 230).

4.1.3.4 Predictive Capacity of WHO Guidelines

The WHO's Integrated Management of Childhood Illness algorithm specifies general danger signs which prompts urgent attention (lethargy, convulsions, the child is unable to take the breast or vomits everything). It further specifies that initiation of antibiotic treatment in the absence of cough, breathing difficulties and diarrhea should be based on fever (235). In malaria-endemic areas, irrespective of whether endemicity is high or low, empiric antibiotic treatment is indicated only when a general danger sign is present or the child has a stiff neck. In the absence of these signs, treatment is limited to antimalarials. Over the last two decades, the incidence of malaria has been declining in many regions in Africa (236) and is often comparable to the prevalence of BSI. In line with a previous study from Guinea-Bissau (213), there was considerable over-diagnosis of malaria (diagnosed in 64% of the study participants, laboratory-verified only in 5%). This indicates a need for future studies to evaluate the appropriateness of subordinating antibacterial treatment to antimalarial treatment in WHO guidelines.

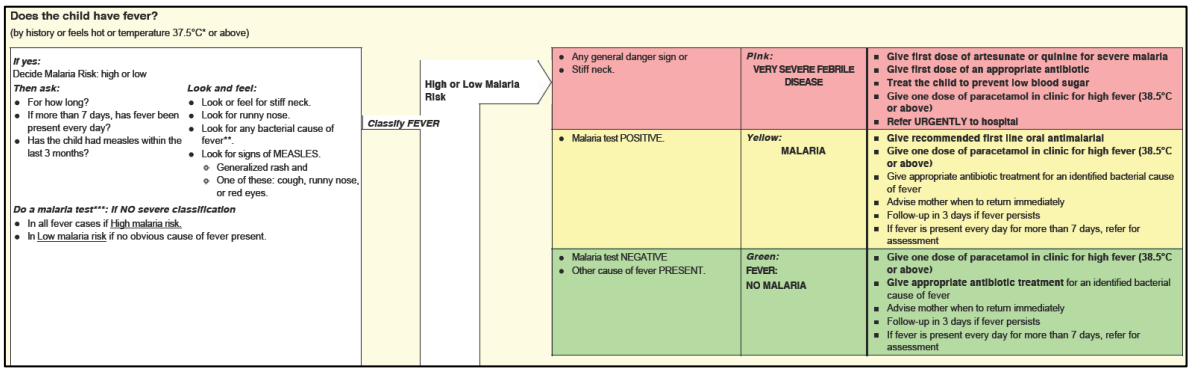

Figure 10. Excerpt from the WHO's Integrated Management of Childhood Illness Chart Booklet (235). In malaria-endemic regions, no antibiotic treatment is indicated for fever in the absence of general danger signs, stiff neck or a positive blood culture.

4.2 EPIDEMIOLOGY OF BSI WITH EPE IN SWEDEN

4.2.1 Risk of EPE BSI After an EPE Finding in Feces or Urine

4.2.1.1 Basic Characteristics

A total of 660 outcomes of BSI with EPE occurred during the follow-up time, which was up to 6 years long. A majority of these (448, 67.9%) occurred within 30 days of the initial EPE finding in urine (urine cohort) or feces (feces cohort) and were classified as *prevalent* outcomes, whereas the 212 events that occurred after ≥30 days were classified as *incident* outcomes. Three of the incident outcomes (1.4%) occurred in individuals from the matched cohorts with individuals with no previously documented findings of EPE. The majority of findings were *E. coli* (522, 79.1%) while *K.*

pneumoniae and *Citrobacter* species numbered 57 (8.6%) and 10 (1.5%), respectively. A subset of the incident events (29/212, 13.7%) were caused by a different species than the one identified in the baseline sample. The mainstay of incident outcomes (167/212, 78.8%) were community-onset.

4.2.1.2 Incidence Rate Compared to the General Population

There were large differences in the cumulative incidence of the outcome based on the source of the baseline specimen: 3.8% in urine, 1.6% in feces and 0.02% in the population-based comparison group (**Figure 11**). The incidence rates of the outcome were 181 and 1081 events per 1000-years within one week of baseline sampling in the feces and urine cohorts, respectively. The rate declined rapidly down to 2.2 events per 100000 person-years in the feces cohort where it remained for three years, thereafter reaching 0. The same pattern of a rapid initial decrease followed by a plateau at 2.3 events per 100000 person-years was seen also in the urine cohort, where that moderate rate remained for most of the 6-year-long follow-up period.

The proportion of EPE BSI out of the total number of ICD-10 diagnoses related to Gram-negative sepsis was 16.5% (83/506), 8.4% (19/225) and 6.9% (40/581) after a follow-up of <6, 6-12 and >12 months, respectively. Adjusting for age, sex, residence county, bacterial species at baseline and Charlson Comorbidity score, the adjusted cause-specific hazard ratios (aCSHRs) for developing the outcome were 392 (CI 120 – 1283) and 118 (CI 35 – 395) among individuals with a previous finding in urine and feces, respectively. When limiting the same analysis to incident outcomes, the corresponding figures were 61 (CI 15 – 247) and 32 (CI 8 – 135).

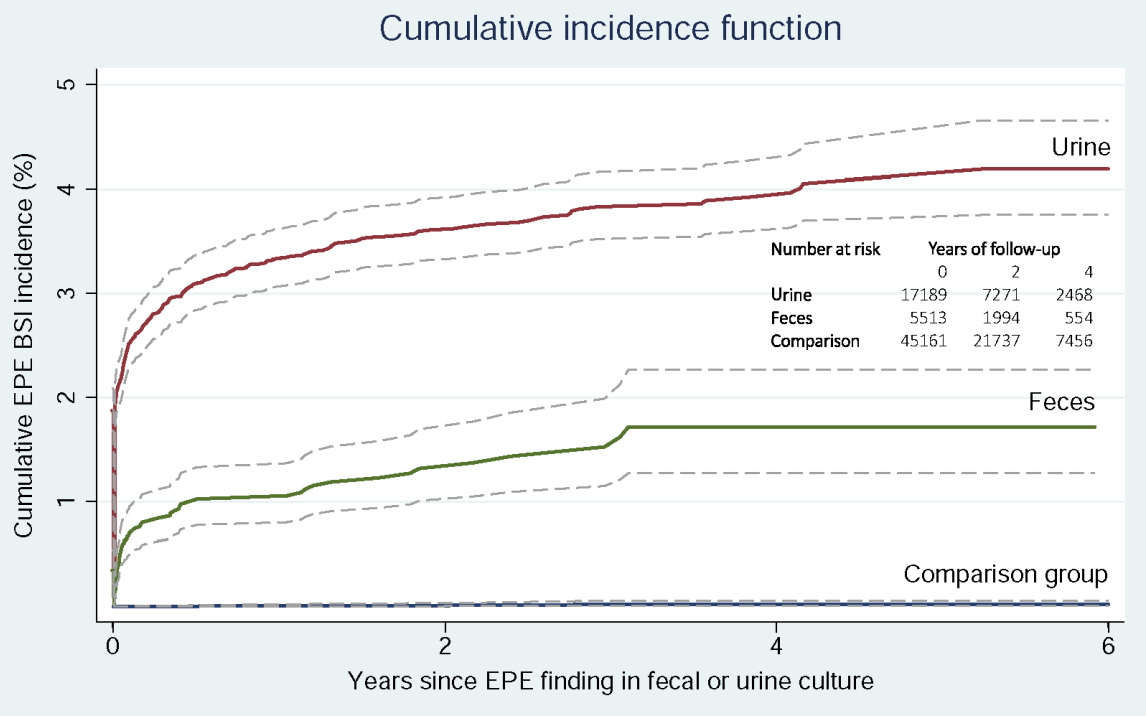

Figure 11. Cumulative incidence of prevalent BSI with EPE over 6 years in relation to source of baseline sample. The incidence peaked at the time of the EPE finding in feces or urine and then declined progressively in these cohorts, while the incidence in the population-based comparison group remained low throughout the follow-up. The incidence function should be interpreted with caution towards the end of the follow-up, since few person-years were available.

4.2.1.3 *Risk Factors*

A previous finding in urine was associated with a doubled risk of BSI with EPE compared to a finding in feces (aCSHR 2.0, CI 1.4 – 3.0). Having *K. pneumoniae* cultured at baseline was associated with a similar risk increase when compared to *E. coli* (aCSHR 1.8, CI 1.2 – 2.7), just as men compared to women (aCSHR 2.2, CI 1.7 – 2.9). Age was not a strong predictor of the outcome, with only weak evidence of an effect between 70 and 80 years of age (aCSHR 1.8, CI 1.1 – 3.2) which disappeared in the ≥80 years category. Similarly, no evidence of increased risk was found based on educational status (aCSHRs 0.8 and 0.9 in the >10-12 and ≥12 years categories, respectively, both CIs including 1). There was no marked risk difference between the large residence counties, with aCSHRs lingering between 0.9 and 1.1 in Västra Götaland and Skåne and in the composite variable of the smaller counties, when compared to Stockholm. There was a general pattern toward increasing risks with higher comorbidity and urological disorders was the disease group associated with the highest risk of developing the outcome (aCSHR 3.4, CI 2.5 – 4.7).

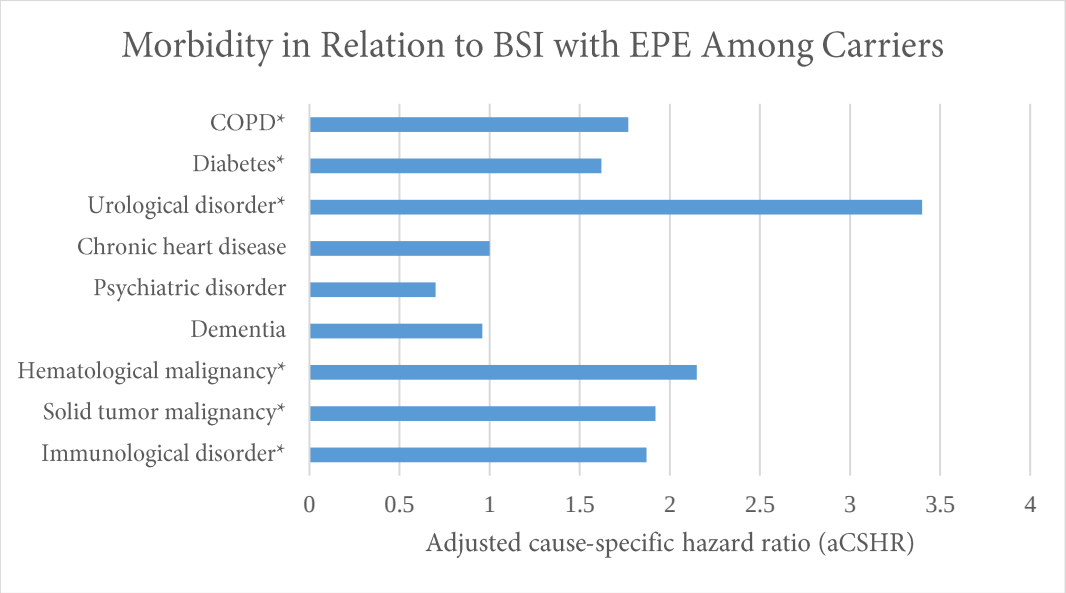

Figure 12. Common disease groups in relation to the study outcome. COPD = Chronic obstructive pulmonary disease. Statistical evidence of an association at the 95% level is indicated with an asterisk.

As regards pharmacy-dispensed antibiotics and the risk of EPE BSI, the risk was 3.1-fold (CI 1.7 – 5.6) between 8 and 30 days after the dispensation of fluoroquinolones, while penicillins with extended spectrum and pivmecillinam were associated with 2-3-fold risk after 30 to 91 days (neither CI included 1). There was no evidence of an association between any of the studied antibiotics and the outcome between 92 and 182 days of follow-up.

4.2.2 Risk of BSI with EPE in Sweden

4.2.2.1 *Basic Characteristics*

After exclusion of 300 nosocomial and 79 recurrent events, a total of 945 cases of first-occurrence, community-onset events of EPE BSI remained. Sixty out of the 9450 controls were excluded since they were hospitalized at the index date (same exclusion criteria as for the cases), resulting in 9390 controls. The matching procedure resulted in well-balanced risk sets in terms of age, sex and county of

residence. The median age was 71 years and 41% of the cases were females. The overall incidence rate of EPE BSI during the study period was 1.7 events per 100 000 person-years, however the incidence increased with calendar time to 2.9 events per 100 000 person-years in 2012. The highest incidence rate was seen in males ≥ 85 years of age, where it peaked at 15.8 per 100 000 person-years. An increasing gradient was seen with higher age and starting from approximately age 50, the risk in males was approximately twice that of females (**Figure 13**).

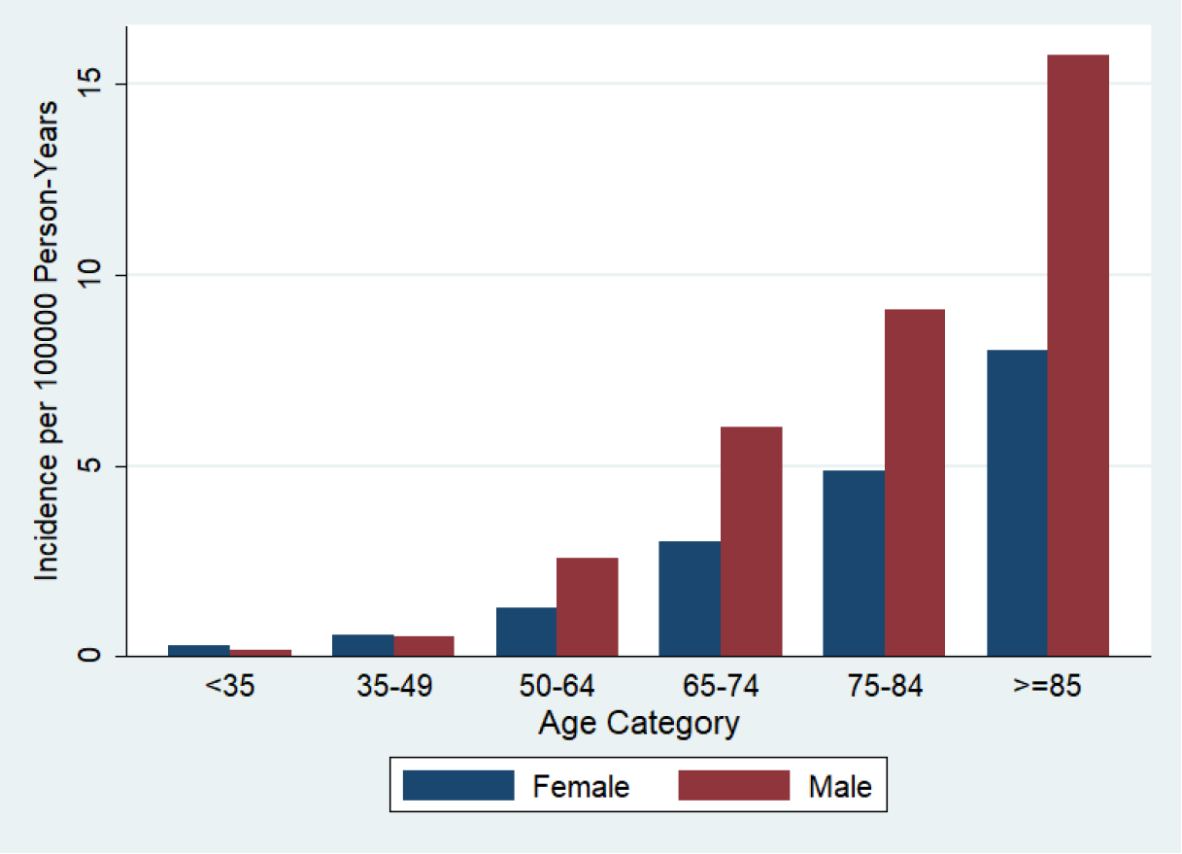

Figure 13. Incidence rates of BSI with EPE in Sweden 2007-12 per age and sex. The incidence rate increased gradually with age and markedly after age 50. Above that age, the incidence rate among males was approximately double that of females.

4.2.2.2 Burden of Disease and Risk Factors

More underlying morbidity was closely associated with increased risk. The adjusted odds ratios (aORs) increased gradually to reach 12.6 (8.4-19.1) for individuals with Charlson Index Scores ≥6. Hospitalization 8-30 days before the event was associated with an aOR of 6.7 (CI 5.0-8.9). Urological disorders and procedures were individually associated with ≥3-fold risk (aORs 4.3 [CI 3.4-5.5] and 3.0 [CI 2.5-3.6], respectively). Immunological disorders and hematological malignancies were also associated with considerably increased risks (aORs 3.5 [CI 2.0-6.2] and 2.8 [1.6-4.9], respectively). Some evidence of associations was also present for COPD, diabetes, dementia and solid tumors, with aORs from 1.61-2.28 (all CIs >1), while no such evidence was present for educational status.

More than half of the cases (59.3%, 378/637) received at least one course of antibiotics during the 6 months that preceded the case event, while the corresponding proportion among controls was 12.3%. The antibiotic was dispensed from a pharmacy in 317 cases, while 162 had it administered in a

hospital (101 received both in- and outpatient antibiotics during that time interval). No effect was present for any course of antibiotics taken more than 3 months before index date.

Receipt of ≥1 antibiotic drug with selective activity against Gram-negative spectrum but mostly not EPE (aminopenicillins, trimethoprim, trimethoprim-sulfamethoxazole, fluoroquinolones or cephalosporins) 8-91 days before index date was associated with an aOR of 3.79 (CI 1.9-7.7). Receipt of fluoroquinolones was associated with an aOR of 5.5 (2.8-11.0) which was the highest of any antibiotic group. However, extended-spectrum penicillins (aOR 2.8, CI 1.0-7.7), trimethoprim-sulfamethoxazole (aOR 2.8, CI 1.1-6.8) and pivmecillinam (aOR 2.6, CI 1.0-6.8) also conferred some but limited evidence of increased risk. In contrast, in adjusted analysis there was no compelling evidence of an increased risk for cephalosporins (aOR 3.3, CI 0.9-11.5). The population attributable fraction (PAF) for receiving a drug with selective activity against Gram-negative bacteria but mostly not EPE 8-91 days before index date was 17% and, for fluoroquinolones, 14%.

In a separate analysis of risk factors for 30-day mortality, a highest attained education of <10 years was associated with aOR 2.4 (CI 1.1-4.9) compared to the most highly educated group, in spite of no such association being seen with regards to the risk of EPE BSI. Other risk factors for mortality for which there were statistical evidence were (in falling order of magnitude): Charlson Index Score (aOR 12.6, CI 8.4-19.1 for a score ≥6 compared to 0), hematological malignancy (aOR 2.9, CI 1.3-6.6), solid tumor malignancy (aOR 2.0, CI 1.2-3.4) and chronic heart disease, aOR 1.8 (CI 1.1-3.0). There was no evidence of an association for urological disorders, which were strongly associated with EPE BSI.

4.2.3 Discussion

These two register-based studies in Sweden are to our knowledge the first nationwide investigations into disease burden and determinants of BSI with EPE. Two approaches were explored: **Paper III** focuses on the risk of and risk factors for the outcome among individuals with a previously reported finding of EPE in urine or feces, and compares the risk with that of the Swedish population as a whole. **Paper IV** instead includes *all* cases of community-onset EPE BSI in Sweden and compares their exposure experience to that of population-based controls. It provides measures of disease burden of and identifies risk factors for EPE BSI, including an analysis of both in- and outpatient antibiotic consumption.

4.2.3.1 Principal Findings

In **paper III**, we report many-fold relative risk after a baseline EPE finding compared to the general population. The risk remained elevated for 3 years in the urine cohort and for at least 5 years in the feces cohort. However, there was a rapid decline in the incidence from 65 and 48 per 1000 person-years in the urine and feces cohorts within the first month of the baseline finding to 2 after 1 year and 6 months, respectively. A caveat is that the person-time at risk decreases with follow-up time, limiting the precision of the incidence rate towards the end of the follow-up time. **Both papers** identified markedly increased risk associated with urological disorders and tumor- and immunodeficiency-related diagnoses. There was no readily discernible association between EPE BSI and highest attained educational level in either paper. In- and outpatient consumption of fluoroquinolones was associated with increased risk in both studies, however the effect was only short-term (≤91 days). **Both studies** also found some weak evidence of short- to medium-term effects for penicillins with extended

spectrum and pivmecillinam (analyzed as separate groups). The overall attribution of the reported in- and outpatient antibiotic consumption was low in **paper III**, where data was limited to outpatient dispensation, and moderate in **paper IV**.

4.2.3.2 Natural History of EPE Carriage and Infection

Paper III corroborates reports from previous case-control studies (137, 148, 237, 238) and the biologically conceivable hypothesis that a trivial EPE finding is a risk factor for subsequent BSI. The steeply elevated adjusted relative risks were 61 and 32 for the urine and feces cohorts, respectively, compared to the general population. The reason behind constructing a population-based comparison group instead of using population figures from Statistics Sweden to calculate relative risk was to be able to individually match the cohorts on the matching variables and to adjust for potential confounders, especially comorbidity. However, since only 3 outcomes occurred in the comparison cohort, the possibility for adjustments was limited to a few covariates, including Charlson score. Consequently, residual confounding may remain in the reported figures.

Arguably the most important finding in **paper III** regards the time-course of EPE BSI risk after a previous EPE finding: We know that individuals with a previous EPE finding are experiencing increased risk, but *how big* is it and *for how long* does it last? As the prevalence of EPE has increased in Sweden, the question of how to interpret a previous positive screening finding has gained importance. *For a patient with a such finding two years back who is now being admitted to hospital for an orthopedic injury, should extra contact precautions be taken and a single room be made available? Should different clinical decisions be made if the same patient instead arrives in ambulance with a suspected severe sepsis?* Our data shows that the rate starts at a high level and falls drastically over the first year, why a reasonable judgment is that there is a considerably increased risk of EPE BSI if a patient presents with sepsis approximately during that first year.

To better answer the questions posed above, risk groups with especially high risk of EPE BSI may be discerned from our model. For instance, a male with a urological disorder and a previous UTI caused by *K. pneumoniae* experienced 14-fold risk of invasive infection when compared to a female with a previous finding of EPE in feces but without a urological disorder. In order to validly assess the predictive capacity of specific time frames since the baseline sample, however, the proper choice of study design is a cohort study based on patients with suspected Gram-negative sepsis. One such study from the Netherlands reported that the PPV of a documented EPE finding within 90 days for BSI resistant to third-generation cephalosporins was 7.4% (149).

The incidence rate of community-onset EPE BSI in Sweden from 2007-12 averaged 1.7 events per 100 000 person-years (**paper IV**) but increased to 2.9 during 2012. The overall incidence rate (including nosocomial events) was 6.0 in 2016-17, which suggests that the upward trend continues. At the time of writing this chapter data is several years old and the timeliness of the risk factor analysis and disease burden estimates can be questioned. Indeed, considering the ever-increasing dissemination of EPE in the community, the proportion of cases directly attributable to individual antibiotic consumption may decrease. Importantly, if antibiotic consumption does not specifically increase in subpopulations which are at higher risk of EPE BSI, the shrinking proportion could occur without changes in the absolute number of attributed cases.

4.2.3.3 Risk Factors for EPE BSI

Identifying risk factors that correlate with EPE BSI can be important for two reasons. First, if the factor causes the outcome and is modifiable, it may be a suitable target for public health policy interventions to reduce disease burden. Second, even if the factor *does not* cause the outcome, the correlation may be used in the clinic to *predict* risk and when necessary modify patient treatment accordingly.

From both **papers III** and **IV** we identified urological disorders as the factor that most increased the risk of the outcome. The causative mechanism is likely to be a propensity to develop UTIs, which both increases the risk of BSI *and* leads to antibiotic exposure that increases the risk of EPE acquisition. Another risk factor was immunological disorders. This factor's risk is probably mediated by lowering the body's ability to fend off disease-causing bacteria, increasing the risk of BSI without notably affecting the risk of EPE acquisition itself. Conversely, fluoroquinolone consumption increases the risk of the outcome selectively by creating an ecological niche for EPE, without affecting BSI risk itself.

4.2.3.4 Methodological Remarks

It is important to point out that EPE BSI comprises two phenomena with a separate set of causes and risk factors. The first phenomenon is *acquisition of EPE*, with risk factors including travel to high-endemic regions, hospitalization and residency at long-term care centers (see 1.5.6). The second phenomenon is *BSI*, which risk factors include conditions such as immune-deficiency, structural deviances in the respiratory or urinary tract and major trauma. As a practical example of this reasoning, patients with immune system deficiencies may be more likely to develop EPE BSI than community controls by virtue of their increased risk of BSI, *irrespective of their risk of acquiring EPE*. Consequently, in both **papers III** and **IV** an association between risk factor and the outcome may *either* be explained by a causal relationship between the studied factor and EPE (re-)acquisition, a causal relationship between the studied factor and BSI, *or both*. As noted previously (see headings 1.5.6.1 and 3.4.2.1), most studies that aim to investigate antibiotic consumption as a risk factor for BSI with EPE use control groups with non-ESBL-producing Enterobacteriaceae (133, 145), effectively removing such confounding by differences in underlying BSI risk. However, this choice of control group may instead introduce selection bias as controls on average may consume less antibiotics than community residents population (145).

Proper adjustment for central risk factors in the analysis is key to effective confounding control in etiological studies. In line with previous studies of antibiotic resistance outcomes (145, 239), we used Charlson Index Score to control for underlying morbidity. However, the population-based comparison cohort (**paper III**) and controls (**paper IV**) had significantly less morbidities than the exposed cohorts and cases, increasing the risk for residual confounding by morbidity in our analysis of antibiotic exposure. As regards the analyses of antibiotic exposures, there is a chance of the antibiotic having been dispensed *because of* an infection which has subsequently resulted in EPE BSI, rather than having caused it. After excluding consumption during the first week(s) in both **papers III** and **IV**, we believe the risk of such reverse causality was limited.

4.2.3.5 Conclusion

Papers III and **IV** were both register-based studies with near-comprehensive nation-wide data coverage which aimed to describe the burden of disease and identify groups at increased risk of severe infections with EPE. In **paper III**, the incidence of EPE BSI among individuals with previous EPE findings in urine or feces was studied for 6 years, providing insights about the natural history of EPE colonization and infection. The risk of EPE BSI was markedly increased for about one year after the initial finding in urine or feces, which is important information that can help direct targeted therapy in antibiotic stewardship programs.

As regards other factors that affect EPE BSI risk in the two different populations, **both papers** identified increased risk for male sex, high age, urological disorders and other underlying morbidities. The relative risk of specific patient subgroups could be discerned by combining risk factors. Furthermore, **both papers** identified consumption of fluoroquinolones, penicillins with extended spectrum or compounds with selective activity against gram-negative bacilli but mostly not EPE during the last 3 months to be associated with a 2 to 5.5-fold increased risk of EPE BSI.

5 CONCLUDING REMARKS

Success represents the 1% of your work which results from the 99% that is called failure

Soichiro Honda (1906 – 1991), Founder of Honda Motor Co.

This thesis provides descriptive and analytical measurements of the EPE dissemination and disease burden in Sweden and Guinea-Bissau. It contributes to previous literature in two different ways. First, by providing descriptive epidemiology and molecular epidemiological data on EPE and bloodstream infection in Guinea-Bissau, where no studies of EPE dissemination have previously been performed and routine laboratory infrastructure for blood cultures is lacking. Second, by comparing a comprehensive, Sweden-wide material on EPE BSI cases to population-based controls in terms of risk factors and measures of disease burden.

- Nearly one third of 370 consecutively enrolled children with fever and/or tachycardia seeking care at a pediatric emergency department in Bissau, Guinea-Bissau, were colonized by EPE in their feces. The large clonal heterogeneity of the isolates indicates widespread community dissemination of ESBLs.

- *S. aureus* and NTS were the commonest bacteria in blood cultures from the above-mentioned children. The prevalence of bacteremia was higher than that of malaria, irrespective of whether *S. aureus* findings were classified as contaminants or pathogens. Yet, a majority of children were diagnosed and treated primarily for malaria. Two out of five Enterobacteriaceae findings (40%) produced ESBLs.

- Nearly half (46%) of the children with a positive blood culture in the same study did not present with fever. This indicates low sensitivity for BSI in current WHO guidelines for management of severe infections in children, which base antibiotic treatment recommendations on fever.

- The risk of BSI with EPE was 30- and 60-fold among individuals with a previous EPE finding in feces and urine, respectively, after controlling for age, sex residence county and underlying morbidity. The largest risk increase occurred in the short term after the baseline finding and thereafter rapidly declined with time.

- Underlying morbidities, in particular cancer diagnoses and urological disorders, were associated with 2- to 4-fold odds of EPE BSI both among documented carriers and in the Swedish population.

- Fluoroquinolone consumption was associated with moderately to strongly increased odds of EPE BSI. The point estimate for cephalosporin consumption was 3.25, however we had insufficient statistical power to confirm the increased risk reported in previous studies.

6 FUTURE PERSPECTIVES

Every morning brings new potential, but if you dwell on the misfortunes of the day before, you tend to overlook tremendous opportunities

Harvey Mackay (born 1932), American author

6.1 SCENARIOS

The ongoing depletion of the antibiotic arsenal has severe consequences for health care and the public. The following four are among those that I find of particular concern.

First and most conspicuously, mortality in the elderly community will increase as the chances of cure for common infections such as pneumonia, pyelonephritis and sepsis decline. These infections, which have often been considered "trivial" during the antibiotic era, drive the disease burden of gram-negative infections in most age groups, but the elderly have both the highest absolute number of cases and the highest mortality. Few years are lost per case, however the total number of years lost in the population is high due to the high incidence. Second, insufficiently effective preemptive antibiotics in orthopedic and general surgery results in higher complication rates, shifting the risk-benefit calculation in favor of conservative treatment. Poorer quality of life ensues as cholecystectomies, hip and knee prosthesis operations and other elective procedures are avoided due to the risk of life-threatening post-operative infections.

Third and perhaps most disturbingly, we will see increased morbidity and mortality due to less common but haphazardly distributed medical perils in younger ages. These include postpartum fever, urinary tract and systemic infections resulting from trauma. The total disease burden is lower than in the elderly, however it will strike individuals in early life and mid-life and project a gruesome imagery of the resistance problem into public view. Fourth, regions with the highest overall burden of bacterial infections, such as economically underdeveloped nations in Africa, will face the direst consequences (to the extent that effective antibiotics have previously been accessible).

6.2 RESEARCH

Very few antibiotics specifically targeting the Gram-negative spectrum have been developed in recent years (191, 196). As the incidence of infections with EPE continues to increase, carbapenems are increasingly become the empirical treatment of choice for severe infections that are presumptively caused by Gram-negative bacteria. (Indeed, retail sales have already spiked in India, Pakistan and Egypt (240).) It is therefore troubling to follow the reports of ever-increasing dissemination and disease burden caused by $ESBL_{CARBA}$-producing bacteria (109, 111, 114, 115). In order to preserve carbapenems as a last-resort treatment, the fraction of ESBL-producing isolates among community-acquired infections needs to be low. Therefore, studies that measure the disease burden of EPE infections that is attributable to consumption of specific antibiotic classes for infections with EPE are needed. Such data can propel informed policy-making on how to better handle our communal antibiotic resource. Antibiotic stewardship programs are already in place across the world and, properly managed, provide a vehicle for implementing sound dispensation practices in many hospitals.

However, limiting the scope for research and interventions to antibiotic stewardship programs and measures within the health-care system will be insufficient. In the light of the successful spread of CTX-M-producing EPE in the community worldwide, a more comprehensive source attribution model that encompasses nosocomial, community and ecological perspectives is needed in order to effectively address the problem (181). It is my belief that in order to understand the determinants of resistance development and dissemination, individual-level studies of factors such as morbidity, socio-economy and antibiotic consumption need to be complemented with ecological studies. These should seek to draw conclusions from macro dimensions such as east-west and south-north, rich-poor, burden of bacterial infections, sanitation systems and use of antibiotics in livestock. Particularly, life-cycle studies of antibiotic drugs that measure the full ecological impact of production, use and disposal of these drugs in different socio-geographies are needed.

6.3 POLICY

Knowing what must be done does away with fear

Rosa Parks (1913 – 2005), American civil rights activist

In "Rose's Strategy of Preventive Medicine", Geoffrey Rose discusses the high-risk prevention strategy as a "targeted rescue operation for vulnerable individuals" (241). However, he argues, "…the burden of ill health comes more from the many who are exposed to a low inconspicuous risk than from the few who face an obvious problem". For example, patients that have cholesterol level above the threshold level are put on statins to treat their hypercholesterolemia, while the contemporary lifestyle has right-shifted the normal distribution of blood cholesterol in the whole population. Even if cardiovascular disease risk surges with decidedly increased cholesterol levels, the right-shift in the normal distribution curve will produce more cases among those with "normal" cholesterol levels, since the vast majority of the population is in that category. This, according to Rose, "sets a limit to the effectiveness of an individual (high-risk) approach to prevention".

Doubtlessly, ESBL dissemination works principally different from cholesterol levels and other non-communicable diseases, for instance due to the fact that the individual-based approach of containment and eradication of ESBLs in an inpatient can spare both that patient *and* his or her fellow inpatients. Yet I believe that the mentality of the high-risk strategy of prevention may result in an overly one-sided focus on efforts to prevent nosocomial spread of pathogens and to reduce in- and outpatient dispensation of antibiotic drugs. These are the actions that *can* be taken within health-care (where problem awareness is high), while actions that *need* to be taken include political decision making and international development cooperation. Examples of such steps include implementation and/or enforcement of antibiotic sales regulations in weak or failed states, investments in sanitation systems and full-scale, improved vaccine coverage for major bacterial diseases and full-scale rollout of antiretroviral therapy and tuberculosis treatment. Finally, a debate on the role of widespread international tourism and travel patterns in dissemination of these pathogens should be encouraged.

In conclusion, I agree with Dr. Rose when he suggests that "a population strategy of prevention is necessary where risk is widely diffused through the whole population".

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Det allra viktigaste är att man inte skriver som man har forskat

Ur ”Den populärvetenskapliga textens struktur” av Jessica Parland-von Essen

Ungefärligen sedan millennieskiftet har så kallade ESBL-bildande bakterier spridits globalt. Namnet ESBL står för *extended spectrum β-lactamase*, den engelska beteckningen för en uppsättning enzymer som på svenska benämns β-laktamaser med utvidgat spektrum. ESBL-enzymer bildas av bakterier och gör dessa resistenta mot antibiotikagruppen tredje generationens cefalosporiner. Dessa antibiotika används idag för att behandla allvarliga infektionstillstånd såsom övre urinvägs-, tarm- och blodinfektioner. Som lök på laxen så har ESBL-bildande bakterier dessutom oftast utvecklat motståndskraft mot andra viktiga antibiotikagrupper, däribland *kinoloner* som innefattar det viktiga preparatet ciprofloxacin. I dagsläget finns endast en antibiotikagrupp med säker effekt mot ESBL-bildande bakterier, men de senaste åren har resistens även mot denna grupp blivit vanligare.

En förutsättning för spridningen av ESBL-bildande bakterier som ofta nämns är samhällets stora *antibiotikabörda*. Detta begrepp innefattar människors och djurs konsumtion av antibiotika, såväl som utsläpp i naturen från preparatens produktion och nedbrytning. I rika länder som Sverige regleras tillgången genom att recept från läkare krävs, å andra sidan är tillgången till sådana recept genom sjukvården god. Djur i de flesta länder med ett industrialiserat jordbruk får antibiotika i tillväxtsyfte och för att bekämpa infektioner. I fattiga länder som Guinea-Bissau säljs antibiotika i tablettform öppet på gatan och antalet bakteriella sjukdomar är stor, vilket ökar antibiotikabördan. Bristfälliga avloppssystem främjar spridning i samhället av både bakterier och nedbrytningsprodukter från antibiotika. Merparten av antibiotikaproduktionen sker i länder som Indien och Kina, där efterlevnaden av regelverk som omgärdar produktionen är skral.

Insatser för att minska antibiotikakonsumtionen pågår men varje minskning måste göras omsorgsfullt, eftersom utebliven behandling i värsta fall är förenat med livsfara. Syftet med den här avhandlingen är att beskriva förekomsten och sjukdomsbördan av ESBL-bildande bakterier i Guinea-Bissau och Sverige. I studierna i Sverige söker vi även att beskriva storleken på betydelsen av bakomliggande sjuklighet, antibiotikakonsumtion och socioekonomi för risken att insjukna i blodinfektion med dessa bakterier. Ansatsen är att vi genom att identifiera ESBL-problemets underliggande orsaker och uppskatta dess storlek kan bistå sjukvårdens beslutsfattare i att identifiera de interventioner som har störst möjligheter att minska de resistenta bakteriernas utbredning.

I Guinea-Bissau samlade vi in prover från avföring och blod hos omkring 400 barn med tecken på allvarliga infektioner. En tredjedel av barnen bar ESBL-bildande bakterier i sin avföring, vilket bör jämföras med andra studier i Afrika där andelen varit lägre eller liknande. Från 13% av barnen växte bakterier i blododlingarna och 2 av 5 fynd av bakteriearter som kan bilda ESBL gjorde det. En stor andel av barnen fick inledningsvis fel antibiotikatyp i förhållande till blododlingsfyndet, delvis på grund av att malaria diagnostiserades i mycket större utsträckning än vad vi senare kunde bekräfta med laboratorieprover. Studieresultaten kan användas vid kliniken för att förändra initial behandling men avsaknaden av grundläggande resurser, inklusive flera antibiotikatyper och laboratoriediagnostik, är exempel på mer akuta behov för att kunna förbättra vården.

I Sverige använde vi Folkhälsomyndighetens register över anmälningspliktiga sjukdomar, SmiNet, dit fynd av ESBL-bildande bakterier är anmälningspliktiga enligt Smittskyddslagen sedan

2007. Alla de omkring 22 000 individer med ett tidigare urin- eller avföringsodlingsfynd följdes över tid i registret för att identifiera vilka som senare även hade ett blododlingsfynd med sådana bakterier (totalt 1245 individer). Totalt insjuknade närmare 4% i gruppen med urinodlingsfynd och omkring 2% i gruppen med avföringsfynd. Dessa gruppers risk jämfördes genom så kallade regressionsanalyser med risken hos en slumpmässigt utvald grupp om cirka 45 000 individer från Sveriges befolkning. Risken var omkring 60 gånger högre hos de med tidigare urinodlingsfynd och 30 gånger högre hos de med avföringsfynd av ESBL-bildande bakterier jämfört med befolkningen i övrigt, efter att hänsyn tagits till bland annat bakomliggande sjukdomar och ålder. Den tydligaste riskökningen sågs direkt efter odlingsfyndet och avklingade därefter successivt. Den fortsatte dock att vara förhöjd i 5 år bland de med urinodlingsfynd och i 3 år bland de med avföringsodlingsfynd.

Därtill undersökte vi betydelsen hos individen som kan påverka risken för blodinfektion med ESBL-bildande bakterier genom att hämta uppgifter från hälso- och arbetsmarknadsregister hos Socialstyrelsen samt Statistiska Centralbyrån. Starkast riskökning efter statistisk justering för viktiga förväxlingsfaktorer sågs för urologiska sjukdomar och blodcancerdiagnoser. Vad gäller antibiotika som riskfaktor för blodinfektion med ESBL-bildande bakterier så var kinolonantibiotika den grupp som var starkast förknippad med ökad risk. Detta liknar många tidiga publicerade studier men våra studier utgör ett viktigt bidrag med tanke på deras stora skala och att de kunde göras på nationellt heltäckande information om såväl blodinfektioner med ESBL-bildande bakterier som antibiotikakonsumtion.

Sammanfattningsvis så visar studierna från Sverige att risken för att få en allvarlig blodinfektion med ESBL-bildande bakterier är kraftigt förhöjd efter ett tidigare fynd av sådana från urin eller avföring jämfört med befolkningen i stort men att risken avtar snabbt och planar ut efter 3–5 år. Studieresultaten stärker även tidigare forskning som pekar på att konsumtion av kinolonantibiotika under de senaste tre månaderna ökar individens risk för att insjukna i blodinfektion med ESBL-bildande bakterier. Multisjuka patienter, framförallt de med underliggande urologiska sjukdomar eller cancersjukdomar, som har konsumerat vissa antibiotikatyper under de senaste tre månaderna har en särskilt hög risk att insjukna i blodinfektion med dessa bakterier. Detta bör tas i beaktning i valet av antibiotikabehandling när dessa patientgrupper söker vård med misstänkta allvarliga infektionstillstånd.

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