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Extended-Spectrum ß-Lactamase-Producing Enterobacteriaceae

Antibiotic Consumption, Detection and Resistance Epidemiology

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Till mormor Anna-Lisa

“Allting är mycket osäkert, och det är just det som lugnar mig”

”All things are so very uncertain, and that’s exactly what makes me feel reassured”

Ur Trollvinter av Tove Jansson

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ABSTRACT

ESBL-producing Enterobacteriaceae are emerging worldwide and they are frequently multi-drug resistant, thus limiting treatment options for infections caused by these pathogens.

The overall aim of the thesis was to investigate ESBL-producing Enterobacteriaceae in a Swedish county.

First, we developed a molecular method, a multiplex PCR assay for identification of SHV, TEM and CTX-M genes in clinical isolates of Enterobacteriaceae with an ESBL phenotype.

From 2002 until the end of 2007 all isolates of ESBL-producing Enterobacteriaceae in Östergötland, Sweden were further investigated. The prevalence of ESBL-producing Enterobacteriaceae was low, <1%, but increasing, while the antibiotic consumption remained unchanged. CTX-M enzymes, particularly CTX-M group 1, dominate in our region as well as in the rest of Europe.

Furthermore, we have investigated antimicrobial susceptibility by performing MIC-testing in a large, well-characterized population of CTX-M-producing *E. coli*. Only three oral antimicrobial agents (fosfomycin, nitrofurantoin and mecillinam) demonstrated susceptibility above 90%. High susceptibility, >90%, was also demonstrated for carbapenems, colistin, tigecycline and amikacin. Sixty-eight per cent of ESBL-producing *E. coli* was multi-resistant, and the most common multi-resistance pattern was the ESBL phenotype with decreased susceptibility to trimethoprim, trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin and tobramycin. Isolates belonging to CTX-M group 9 are generally more susceptible to antibiotics than the CTX-M group 1-producing *E. coli*.

Finally, a prospective multicentre case-control study examined the prevalence of ESBL-producing Enterobacteriaceae in faecal samples before and after travel abroad and the risk factors of acquisition. Sixty-eight of 226 travellers (30%) had ESBL-producing Enterobacteriaceae in the faecal flora. The geographical area visited had the highest impact on acquisition, with the highest risk for travellers visiting the Indian subcontinent, followed by Asia and Africa north of the equator. Also, acquisition of ESBL-producing Enterobacteriaceae during travel is associated with abdominal symptoms such as diarrhoea. Age also seemed to affect the risk of acquiring ESBL-producing Enterobacteriaceae, the highest risks were found among travellers ≥ 65 years.

This thesis has contributed to increased understanding of the epidemiology of ESBL-producing Enterobacteriaceae and their susceptibility to both beta-lactam and non-beta-lactam agents.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Gramnegativa tarmbakterier tillhörande familjen Enterobacteriaceae kan orsaka urinvägsinfektioner men även allvarligare infektioner såsom till exempel pyelonefrit, sepsis och postoperativa bukinfektioner. De orsakande bakterierna kommer ofta från patientens egen tarmflora. Under de senaste åren har produktion av ”extended spectrum beta lactamases” (ESBL) hos gramnegativa bakterier blivit ett snabbt växande antibiotikaresistensproblem över stora delar av världen. Ofta har bakterier med ESBL även förvärvat resistensmekanismer mot andra antibiotikagrupper, så kallad multiresistens. Patienter infekterade med dessa bakterier riskerar att svara sämre eller inte alls på behandling med antibiotika.

I denna avhandling beskrivs ESBL-producerande Enterobacteriaceae ur flera aspekter.
I det första delarbetet utvecklade vi en molekylärbiologisk analys för att påvisa ESBL-gener hos Enterobacteriaceae i kliniska prover.
I delarbete 2 studerades förekomsten av ESBL-producerande Enterobacteriaceae i kliniska odlingar under perioden 2002-2007 i Östergötland och dessa var fortfarande på en låg nivå och under samma period var förbrukningen av antibiotika oförändrad. I vår region, precis som i övriga Europa, dominerar ESBL-enzymer tillhörande CTX-M grupp 1.
I delarbete 3 och 4 testades olika antibiotika för att se vilka preparat som ESBL-producerande *E. coli* bakterier är känsliga för och som skulle kunna vara möjliga behandlingsalternativ. Bland antibiotika som kan tas peroralt var det fosfomycin (finns enbart att tillgå på licens), nitrofurantoin och mecillinam som mer än 90 % av bakterierna var känsliga för. Ytterligare behandlingsalternativ är karbapenemer, colistin, tigecyclin, och amikacin men nackdelen med dessa är att de måste ges parenteralt. Det framkom skillnader i känslighet mellan olika grupper av ESBL-enzymer där CTX-M grupp 9 är generellt mer känsliga för antibiotika än CTX-M grupp 1-producerande bakterier. Sextioåtta procent av alla ESBL-producerande *E. coli* som vi undersökte från Östergötland var multiresistenta det vill säga resistenta mot flera olika grupper av antibiotika och det medför att infektioner orsakade av dessa bakterier blir svårbehandlade.
I delarbete 5 undersökte vi huruvida resande till länder med hög förekomst av ESBL-producerande tarmbakterier medför en risk för förvärvande av sådana bakterier till tarmfloran, och vi fann att ca 30 % av studiepersonerna förvärvade ESBL-producerande tarmbakterier när de reste utanför Norden. Risken var störst vid resor till den indiska subkontinenten, följt av Asien och Nordafrika. Resenärerna som blev bärare av ESBL-producerande bakterier i tarmfloran under resan rapporterade oftare besvär från buken såsom exempelvis diarré i samband med utlandsresan och dessutom innebar ålder 65 år eller äldre en ökad risk för bärarskap.

Det finns ett stort behov av nya antibiotika utvecklas för att kunna behandla multiresistenta gramnegativa infektioner samtidigt som det bör betonas att det inte är lösningen på problemet med antibiotikaresistens. Det krävs åtgärder på global nivå med tillgång till rent vatten och fungerande avlopp, dessutom krävs förbättrad mikrobiologisk diagnostik, hygien och rationell användning av antibiotika inom både human- och veterinärmedicin.

LIST OF PAPERS

This thesis is based on the following original papers:

I. Monstein H-J, Östholm-Balkhed Å, Nilsson M.V, Nilsson M, Dornbusch K, Nilsson L.E. Multiplex PCR amplification assay for the detection of ^{bla}SHV, ^{bla}TEM and ^{bla}CTX-M genes in Enterobacteriaceae. APMIS 2007, 115:1400-8

II. Östholm-Balkhed Å, Tärnberg M, Nilsson M, Johansson AV, Hanberger H, Monstein H-J & Nilsson LE. Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae and trends in antibiotic consumption in a county of Sweden. Scand J Infect Dis 2010, 42:831-8.

III. Tärnberg M, Östholm Balkhed Å, Monstein H-J, Hällgren A, Hanberger H & Nilsson LE. In vitro activity of beta-lactam antibiotics against CTX-M-producing *Escherichia coli*. Eur J Clin Microbiol Infect Dis 2011, 30:981-7.

IV. Östholm Balkhed Å, Tärnberg M, Monstein H-J, Hällgren A, Hanberger H & Nilsson LE. High frequency of co-resistance in CTX-M-producing *Escherichia coli* to non-beta-lactam antibiotics, with the exceptions of amikacin, nitrofurantoin, colistin, tigecycline, and fosfomycin, in a county of Sweden. Scand J Infect Dis 2013, 45:271-8.

V. Östholm-Balkhed Å, Tärnberg M, Nilsson M, Nilsson L.E, Hanberger H, Hällgren A. Travel-associated faecal colonization with ESBL-producing Enterobacteriaceae: incidence and risk factors. J Antimicrob Chemother 2013, 68: 2144-53.

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ABBREVIATIONS

AmpC Ampicillinase C
ATC Anatomical Therapeutic and Chemical Classification system
bla Gene encoding beta-lactamase
BSAC British Society for Antimicrobial Chemotherapy
CTX-M Cefotaximase Munich
DDD Defined Daily Dose
DID Defined Daily Doses per 1 000 inhabitants and day
EARS-Net European Antimicrobial Resistance Surveillance Network
ECOFF Epidemiological cut-off value
ESBL Extended-spectrum beta-lactamases
$ESBL_A$ "Classical" ESBL (SHV-, TEM-variants and CTX-M)
$ESBL_{CARBA}$ Carbapenemases (KPC, NDM, VIM and OXA-variants)
$ESBL_M$ Miscellaneous ESBL (plasmid-mediated AmpC)
EUCAST European Committee on Antimicrobial Susceptibility Testing
KPC *Klebsiella pneumoniae* carbapenemase
MBL Metallo-beta-lactamases
MDR Multi-drug Resistance
MIC Minimal inhibitory concentration
MDA Multiple displacement amplification
MLST Multilocus sequence typing
NDM New Delhi Metallo-beta-lactamase
NordicAST Nordic Committee on Antimicrobial Susceptibility Testing
OXA Oxacillinase-type beta-lactamase
PBP Penicillin binding protein
PCR Polymerase chain reaction
PFGE Pulsed-field gel electrophoresis
Qnr Plasmid-mediated quinolone resistance
SHV Sulfhydryl variable, a type of beta-lactamase
SMART Study for Monitoring Antimicrobial Resistance Trends
SMI Swedish Institute for Infectious Disease Control
SRGA Swedish Reference Group of Antibiotics
STRAMA Swedish Strategic Programme against Antibiotic Resistance
ST131 Sequence type 131, an international clone of *E. coli*
TEM Temoneira, a type of beta-lactamase named after the first patient
TMP Trimethoprim
TMP-SMX Trimethoprim-sulfamethoxazole or co-trimoxazole
UTI Urinary tract infection
VIM Verona integron-encoded metallo-beta-lactamase

INTRODUCTION

The pathogens- Enterobacteriaceae

The Enterobacteriaceae are Gram-negative bacteria that are natural inhabitants of the intestinal flora, but some of them are human intestinal pathogens. Enterobacteriaceae can also be found in the environment, soil, and on plants.
Escherichia coli is the most frequent cause of some of the most common bacterial infections, including urinary tract infections, bacteraemia and bacteria-related traveller's diarrhoea. It can also cause other clinical infections such as meningitis and pneumonia. *Klebsiella pneumoniae* is associated with pneumonia in immunocompromised hosts, but can also cause urinary tract infections, abdominal infections and bloodstream infections. [1, 2]

Antimicrobial agents and corresponding resistance mechanisms in Enterobacteriaceae

An overview of antibiotics used for treatment of infections caused by Enterobacteriaceae and a more specific description of associated mechanisms for resistance in *E. coli* and *K. pneumoniae*.

Beta-lactam antibiotics
Cell wall active agents, especially beta-lactam antibiotics, play a crucial role in the treatment of bacterial infections, but the emergence of multidrug resistant bacterial strains is alarming.
Alexander Fleming discovered penicillin in 1928 and during World War II penicillin G became available for treating infections, which represented a major advancement in medical history. Beta-lactam antibiotics are still the cornerstones of most therapeutic regimens, used in both common- as well as life-threatening infections. The beta-lactam ring is the characteristic of all beta-lactams and a condition for their antibacterial effect by destruction of the bacterial cell wall by binding to penicillin-binding proteins PBPs, which are the enzymes necessary for the formation of bacterial cell walls. Thus, only growing bacterial cells are affected by beta-lactam antibiotics.[2-4]
The most significant and prevalent beta-lactam resistance mechanism in Enterobacteriaceae consists of beta-lactamases, different enzymes encoded by genes either chromosomally located or carried in plasmids. Moreover, beta-lactam resistance due to efflux pump and porin loss is described. [5, 6] [7, 8]
A recent study indicates that mutations in a penicillin-binding protein may also play a role in carbapenem-resistance among *E. coli*.[9]

The beta-lactam antibiotics include four main groups: penicillins, cephalosporins, monobactams and carbapenems.

Penicillin G and V both have a narrow antibacterial spectrum, targeting preferably gram-positive cocci, due to the presence of an outer membrane in gram-negative bacteria that penicillin G or V cannot easily penetrate.

In contrast to penicillin G, ampicillin and ampicillin-like agents, such as amoxicillin, are active against some gram-negative organisms. However, ampicillin-resistant *E. coli* are common and resistance is nearly always due to beta-lactamase production.[10] Beta-lactamase inhibitors exhibit weak antibacterial activity against most bacteria but they bind irreversibly to the beta-lactamase. Consequently, the bound beta-lactamase cannot hydrolyse the penicillin and the penicillin is free to bind to the PBP and exert its antibacterial effect. Amoxicillin combined with clavulanic acid and piperacillin in a fixed combination with tazobactam are commonly used, of which the latter has the broadest antibacterial spectrum. Hyperproduction of plasmid-encoded beta-lactamases usually results in cross-resistance to all inhibitor-penicillin combinations.[11-14] Mecillinam differs in its antibacterial activity from other penicillins, being much more active against gram-negative than against gram-positive organisms. It is highly active against most Enterobacteriaceae and resistance to mecillinam is much less common than to ampicillin and even ESBL-producing strains tend to be mecillinam-sensitive.[15] Temocillin has only antibacterial activity against gram-negative organisms, with the advantage of high stability to beta-lactamases, including ESBLs. The drug is only available in Belgium and the UK.[16]

Cephalosporins are bactericidal and have the same mode of action as other beta-lactam agents. They are commonly classified into generations and the antibacterial spectrum differs among them. The first generation, e.g. cefadroxil, have the strongest activity against gram-positive bacteria and some activity against Enterobacteriaceae, although they are hydrolysed by ESBL- and AmpC-producing bacteria. The second, e.g. cefuroxime and third, e.g. cefotaxime, ceftibuten, ceftazidime, generations exhibit more activity against gram-negative pathogens and some of them have less gram-positive antibacterial activity. They are inactivated by ESBLs, AmpC beta-lactamases and most metallo-beta-lactamases. The fourth generation, e.g. cefepime, has a wider spectrum than the third-generation cephalosporins, and they are effective against both gram-positive as well as gram-negative organisms. Cefepime is hydrolysed by CTX-M type ESBLs and by most carbapenemases, but in general is not hydrolysed by AmpC beta-lactamases. Finally, the fifth generation, ceftobiprole, which is not yet registered in Sweden, exhibits potent gram-positive activity even against methicillin- and vancomycin-resistant Staphylococcus aureus and penicillin-resistant pneumococci and its gram-negative activity resembles that of cefepime. Ceftaroline was registered in Sweden 2012 and has similar gram-positive activity but slightly less gram-negative activity. [17-24]

Monobactams, with the commercially available aztreonam, are only active against gram-negative bacteria. Aztreonam has the same resistance profile as other beta-lactam agents and is hydrolysed by beta-lactamases with an extended-spectrum.[25]

Carbapenems (e.g.imipenem, meropenem, doripenem, ertapenem) cover a broad antibacterial spectrum with activity against both gram-positive and gramnegative aerobic and anaerobic bacteria. Enterobacteriaceae are generally carbapenem-susceptible, including ESBL- and AmpC-producing isolates. Unfortunately, in recent years emerging resistance to carbapenems has been observed. Most often, multiple mechanisms are responsible for the final resistance phenotype. It is likely that the interplay between efflux pumps, beta-lactamases and altered membrane permeability through porin loss is what mediates the resistance. Beta-lactamases that are able to hydrolyze carbapenems are categorized as class A carbapenemases consisting of plasmid-encoded KPC, class D

carbapenemases also known as OXA-type (mainly in Acinetobacter), and class B metallo-beta-lactamases MBLs, including IMP, VIM.[26-29]

Quinolones

Nalidixic acid is the original substance in the group of quinolones, which were discovered in the early 1960s. Gradually new generations of quinolones, fluoroquinolones (mainly ciprofloxacin) with a broader antibacterial spectrum and extended spectrum quinolones (moxifloxacin) were developed. Quinolones have a selective and bactericidal effect by interfering with bacterial DNA replication as they target the enzymes that control DNA supercoiling, DNA-gyrase and topoisomerase IV.
Several mechanisms of quinolone resistance in Enterobacteriaceae are known. Mutations in genes coding for DNA-gyrase or topoisomerase IV, resulting in reduced binding affinity to quinolones, porin changes and active efflux pumps that decrease intracellular quinolone concentration are described resistance mechanisms. Moreover, plasmid-borne resistance mediated by qnr-genes which produce proteins that can bind to DNA-gyrase or topoisomerase IV thus minimizing opportunities for quinolones to act upon these enzymes, has been reported. Another transferable mechanism of quinolone resistance is a variant of the *aac(6')- Ib* gene that, in addition to inactivation of aminoglycosides, also inactivates quinolones.[30, 31]

Aminoglycosides

Streptomycin was discovered at the beginning of the 1940s, and it was the first antibacterial agent that could be used to treat tuberculosis. The aminoglycosides work by binding the bacterial 30S ribosomal subunit and thereby inhibit protein synthesis. They have a broad spectrum of activity with a bactericidal effect against both gram-positive and gram-negative bacteria. Other antibiotics that affect bacterial protein synthesis are usually bacteriostatic, thus the bactericidal effect is notable and unexplained.
Streptomycin has been deregistered due to side effects, and four other aminoglycosides-tobramycin, gentamicin, amikacin and netilmicin are in clinical use nowadays in Sweden.[32] Aminoglycosides and beta-lactam antibiotics have a synergistic effect, due to the beta-lactam facilitating penetration of the aminoglycoside into the cell. [33] In addition, for treatment of gram-negative organisms, aminoglycosides have been shown to significantly reduce beta-lactam-induced endotoxin release.[34]
There are four known resistance mechanisms by which Enterobacteriaceae develop resistance to aminoglycosides: alteration of the ribosomal site of action by plasmid-mediated methylase genes, decreased cell permeability, overexpression of efflux pumps, and production of aminoglycoside-modifying enzymes. In amikacin the presence of a side-chain gives the drug stability against most of the bacterial plasmid-mediated enzymes. For this reason, amikacin is active against many gentamicin- and tobramycin-resistant gram-negative organisms.[32]

TMP / TMP-SMX

Sulfonamides were the first effective systemic antimicrobial agent used during the 1930s. They exert an anti-metabolic effect as they inhibit an enzyme involved in the synthesis of folic acid in bacteria. Trimethoprim was first used for treatment of infections in humans thirty years later. The combination of trimethoprim and sulfamethoxazole TMP-SMX (or co-trimoxazole) blocks sequential steps in the pathway of folic acid synthesis, thus they potentiate the antibacterial activity of one another and act synergistically against a wide variety of organisms. [1, 2] Resistance to both trimethoprim and TMP-SMX are common. The mechanisms for resistance in Enterobacteriaceae are several mutations in genes

encoding DHFR (dihydrofolate reductase, which is the target enzyme of trimethoprim) and these genes can be located either in chromosomes or plasmids. This implies reduced trimethoprim binding or overproduction of DHFR.[35, 36] Sulfonamide resistance in clinical isolates of Enterobacteriaceae is mediated by plasmid-borne genes encoding alternative drug-resistant variants of the DHPS (dihydropteroate synthase) enzyme.[37]

Tigecycline

Tigecycline, a glycine derivative of tetracyclines, has been developed as an antibacterial agent to circumvent the tetracycline resistance mechanisms (tet-genes expressing proteins for efflux pumps and ribosomal protection proteins). Tigecycline acts bacteriostatically by reversibly binding to the 30S ribosomal subunit and preventing protein synthesis. Furthermore, the spectrum of antibacterial activity is broad. However, there is evidence for tigecycline resistance in Enterobacteriaceae caused by multidrug efflux pumps.[38]

Polymyxins

The polymyxins are a group of antibiotics; among these only polymyxin B and E (colistin) are used clinically. The detailed mechanism of action is not clear, however they are believed to interact with lipopolysaccharide, LPS, on the outer membrane of gram-negative bacteria and thereby cause permeability changes in the cell envelope, leakage of cell contents and, subsequently, cell death. In addition, the polymyxins have anti-endotoxin activity.

The polymyxins are bactericidal drugs and as a consequence of their mode of action the antibacterial spectrum is limited to gram-negative bacteria. They are active against most Enterobacteriaceae, but Proteus spp. and *Serratia marcescens* are usually resistant. Resistance to polymyxins is not very common and the mechanism of resistance is poorly defined. Resistance in *E. coli* may be based on modification of lipid A of the LPS, thus preventing colistin binding. In polymyxin-resistant strains of *K. pneumoniae* an increased production of capsule polysaccharide has been observed. Almost complete cross-resistance exists between colistin and polymyxin B. The revival of colistin, an old antibiotic discovered in the late 1940s, is due to its role as last-line therapy against infections caused by carbapenem- or multidrug- resistant gram-negative bacteria. [39-41]

Nitrofurantoin

Nitrofurantoin has been available since the middle of the 1950s for treatment of urinary tract infections, it has a broad antibacterial spectrum covering both gram-positive and gram-negative bacteria. The precise mechanism of action of nitrofurantoin is not known, but it has several mechanisms of action on bacteria and this may explain why bacterial resistance to this drug is uncommon. The drug inhibits a number of bacterial enzymes and attacks ribosomal proteins which causes inhibition of protein synthesis and may also damage DNA.

A reduction of nitrofuran reductase activity was found in nitrofurantoin-resistant strains of *E. coli* conferred by mutations in the nfsA and nfsBgenes. There is evidence that suggests a reduction in fitness in nitrofurantoin-resistant strains in the absence of antibiotics. In the presence of therapeutic levels of nitrofurantoin even resistant mutants had altered growth.[42, 43]

Fosfomycin

Fosfomycin has a mechanism of action similar to that of the betalactams as it interferes with bacterial cell wall synthesis. It binds to and inactivates transferase, an enzyme necessary for the formation of bacterial cell walls. The substance has bactericidal

properties against both gram-positive and gram-negative bacteria. Fosfomycin is particularly used for treatment of lower UTI. In uncomplicated cases; treatment with a single dose fosfomycin may be sufficient. Several mechanisms conferring resistance to fosfomycin have been described. These can broadly be divided into two types: mutational changes related to the transport of fosfomycin into the bacterial cell and a plasmid-encoded enzyme that causes inactivation of fosfomycin. As in the case of nitrofurantoin resistance, an in-vitro study demonstrated that fosfomycin mutations reduced the fitness of the *E. coli* strains. Fosfomycin resistance levels are quite low even in countries with more frequent use of the drug.[44, 45]

Chloramphenicol

The mechanism of action by chloramphenicol is to stop bacterial growth by inhibiting protein synthesis. The drug inhibits an enzyme, peptidyl transferase, in the bacterial ribosome.

Chloramphenicol is a reversible bacteriostatic agent with a broad antibacterial spectrum. It has been in clinical use since 1949. Nonetheless, it is not widely used because of feared side effects such as, reversible bone marrow depression or fatal aplastic anaemia.

There are several mechanisms by which gram-negative organisms can acquire chloramphenicol resistance. Reduced membrane permeability prevents the drug from entry and transmembrane efflux pumps export the drug from the bacteria. However, the most important mechanism is the enzyme called chloramphenicol acetyltransferase, which inactivates chloramphenicol and prevents chloramphenicol from binding to the ribosome. The cat-genes are plasmid-borne and thus transferable to other bacteria.[46]

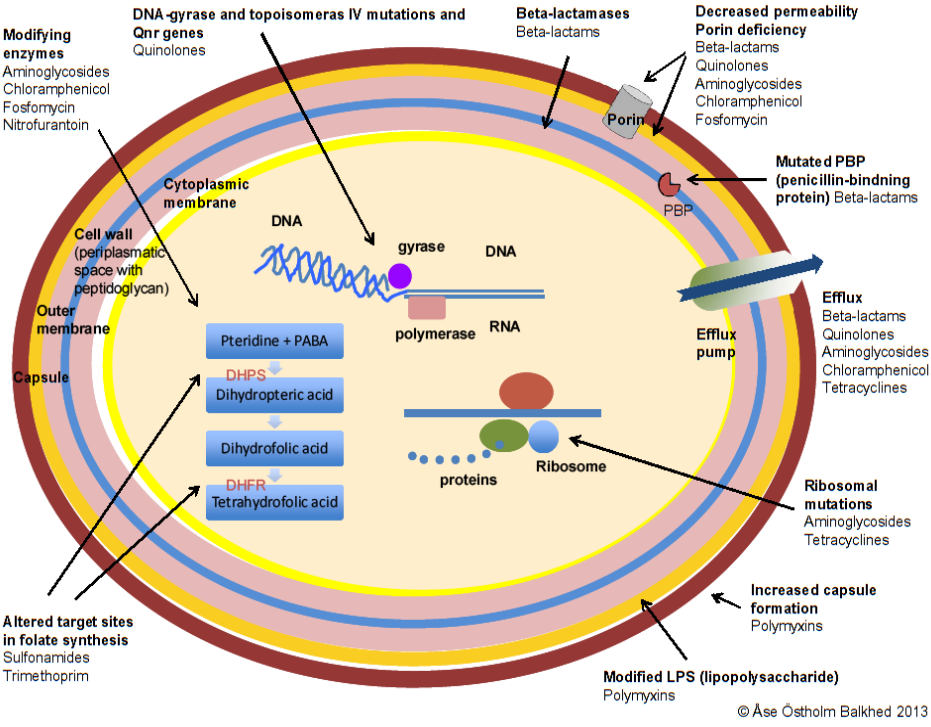

Figure 1. Resistance mechanisms in Enterobacteriaceae

Antibiotic consumption

The ATC (Anatomical Therapeutic and Chemical Classification system) /DDD (Defined Daily Doses) system is a tool for drug utilization research developed by WHO. Drug consumption figures should preferably be presented as numbers of DDD/1000 inhabitants/day or, when in-hospital drug use is considered, as DDDs per 100 bed days. Sales or prescription data presented in DDD/1000 inhabitants/day may provide a rough estimate of the proportion of the population within a defined area treated daily with certain drugs.
For anti-infectives (or other drugs normally used for short periods) it is often considered most appropriate to present the figures as numbers of DDDs per inhabitant per year, which will give an estimate of the number of days for which each inhabitant is, on average, treated annually. Alternatively, if the standard treatment period is known, the total number of DDDs can be calculated as the number of treatment courses, and the number of treatment courses can then be related to the total population. [47]
Antibiotic consumption is a major risk factor for the development of antimicrobial resistance. For *E. coli* a correlation with increased utilization of quinolones and trimethoprim-sulfamethoxazole (TMP-SMX), and developing resistance has been shown. The increased incidence of ESBL-producing *K. pneumoniae* has been associated with increasing utilization of third-generation cephalosporins. Outpatient antibiotic use differs between European countries with a general trend of a growing use of broad-spectrum antibiotics. From Scandinavia low antibiotic use and more prescriptions of narrow-spectrum penicillins and first generation cephalosporins have been reported. Livermore et al. reported from the UK that non-susceptibility to cephalosporins and quinolones among invasive isolates of Enterobacteriaceae has declined, probably reflecting a prescription shift in which cephalosporins and quinolones have been largely replaced by piperacillin-tazobactam. It is noteworthy that, the total number of *E. coli* bacteraemia increased during the study period. The altered prescription pattern in the UK was mainly due to the problem with *Clostridium difficile*. [48-54]

Antimicrobial susceptibility testing and breakpoints

The success or failure of antimicrobial therapy in bacterial infections is predicted by antimicrobial susceptibility testing, in which microorganisms are divided into treatable or non-treatable categories based upon MIC breakpoints. Nowadays, breakpoints are also used to facilitate the important task of discovering and monitoring phenotypical resistance. [55]
Thus, breakpoints are helpful both for guiding therapy and for surveillance of emerging resistance. Dosages, clinical indications, pharmacokinetics, resistance mechanisms, MIC distribution, zone diameter distributions and more recently, pharmacodynamics and epidemiological cut-off values are used in the breakpoint setting process. When any of these background data change, antimicrobial breakpoints may require revision. The epidemiological cut-off is related to the distribution of MICs of wild type organisms lacking acquired or mutational resistance to the antimicrobial agent in question. The epidemiological cut-off values are essentially the upper MIC values of the wild type distribution. The S/I breakpoint is normally based on the standard dose, and the I/R breakpoint on the maximum dose.[1, 56, 57]
MIC breakpoints are harmonised in Europe by EUCAST (The European Committee on Antimicrobial Susceptibility Testing). [55] Through the EUCAST MIC distribution

database, full MIC distributions of antimicrobial agents for individual species are available, both for wild type and non-wild type organisms.

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MIC is a fundamental measurement that forms the basis for most susceptibility testing methods and against which the levels of drug achievable in body fluids may be compared to determine breakpoints for defining susceptibility. [1]
According to EUCAST a microorganism is defined as susceptible if it has a level of antimicrobial activity associated with a high likelihood of therapeutic success. A microorganism is categorized as susceptible by applying the appropriate breakpoint in a defined phenotypic test system. Conversely, resistance is defined as a high likelihood of therapeutic failure. In the category intermediate, the antimicrobial activity is associated with an uncertain therapeutic effect.[55]
Determination of MIC can be performed with different methods. Disk diffusion is one of the oldest approaches to antimicrobial susceptibility testing and remains one of the most widely used methods in routine clinical laboratories. A disk containing a defined amount of antibiotic is applied to an agar surface and then incubated at 35+/-1 ^{o}C for 16-20 hours. The growth of microorganisms is inhibited by the presence of the antibiotic. The diameter of zone of inhibition is measured and this correlates to the MIC of the isolate, a small zone indicates a higher MIC. The disc diffusion method for antibiotic susceptibility testing according to EUCAST/NordicAST (Nordic Committee on Antimicrobial Susceptibility Testing) is commonly used in routine analysis in the Swedish microbiological laboratories. The method is quantitative but the results are normally interpreted to give a qualitative "recommendation": **S** (susceptible, sensitive), **I** (intermediate) and **R** (resistant).[58]The agar dilution method can be used for determining the *in vitro* activity of an antibiotic against a bacterial isolate. The bacterium is allowed to grow on a series of agar plates containing twofold dilutions of the antibiotic, and the result, defined as the MIC, lowest concentration with no visible growth, is most often read after incubation at 35-37 ^{o}C for 16 - 18 hours. Dilution methods can also be performed in broth or as microdilution in a microtitre tray. Another method is the Etest (BioMérieux) with a predefined, stable gradient of antibiotic concentrations on a plastic strip. It has the advantages that it is rapid and easy-to-use.

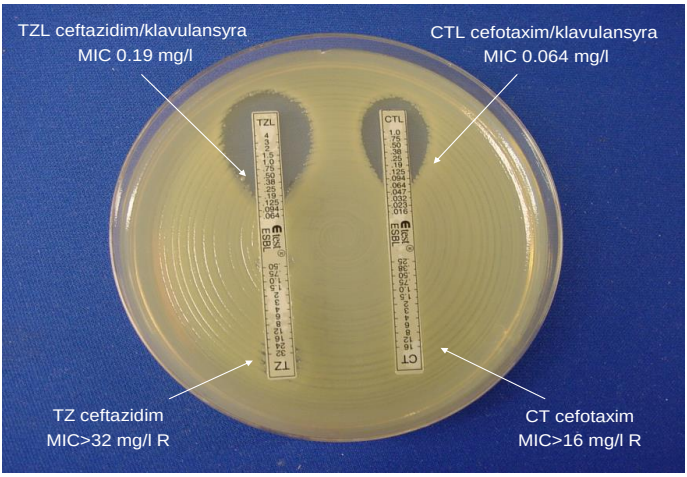

Photo Lennart Nilsson

Classification and detection of ESBL

The widespread use of antibiotics has caused the elaboration of resistant bacteria. In Gram-negative bacteria many different resistance mechanisms have emerged. In these, the most important mechanism of resistance to beta-lactam antibiotics involves the production of beta-lactamases. Such bacterial enzymes may predominantly cleave penicillins (penicillinases), cephalosporins (cephalosporinases), and carbapenems (carbapenemases) but cephalosporinases tend to degrade both penicillins and cephalosporins, and some carbapenemases degrade all of the above-mentioned agents. [59]

Three different classification systems for beta-lactamases exist, the Bush-Jacoby-Medeiros functional classification, the Ambler structural classification and the Giske classification. Most frequently used in Scandinavia is the ESBL classification, according to Giske et al, 2009 where extended-spectrum beta-lactamases are divided into three main groups $ESBL_A$, $ESBL_M$ and $ESBL_{CARBA}$ as illustrated in table 1. [60]

Table 1. Classification ß-lactamases

Beta-lactamases in Enterobacteriaceae				
Class	**Subgroups**	**Ambler class**	**Phenotypic test**	**Hydrolytic activity against**
Penicillinases	TEM-1, TEM-2 SHV-1	A	Inhibited by clavulanic acid	Penicillins
Cephalosporinases $ESBL_A$	TEM-ESBLs SHV-ESBLs CTX-M			Penicillins Cephalosporins
Cephalosporinases non-ESBL	Chromosomal Amp C	C	Inhibited by cloxacillin	Penicillins Cephalosporins
Cephalosporinases $ESBL_M$	Plasmid-mediated Amp C CIT (CMY variants), MOX, FOX, DHA, ACC, EBC			
	OXA-ESBL	D		
Carbapenemases $ESBL_{CARBA-A}$	KPC	A	Synergy with boric acid	Penicillins Cephalosporins Carbapenems
Carbapenemases $ESBL_{CARBA-B}$	Metallo-beta-lactamases NDM, VIM, IMP	B	Synergy with dipicolinic acid/ EDTA	
Carbapenemases $ESBL_{CARBA-D}$	OXA-48-like	D	Temocillin MIC>32 mg/L	Penicillins Carbapenems

Penicillinases, TEM-1, TEM-2 and SHV-1, are enzymes causing resistance only to penicillins, but point mutations have occurred which broaden their spectrum to include beta-lactamase stable penicillins and cephalosporins.

$ESBL_A$, often referred to as "classic" ESBLs, are enzymes capable of cleaving cephalosporins as well, and the most prevalent enzymes among these are TEM-ESBLs, SHV-ESBLs and CTX-M. Today more than 200 TEM and SHV variants are described. In the 21st century the cause of ESBL-production has altered to enzymes of CTX-M type, with more than 90 different enzymes described and divided into five different clusters according to similarities in the amino-acid sequence level; CTX-M-1, CTX-M-2, CTX-

M-8, CTX-M-9 and CTX-M-25. These enzymes are mediated by plasmid located genes, which have a risk of transmission within and between species. In vitro, inhibition by clavulanic acid has been demonstrated and this phenomenon is used as a phenotypic test for $ESBL_A$. Several genotypic tests for detection of ESBL genes are in use at present. Most commonly, PCR based methods are used. For characterizing subtype sequencing is essential to discriminate between the non-ESBL genes (TEM-1, TEM-2 or SHV-1) and ESBL genes, and to identify genotypes within each ESBL group. More recently integrated microarray methods for rapid genotyping of TEM, SHV and CTX-M have been developed.[61-67]

$ESBL_M$ consists of some OXA-ESBLs and AmpC cephalosporinases, which are plasmid-mediated. The AmpC confirmation test is based upon an inhibitory effect of cloxacillin. To determine whether Amp C is chromosomally or plasmid-mediated a genotypic verification is required. Plasmid-mediated AmpC enzymes are commonly divided into the following subgroups: CIT (including CMY variants), MOX, FOX, DHA, ACC and EBC. [67-69]

$ESBL_{CARBA}$ consists of carbapenemases and these confer resistant to all beta-lactam antibiotics. Further subdivision of this group into class A, B and D is commonly done. $ESBL_{CARBA\ A}$ consists mainly of Klebsiella pneumoniae carbapenemase KPC, $ESBL_{CARBA\ B}$ are metallo-beta-lactamases MBL, and $ESBL_{CARBA\ D}$ is mainly OXA-48-like enzymes. Detection of carbapenemase production is complicated because some carbapenemase-producing isolates demonstrate slightly elevated, but yet susceptible, carbapenem MICs. The phenotypic test for KPC is based on observed synergy with boronic acid and for MBL a synergy with dipicolinic acid/EDTA is demonstrated. When no synergy is detected with either test, further investigation of temocillin MIC is performed and a MIC>32 mg/L indicates OXA-48. OXA-48 enzymes hydrolyse penicillins at a high level, carbapenems at a low level. They exhibit weak activity against third and fourth generation cephalosporins and are not susceptible to ß-lactamase inhibitor combinations. MBLs are of VIM and IMP types and, more recently, of New Delhi metallo-beta-lactamase-1, NDM-1 type. NDM-1 was discovered in 2008 in Sweden, the isolate came from a patient previously hospitalized in New Delhi, India. In addition to acquisition of carbapenemases, carbapenem resistance in Enterobacteriaceae can occur when an isolate expresses an $ESBL_A$ or an $ESBL_M$ in combination with porin loss.[67, 70-74]

ESBL epidemiology

The types of ESBL enzymes and prevalence rates vary in different parts of the world. In the SMART study, which studied resistance in gram-negative bacteria from intra-abdominal infections in the Asia-Pacific region in 2007, 42.2% and 35.8% of *E. coli* and Klebsiella spp. were ESBL-positive. Moreover, ESBL rates in India for *E. coli* and *K. pneumoniae* were 79.0% and 69.4%, respectively. Among *E. coli* isolates from China, 55.0% were ESBL-producers and the corresponding figure from Thailand was 50.8%. A couple of years later, in 2009-2010 a SMART investigation of urinary tract isolates of Enterobacteriaceae from hospitalized patients detected ESBL-production in 8.5% and 8.8% of *E. coli* and *K. pneumoniae*, respectively, in North America and in 17.6% and 38.9% for European isolates, respectively. This difference between continents, with higher prevalence of ESBL-producers in Europe than in North America has previously

been noted. According to EARSS data from 2011 the percentage of resistance to third-generation cephalosporins in invasive *E. coli* isolates differs between European countries, with higher resistance rates in southern and eastern parts - generally 10-25% with the exceptions Slovakia 30.9% and Cyprus 36.2% - than in Scandinavia < 5%. [75-77] Scandinavia is regarded as a low-prevalence area, but outbreaks were reported from Uppsala of CTX-M-15-producing *K. pneumoniae* in 2005, from Kristianstad in 2005-2006 of CTX-M-15-producing *E. coli*, and in recent years several outbreaks of CTX-M-producing *K. pneumoniae* from neonatal care units in Västerås, Växjö, Sundsvall and Stockholm. [78-80]

During the 1990s, most reports of ESBLs concerned TEM/SHV types, with the exception of the CTX-M-2 genotype from South America, but since the turn of the century a dramatic shift in the types of ESBLs has been reported, with CTX-M dominating.[81] The spread of ESBL is now dominated by the CTX-M-15 enzyme worldwide and causes both community-onset and hospital-acquired infections. CTX-M-15 belongs to the CTX-M-1 cluster and was first detected in *E. coli* from India in 2001. [82-86] With multilocus sequence typing (MLST) a clone named ST131 has been identified among CTX-M-15-producing *E. coli* and this clone has emerged worldwide. [84]

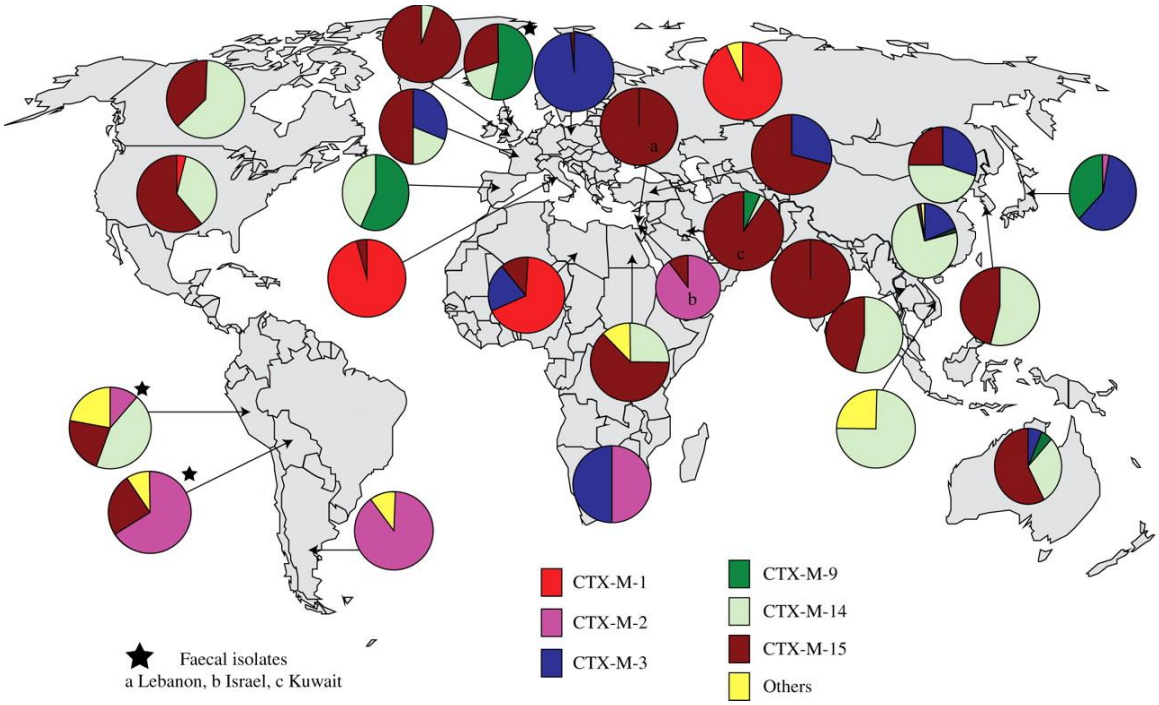

Figure 2. Global distribution of CTX-M genotypes. Hawkey and Jones 2009. With permission from Oxford University Press.

Clonal outbreaks of CTX-M-producing strains have been reported from many parts of the world. In Argentina, South America, the CTX-M-2 group has been the most prevalent ESBL but CTX-M-15-producing clones have also started to emerge. CTX-M-14 occurred in a large outbreak in Calgary, Canada in 2000-2002. From Israel, the emergence of both CTX-M-14 and CTX-M-15 producing *E. coli* ST131 has been reported. In Indonesia the CTX-M-15 gene was highly prevalent among ESBL-producing E. coli. In the early 2000s in Japan the majority of CTX-M-producing *E. coli* harboured CTX-M-group 9 genes, mainly due to the clonal spread of two strains. From neighbouring China, a study conducted in the mid-2000s demonstrated an overall ESBL rate of 33.4% among clinical isolates. These were carrying predominantly CTX-M-14, CTX-M-3 and CTX-M-15 genes (in order of decreasing prevalence).[87-92]

The emergence of ESBLs and carbapenemases began from different epicentres but lately they have spread to almost all over the world. Regarding $ESBL_{CARBA}$, so far a low incidence is reported in clinical invasive samples from Europe. However, there is a major difference between the low-prevalence in Nordic countries and the endemic situation in Southern Europe, e.g. Greece and Italy. The situation in Greece, where 49% of invasive Klebsiella pneumoniae isolates exhibit resistance to carbapenems, is alarming. The actual prevalence of carbapenemase producers is still unknown or underestimated in many areas, since several countries, especially these that are likely to be reservoirs, have not established detection strategies. Although dissemination of carbapenemases mainly occurs in *K. pneumoniae* among hospitalized patients, community acquisition is increasing especially for OXA-48 producers. The estimated occurrence of carbapenemase-producing Enterobacteriaceae in different parts of Europe is illustrated in figure 3.[93-96]
According to the Swedish Communicable Disease Act of February 1st 2007, ESBL-producing isolates of Enterobacteriaceae have to be notified to the Swedish Institute for Infectious Disease Control, and they are nowadays the most commonly notified resistant bacteria. A particular threat is the dissemination of $ESBL_{CARBA}$, therefore the Communicable Disease Act was reinforced in 2012 and since then infection tracing has been mandatory. The number of cases of $ESBL_{CARBA}$ in Sweden still remains low but is steadily increasing and 42 cases were reported in 2013, mostly associated with import cases.[97]

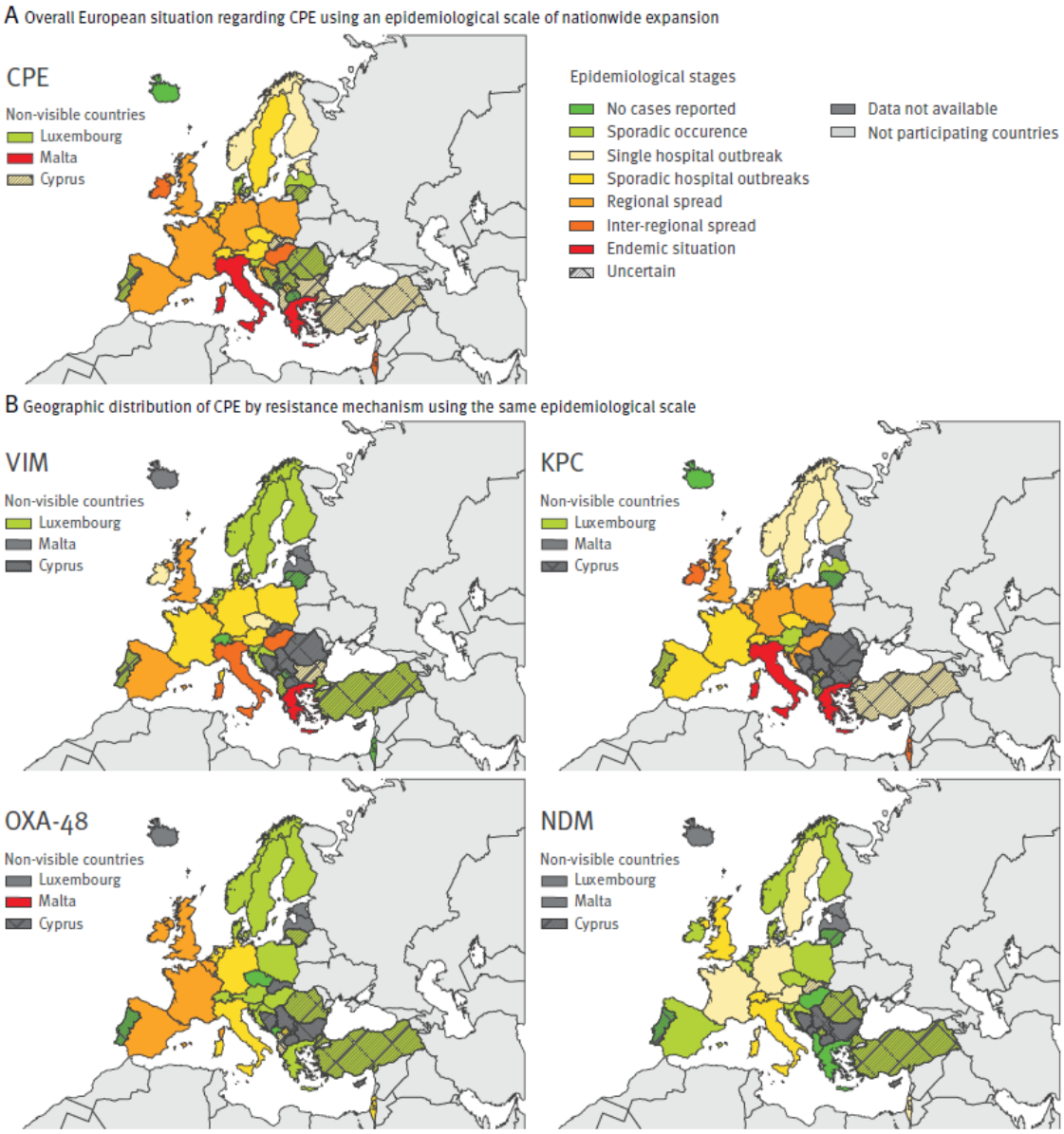

Figure 3. Occurrence of carbapenemase-producing Enterobacteriaceae in 39 European countries based on self-assessment by respective national experts, 2013.
Glasner et al. 2013. Courtesy of Eurosurveillance.

Faecal carriage and duration

Antimicrobial resistance is rapidly spreading across the globe and entails a significant threat to public health. Antibiotic resistance increases the morbidity, mortality and costs of treating infectious diseases. [81, 98] The gut plays a prominent role in the development of antibiotic resistance, and the emergence of resistant microorganisms in the gut may be related to ingestion or antibiotic-induced alterations in microorganisms. The resistant organisms then contaminate the environment via the faeces.[99]

Asymptomatic faecal carriage of ESBL-producing bacteria in the community has been reported from several countries and continents with wide differences in carriage rates between geographic areas, as illustrated in Figure 2. The highest prevalence levels have been reported from Thailand 65.7%/2010, Egypt 63.3% /2010-2011, Ghana 46%/2011-12 and China 50.5% /2009. In contrast, in a previous study conducted in China in 2007 the prevalence of rectal carriage was only 7% in an elderly population. Studies from Portugal, France 2010-2011, 2012 Sweden 2010, Bolivia and Peru have focused on faecal carriage among children. Pre-travel carriage among adults was investigated in New York USA, the Netherlands and in two studies from Sweden in 2007-2008 and 2008-2009. In the prevalence studies from the Czech Republic, Madagascar and Sweden 2010 the study subjects were recruited from primary health care centres. In Denmark army recruits were tested for faecal carriage and both ESBL-producing and AmpC-producing bacteria were reported together. Study subjects who had recently used antibiotics were not consistently excluded in some of these studies. In Germany the study participants were screened after close contact to patients with bacterial gastroenteritis. The Swiss study claims a relative high rate of intestinal carriage of ESBL-producers in the general healthy human public without considering whether it is representative with faecal samples from a study population consisting of staff members in meat-processing companies.[100-133]
Thus, the reported prevalence of carriage of ESBL-producing bacteria may be influenced by study population characteristics such as the geographic area, previous use of antibiotics, healthcare environment and also by screening method. The different screening methods used have variable sensitivities and specificities, and chromogenic agar media is advantageous over Mac-Conkey agar supplemented with ceftazidime.[134]

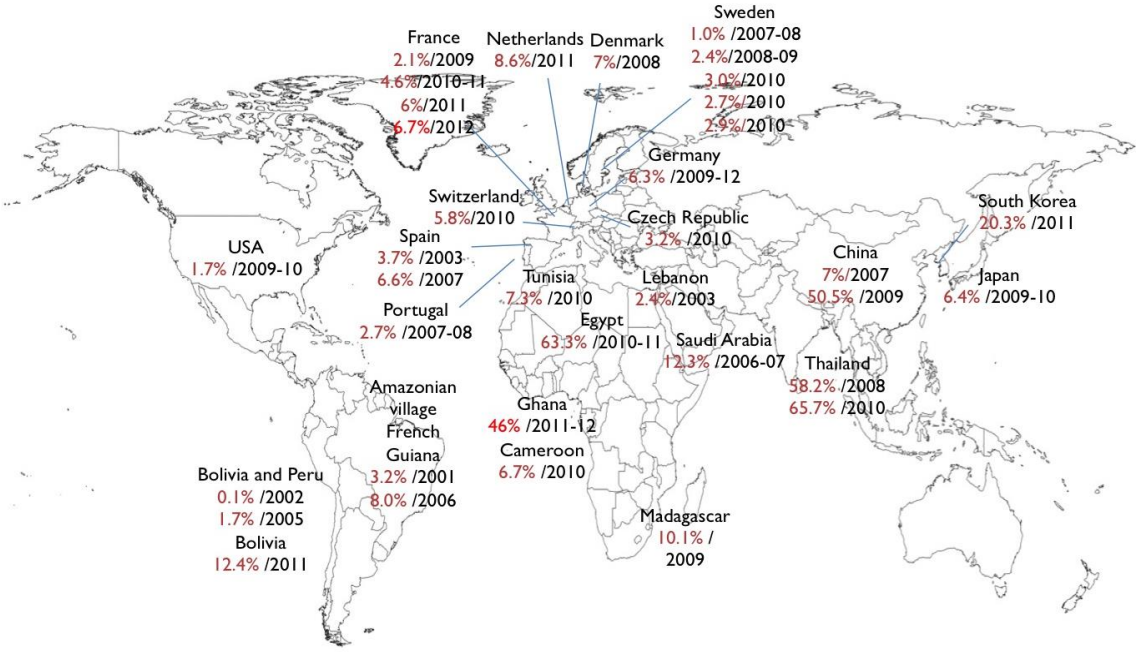

Figure 4. Community carriers of ESBL-producing bacteria in different countries, prevalence (%) /year of sampling

The duration of carriage of ESBL-producing bacteria in the gastrointestinal tract may constitute a critical factor in the epidemiology of ESBL-producing bacteria in hospitals and within the community. Duration of carriage following infection, hospital discharge and international travel is illustrated in Figure 3. Duration data are more or less indirect because it is the clearance of ESBLs that is reported. Comparison of data is difficult as the study subjects and methodology varies considerably between the studies. Dropout frequencies are high in some of the studies. Investigations of isolates regarding genetic relatedness have only been performed in the two studies of carriage among neonates by Löhr and Strenger, and in two Swedish studies by Alsterlund and Andersson.
From India, a study by Kathari et al. shows that in 14.3% of newborns their stool is colonized with ESBL-producing Enterobacteriaceae on day 1, followed by 27.1% on day 21 and 41.5% on day 60. These babies were vaginally delivered, healthy, breast fed with no history of hospitalization or antibiotic use by either babies or mothers, but they were given a probiotic supplementation. Early transmission of ESBL-producing *E. coli* between mothers and neonates has also been demonstrated in a French population. [135-149]
A clinical approach to faecal carriage may be screening of immunocompromised patients, such as patients with haematological and oncological malignancies as well as transplant recipients, to identify risk factors for subsequent infection caused by ESBL-producing Enterobacteriaceae and thereby prescribe directed empirical treatment.[150, 151]

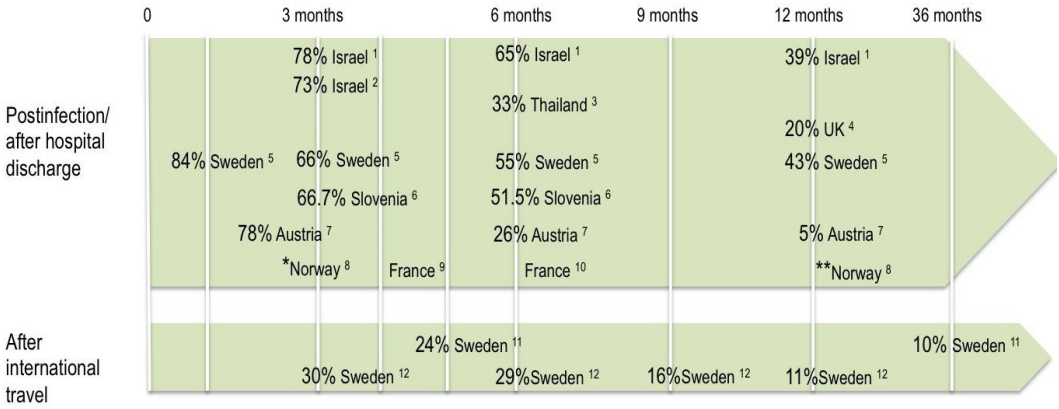

Figure 5. Duration of carriage of ESBL-producing Enterobacteriaceae.
Zimmerman[1] and Schecher [2] both investigated duration of faecal carriage of carbapenem-resistant Enterobacteriaceae following hospital discharge in Israel. Titelman [5] followed colonization in Swedish patients after infection with ESBL-producing Enterobacteriaceae. Warren [4] described faecal carriage for more than one year in UK patients.
Apisarnthanarak [3] screened patients after hospital discharge in Thailand. Papst [6] collected samples from patients infected or colonized with ESBL in Slovenia. Strenger [7] et al. screened faecal samples from neonates in Austria.
Median duration of ESBL carriage was reported by Löhr [8] from Norwegian neonates** and their parents*, and in French patients after discharge as reported by Zahar [9] and Birgand [10].
Tärnberg [12] investigated returning travellers in Sweden for ESBL prevalence in stool samples, Tham[11] et al. from Sweden examined ESBL carriage duration in foreign travellers presenting with diarrhoea.

Environmental dissemination

There is a global dissemination and distribution of ESBL-producing bacteria in community, even in countries with low antibiotic consumption. More recently, environmental reservoirs have received growing attention.
Long-term care facilities may represent a significant reservoir for ESBL-producing bacteria. By investigating faecal samples from nursing home residents in Ireland 2004-2006 a 40.5% carriage rate of ESBL-producing *E. coli* were found. In Italy 2008, 64% of residents were colonized with ESBL of these 5.4% with metallo-beta-lactamase producers. Corresponding rates among staff members were 14.5% and 1.5%, respectively.[152, 153]
Gut colonization with ESBL-producing bacteria in animals and contamination of retail meat may contribute to the increased prevalence of ESBL-producing bacteria in humans. In a Danish intervention study the occurrence of ESBL-producing *E. coli* in pigs at slaughter was 11.8% in 2010 and after a ban on cephalosporin use in pig production the occurrence in 2011 was significantly decreased to 3.6%. A French study reports ESBL-producing, mainly CTX-M, *E. coli* in livestock (5%) as well as in the contaminated farm environment.[154, 155] A study from Spain assessed the prevalence of retail chicken and turkey meat colonized by ESBL-producing *E. coli* and found an increase from 62.5% in

2007 to 93.3% in 2010. Of Dutch retail meat samples 94% contained ESBL-producing bacteria of which 39% belonged to *E. coli* genotypes also present in human samples. In a Swedish survey from 2011 ESBL-producing *E. coli* were found in imported meat samples, particularly in broiler meat from South America (95%), Europe (61%) and Denmark (15%), whereas the overall ESBL frequency was 44% in broiler meat. Unlike the Dutch study the overlap between gene variants in bacteria isolated from meat and humans was limited. A multicentre study in Germany, Netherlands and UK investigated ESBL-producing *E. coli* from humans, animals and animal food products with a microarray and multi-locus sequence typing, MLST. The gene profiles from humans were generally different from those isolated from animals, while many human isolates from the three countries were highly similar in both array profiles and MLST-types. A small number of ESBL genes have also been demonstrated in gut samples from farmed fish in China. A recent Swiss study of freshwater fish detected ESBL-or AmpC producing isolates in 18.7% of fish gut samples and as *E. coli* is not a permanent inhabitant of intestinal tract of fish this finding probably reflects the features of the aquatic habitat and bacterial load in the water. These studies raise serious food safety questions. Furthermore, a German study of faecal samples from vegetarians could not show any protective effect with a vegetarian or vegan diet as they demonstrated nearly the same colonization rate of ESBL-producing *E. coli* as in meat-eaters.[156-162] The following measures to control the selection and dissemination of ESBL/Amp C producing bacteria in food-producing animals have been proposed: restrict cephalosporin use in food animals and minimize off-label use, decrease the total antimicrobial use in animal production, increased farm biosecurity and controls on animal trade, and by improving hygiene.[163] Companion animals may represent potential sources of spread of antimicrobial resistance, as there is a use of antibiotics in veterinary medicine and also their close contact with humans. A Dutch study reports a high prevalence of ESBL /Amp C-producing Enterobacteriaceae among companion animals, from healthy and diarrheic dogs respectively 45% and 55% were carriers, from healthy and diarrheic cats the prevalence was respectively 0% and 25%. From Switzerland the overall prevalence among cats and dogs were 2.5%. [164, 165] Furthermore, ESBL-producing *E. coli* is spread in the environment beyond human and domesticated animal populations into the wildlife, in soil and wastewater/sewage sludge and migrating birds may play a role in transmission into remote areas. Among urban brown rats in Germany, 16% carried an ESBL *E. coli* strain. As vectors, flies may also play an important role in spreading ESBL-producing bacteria from animal faeces.[166-169] Money is also a potential pathway for transmission of multi-drug resistant microorganisms, for instance ESBL-producing *E. coli*.[170] In a study of water from rivers and lakes in Switzerland, ESBL-producing Enterobacteriaceae were detected in 21 (36.2%) of the 58 bodies of water sampled and one river sample tested positive for a carbapenemase-producing *K. pneumoniae*. Even in water sampled from the Antarctic has CTX-M-producing *E. coli* been found, an indication of how widespread the antibiotic resistance problem is.[171, 172] The main digestive and environmental reservoirs of ESBL-producing Enterobacteriaceae are illustrated in Figure 6.

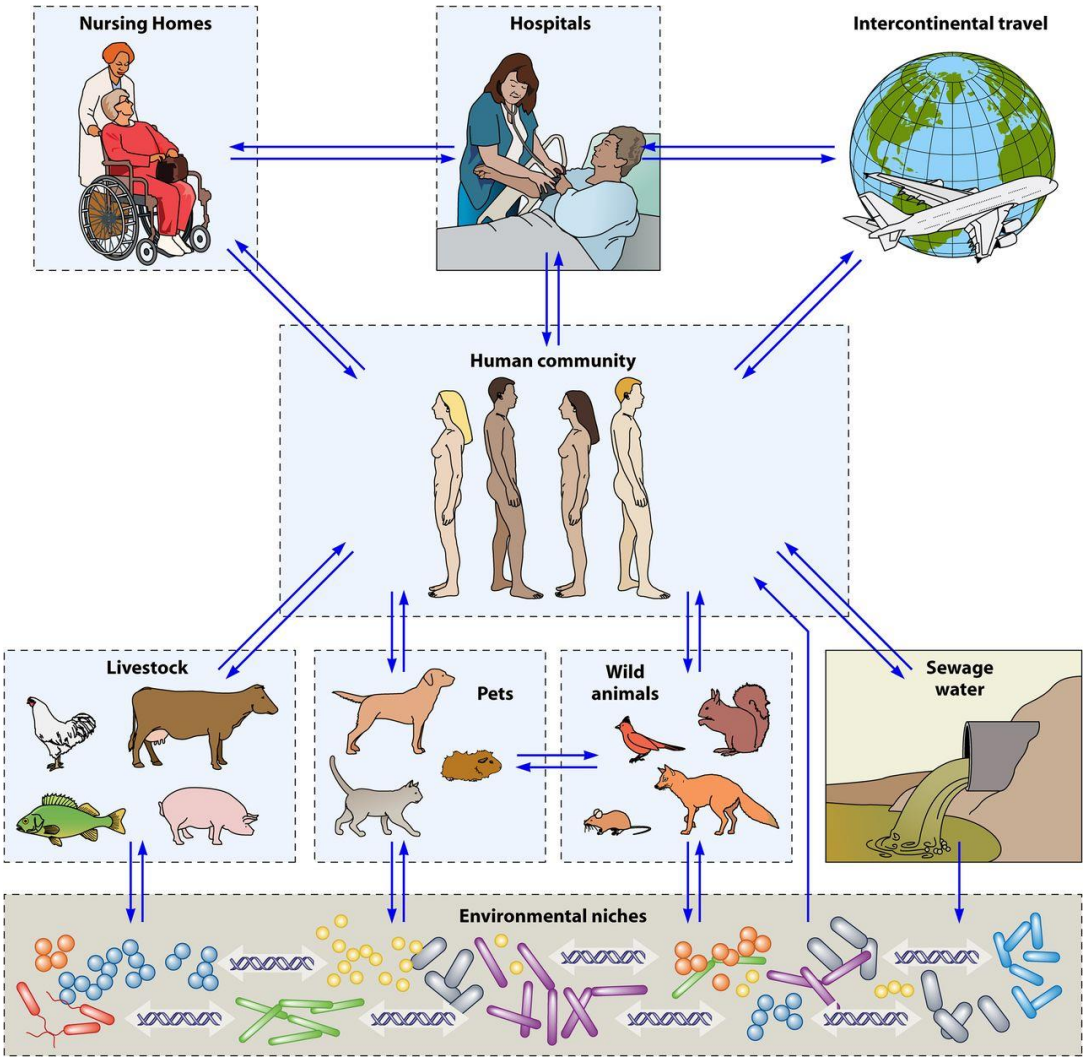

Figure 6. The main digestive or environmental reservoirs of ESBL-producing Enterobacteriaceae to which the worldwide human community belongs and is also exposed. Woerther et al. 2013. With permission from Clinical Microbiology Reviews

Risk factors and nosocomial aspects / infection control

Antibiotic stewardship and infection control are considered as the two cornerstones in attempts to control the spread of resistant bacteria, including ESBL-producing Enterobacteriaceae, in health care. Various risk factors for colonization and/or infection by ESBL-producing bacteria are identified: hospitalization in the previous year, nursing home residency, urinary catheter use, mechanical ventilation, previous antimicrobial use especially beta-lactams or fluoroquinolones, old age, comorbidities, prior ESBL carriage and coming from or travel in high-prevalence countries. The identification of ESBL carriers upon hospital admission is not only important for infection control measures but it is crucial, in case of severe infection, to treat patients with antibiotic therapy that is effective against ESBL-producing bacteria. Recently, data was presented from a low prevalence country, Norway, concerning community- acquired urinary tract infections, UTI, caused by ESBL-producing Enterobacteriaceae where identified risk factors were recent travel to Asia, Middle East or Africa, recent antibiotic use and recreational swimming in freshwater. On the other hand eating fish regularly was associated with a protective effect against ESBL-associated UTI, however the relationship is not clear.[173-180]

Hospital environmental contamination is more frequent with ESBL-producing Klebsiella spp. than ESBL-producing *E. coli*, but in experimental studies both bacteria have exhibited prolonged survival in the environment.[181-185]

There is limited research regarding the optimal approach of infection control interventions in order to prevent transmission of ESBL-producing Enterobacteriaceae. A Swiss observational study was performed during 11 years, all patients who were hospitalized in the same room as a patient colonized or infected with an ESBL-producing Enterobacteriaceae for at least 24 hours were screened for ESBL carriage and transmission, confirmed by PFGE, occurred in 1.5% of contact patients after a mean exposure to the index case of 4.3 days. Standard precautions, including proper use of hand hygiene and the use of personal protective equipment for procedures involving contact with body fluids, were performed and the low rate of transmission may indicate that these measures are sufficient. [186, 187]

In outbreak situations many different infection control measures are considered to reduce further transmission. During an outbreak of multidrug-resistant CTX-M-15-producing *K. pneumoniae* in Uppsala University Hospital between 2005 and 2007 several interventions were performed such as formation of a steering group with economic power, increased bed numbers, less overcrowding and understaffing, more frequent bathroom cleaning, better compliance with hospital dress code and improved hand hygiene for staff as well as patients. Also an antibiotic intervention was performed and a reduction of cephalosporin use was demonstrated, whereas consumption of piperacillin/tazobactam and penicillin G increased and both fluoroquinolone and carbapenem use remained unaffected. The cost of the interventions was estimated to be 3 million euro.[188, 189]

Clinical impact and treatment options

Antimicrobial resistance is a major threat to public health, and the emergence of multi-resistant or nearly pan-resistant gram-negative bacteria is worrisome, as in a near future we may lack therapeutical options to treat both common and serious infections and to manage infectious complications after surgery. This may also lead to a revival and reuse of older, more toxic antibiotics. Moreover, a loss of effective antibiotic treatments jeopardizes the abilities to perform major surgery, organ transplants and cancer therapy. Empirical therapy is prescribed at the time when an infection is clinically diagnosed while waiting for the results of cultures and antimicrobial susceptibility tests. For serious infections cephalosporins are often prescribed and these are often not effective against ESBL-producing bacteria. Inadequate initial antimicrobial therapy for bacteraemia caused by ESBL-producing *E. coli* and *K. pneumoniae* is associated with increased mortality as described in several retrospective studies. However, recently a Dutch study of ESBL bacteraemia found no association between inappropriate therapy <24h and 30-day mortality, this finding is supported by another study from Korea. [190-194]
As a matter of growing concern, therapeutically options are limited as ESBL-producing Enterobacteriaceae frequently co-expresses resistance to other classes of antibiotics such as tetracyclines, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole.[195, 196] Several studies have shown in-vitro effect of different antibiotics but clinical studies are few. Carbapenems (imipenem, meropenem and ertapenem) have, based on observational studies, been regarded as the drug of choice for treatment of serious infections caused by $ESBL_A$-producing bacteria but these have to be administered parenteral and another disadvantage is the broad-spectrum and potential selection of carbapenem-resistance. The actual carbapenem MIC value is probably important for clinical outcome in bacteraemia caused by gram-negative bacteria including ESBL-producers, a study indicates that MIC≥4 mg/L is associated with worse outcome than MIC≤2mg/L. [195, 197]
Beta-lactam antibiotic combined with a beta-lactamase inhibitor, piperacillin-tazobactam, may be a useful agent for treatment of infections caused by susceptible isolates of ESBL-producing bacteria. A study from Spain of patients with bacteraemia with ESBL-producing *E. coli* treated with piperacillin-tazobactam described no mortality among patients with urinary tract infections but for other sources, e.g. intra-abdominal infections, 30-day mortality was lower for isolates with low MIC (≤2 mg/L) than intermediate (4-8 mg/L) and high (≥16 mg/L) MIC values for piperacillin-tazobactam. New combinations of ß-lactam-ß-lactamase inhibitors are under development with the hope that these will be potent and overcome several resistance mechanisms in gram-negative bacteria. [193, 198-202]
Tigecycline has good in-vitro activity against ESBL-producing *E. coli* and *K. pneumoniae* isolates but clinical data from treatment of infections caused by ESBL-producing bacteria are limited. Agents that may be useful for the treatment of ESBL-associated lower urinary tract infections include fosfomycin, nitrofurantoin, pivmecillinam, temocillin, and amoxicillin/clavulanic acid. [203, 204]

KPC producers, mainly nosocomial *K. pneumoniae* isolates, are usually multidrug-resistant and therapeutic options for treating infections with KPC-producers remain limited, and this results in high mortality rate among patients with bloodstream infections caused by these bacteria. Many bacteria with these enzymes remain susceptible to colistin, tigecycline and one or more aminoglycoside, but some are resistant even to these drugs. Various combinations of these antibiotics have been used as treatment.

Retrospective studies have reported higher treatment failure rates with monotherapy than with combination therapy. Recent findings suggest that combination treatment colistin, tigecycline and meropenem might improve survival among bacteraemic patients. A major concern is the emergence of colistin-resistant KPC-producing *Klebsiella pneumoniae* isolates, which is worrying since colistin is essential in treatment combinations. In-vitro data demonstrates a synergistic effect when combining colistin with rifampicin, thus reducing colistin MIC values, which can be useful against colistin-resistant isolates. Again the association between selective pressure, as colistin has been increasingly used in areas with KPC-producers, and the appearance of resistance is likely.[93, 205-207]
Regarding treatment of NDM-producing Enterobacteriaceae only case reports have been published yet. Two cases of UTI with NDM-producing *E. coli* and *E. cloacae* were reported from Australia, both acquired during travel to India. These patients were treated with nitrofurantoin and colistin/rifampicin, respectively.[208]
Another carbapenemase is OXA-48 and isolates harbouring this gene are often also multidrug-resistant. Even if OXA-48 has weak activity against third and fourth generation cephalosporin it is frequently associated with other ESBLs so cephalosporins cannot be considered as a therapeutical option. During a hospital outbreak in Spain there was forty cases of bacteraemia, predominantly of urinary origin, with OXA-48-producing Enterobacteriaceae and different combinations of amikacin, fosfomycin, colistin, tigecycline and meropenem were prescribed, nonetheless was 30-day mortality 50%. Median delay in administration of clinically and microbiologically appropriate treatment was 3 days.[209]
Although carbapenemase –producing Enterobacteriaceae often demonstrates high resistance to carbapenems, a proportion of these have relatively low MICs and if MICs are ≤4 mg/L imipenem or meropenem has been suggested as reasonable therapeutical options if administered in high-dose prolonged infusion regimen and in combination with another active antibiotic.[210]
When making decisions on empirical antibiotic treatment, there are several variables to consider such as focus of infection, whether it is community or hospital acquired, local epidemiology as well as the individual risk factors for ESBLs, co-morbidities and finally the ecological impact of chosen antimicrobial agent. [211]

Since the gut is an important reservoir for resistant Enterobacteriaceae, measures to affect the composition of the intestinal flora could be one way to overcome resistance and prevent spread of resistant bacteria. For this purpose selective digestive decontamination (SDD), either solely by selective oropharyngeal decontamination (SOD), which is antibiotics or antiseptics such as chlorhexidine applicated to the oropharynx, or combined with systemic antibiotic, has become of interest. In France, a randomized, double-blinded, placebo-controlled study of a small cohort with faecal ESBL carriage was performed; the treatment arm received oral colistin and neomycin plus nitrofurantoin in the presence of bacteriuria with ESBL-producing Enterobacteriaceae. During and shortly afterwards there was significantly lower rate of ESBL-carriage in the active treatment group versus placebo-group but this effect disappeared after one week. Attached with the concept of SDD are concerns that the use could promote further resistance among intestinal bacteria and emergence of colistin resistance among ESBL-producing *K. pneumoniae* has been observed. However, a recently published meta-analysis failed to show an increased incidence of colonisation or infection with antimicrobial resistant pathogens in recipients of selective decontamination. Another possible approach to influence the intestinal flora is with probiotics or faecal transplantation.[212-217]

AIMS

The general objective of the thesis was to investigate ESBL-producing *Enterobacteriaceae* and their susceptibility pattern in a Swedish county.

Specific aims of this project were:

1. To develop molecular methods for detection of ESBL-encoded enzymes in Enterobacteriaceae. (Paper I)

2. To survey the antibiotic consumption and occurrence of ESBL-producing Enterobacteriaceae in Östergötland. (Paper II)

3. To investigate the antibiotic susceptibility patterns of beta-lactam antibiotics (Paper III) and non-beta-lactam antibiotics (paper IV) among clinical isolates of ESBL-producing Enterobacteriaceae.

4. To study the prevalence of ESBL-producing Enterobacteriaceae in faecal samples before and after travel abroad, examine rate and risk factors of acquisition. (Paper V)

MATERIALS AND METHODS

Study designs

Paper I: Methodological paper
Paper II, III and IV: Descriptive studies
Paper V: Prospective observational multicentre case-control study

Setting

The county of Östergötland is situated in southeast Sweden and had a population with 411 000 inhabitants 2002 and ten years later the population had increased to almost 434 000. Within Östergötland County are three hospitals, one tertiary care hospital with approximately 600 beds and two smaller secondary care hospitals with 300 and 200 beds respectively. There are also more than 40 primary care centres and around 40 private practitioners in the county.
The county borders Kalmar County to the southeast and Jönköping County to the southwest and in the last study, paper V, travellers from vaccination clinics of the Infectious disease departments in all three counties were recruited.

Bacterial isolates

Control strains
In paper I reference strains *E. coli* ATCC 35218 ($^{bla-}$TEM-1), *K. pneumoniae* ATCC 700603 ($^{bla-}$SHV-1) and *E. coli* strains with $^{bla-}$CTX-M-1, $^{bla-}$CTX-M-2, $^{bla-}$CTX-M-9, J62-$^{bla-}$TEM-1, J53-$^{bla-}$TEM-2 and *K. pneumoniae* 1204-$^{bla-}$SHV-2, J53-$^{bla-}$SHV-2 were all used. Dr D. Livermore, Health Protection Agency, Antibiotic Resistance Monitoring and Reference Laboratory, London, UK provided the latter non-ATCC strains.
E. coli ATCC 25922 was used as a reference strain in paper II, III, IV and V for control of E-test batches and agar plates.
In paper IV control strains with *qnrB1* and *qnrS1/ aac (6')-Ib-cr* were obtained from the Culture Collection, university of Gothenburg, Sweden and *E. coli* with *qnrA* from Prof. P. Nordmann, Hôpital Bicêtre, France and *E. coli* with *qnrC* and *qnrD* from dr L. Cavaco, National Food Institute, Denmark.

Clinical isolates
In paper II-IV clinical isolates were collected at the Clinical Microbiology Laboratory, Linköping University Hospital, Sweden from 1 January 2002 until 31 December 2007.
In paper I, 24 clinical isolates were collected from the same laboratory during 2001-2003, and additional 13 clinical isolates were provided by SMI, the Swedish Institute for Infectious Disease control. In the paper V, faecal samples were obtained from participants before and after travel outside Scandinavia during the study period from 1 September 2008 through April 2009, and cultured at the Clinical Microbiology Laboratory, Linköping University Hospital, either on arrival or stored at -70°C before culture.

In all studies, the isolates were characterized to the species level by conventional biochemical typing methods.[218]

Table 2. Species distribution, number of isolates and origin of isolates studied in each of the papers in the present thesis.

Paper	Isolates	Origin	Year of sampling	Aims
I	23 *E. coli* 7 *K. pneumoniae* 4 *Klebsiella oxytoca* 1 *Citrobacter freundii* 2 *Enterobacter cloacae*	Clinical isolates from Östergötland and from SMI, Sweden	2001-2003	Automated DNA extraction and multiplex PCR amplification assay for TEM, SHV and CTX-M genes
II	224 *E. coli* 23 *K. pneumoniae* 1 *Citrobacter koseri* 1 *Shigella sonnei*	Clinical isolates from Östergötland	2002-2007	Occurrence of ESBL-producing Enterobacteriaceae, multi-resistance and antibiotic consumption
III	198 *E. coli*	CTX-M-producing *E. coli* from paper I	2002-2007	Susceptibility of beta-lactam antibiotics
IV	198 *E. coli*	CTX-M-producing *E. coli* from paper I	2002-2007	Susceptibility of non-beta-lactam antibiotics, occurrence of multi-resistance and plasmid-mediated quinolone resistance
V	104 *E. coli* 10 *K. pneumoniae* 1 *E. cloacae* 1 *Proteus vulgaris*	Faecal samples from travellers attending vaccination clinics in Linköping, Jönköping and Kalmar	Sep 2008-April 2009	Faecal colonisation by ESBL-producing Enterobacteriaceae during travel, associated risk factors, antibiotic susceptibility and ESBL genes

Definitions

The ESBL definition has changed over time. In paper I-IV isolates with $ESBL_A$, classical ESBL enzymes such as CTX-M, TEM and SHV were included. In paper V the wider ESBL definition according to Giske et al, including $ESBL_A$, plasmid-mediated Amp C $ESBL_M$ and carbapenemases $ESBL_{CARBA}$.

Multi-resistance was defined as reduced (i.e. intermediate or resistant) susceptibility to a minimum of 2 antimicrobial agents with different modes of action in addition to the ESBL phenotype.[219]
In paper V the countries visited were categorized into geographic regions: Africa North (including countries north of the equator), Africa South (countries south of the equator, including the Horn of Africa), Asia (except the Indian subcontinent), Europe, the Indian subcontinent (Bangladesh, Bhutan, India, Nepal, Pakistan and Sri Lanka), Oceania and Australia, America North (USA, Canada, Mexico) and America South (including South America, Central America and the Caribbean).

Participants

In paper V individuals of legal age, 18 years or above, attending the vaccination clinics of the Infectious Disease Departments in Linköping, Jönköping or Kalmar and planning a travel outside Scandinavian countries for a maximum of 3 months were asked about participation. After written informed consent had been submitted, the participants completed questionnaires and provided stool samples before and after travelling abroad. The study was performed with permission from the Regional Ethical Review Board in Linköping, Sweden.

Antibiotic consumption data

Antibiotic consumption data was registered according to the anatomic therapeutic chemical (ATC) classification and we used the defined daily doses (DDD) measurement unit.
The National Corporation of Swedish Pharmacies (Apoteket AB), the sole pharmaceutical distributor in Sweden during the study period 2001 – 2009, assisted with sales data covering both outpatient and hospital care. As a measure of antibiotic consumption DID was used, which corresponds to the DDD per 1000 inhabitants and day.

Phenotypic ESBL detection

Phenotypic tests consist of screening and a following confirmation.
ESBL screening of clinical isolates in paper I and II was performed with cefotaxime and ceftazidime by disc diffusion according to the guidelines of the Swedish Reference Group of Antibiotics. Isolates with reduced susceptibility to these cephalosporins were further analysed. A following confirmation test was performed by Etest (BioMerieux) with cefotaxime and ceftazidime with and without and clavulanic acid. An ESBL phenotype was designated when an at least 8-fold reduction of MIC in the presence of clavulanic acid was observed, or if there were any signs of phantom or deformation zones.
In paper V screening was performed by inoculating faecal samples directly on agar plates containing medium selective for cephalosporin resistant bacteria ChromID ESBL agar (BioMerieux) and chromogenic UTI agar (BioMerieux). Thereafter antibiotic discs containing ceftazidime, cefotaxime, piperacillin-tazobactam, cefepime or linezolid were dispersed over the agar plate. Colonies growing on the ChromID ESBL agar plate and colonies that were not inhibited by cephalosporin and/or piperacillin-tazobactam upon the chromogenic UTI agar were further analysed with both species identification and

phenotypic ESBL Etest (BioMerieux). For $ESBL_A$ confirmation Etests with ceftazidime, cefepime and cefotaxime with and without clavulanic acid were used. Confirmation of ampC production, $ESBL_M$, was performed with a combinationtest of cefotetan and cefotetan with cloxacillin.

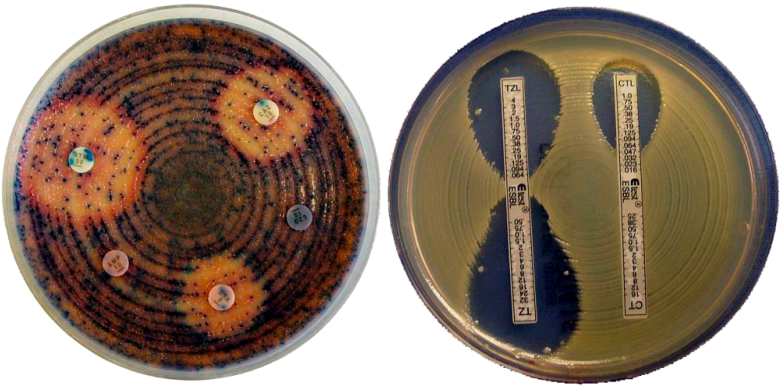

Photo: Lennart Nilsson

Detection of resistance genes

Isolates with an ESBL phenotype were screened for ESBL genes with end-point PCR.
In paper I automated DNA extraction was followed by multiplex PCR assay with primer-pair sequences ^{bla}SHV.SE/AS, TEM-164.SE and TEM-165.AS, universal CTX-M-U1/U2. The results obtained by multiplex PCR were confirmed by three single PCR amplification assays targeting SHV, TEM and CTX-M genes, respectively. DNA sequence analysis of cloned amplicons using gene-specific primers confirmed their identity.
In paper II –V bacterial DNA preparation was amplified by amplification as described elsewhere followed by CTX-M gene specific PCR and subsequent amplicon sequencing. Obtained gene sequences were edited and compared with ^{bla}CTX-M DNA and ^{bla}CTX-M-like DNA sequences using the bioinformatics freeware CLC in order to subgroup isolates into CTX-M groups 1, 2, 8, 9 or 25. In paper II additional PCR amplification assays were carried out for detection of TEM- and SHV-genes. In paper V PCR assays for CTX-M, TEM and SHV were performed in same way. Isolates in paper V without classical ESBL genes or with an AmpC phenotype were screened for the presence of AmpC (^{bla}CIT, ^{bla}MOX, ^{bla}FOX, ^{bla}DHA, ^{bla}EBC and ^{bla}ACC).
Moreover, in paper IV an in-house developed PCR was used to screen CTX-M-producing *E. coli* for both *qnr*- genes (*qnrA, qnrB, qnrC, qnrD* and *qnrS*) and modified aminoglycoside-modifying gene *aac(6')-Ib-cr*.[64, 65, 69]

Antimicrobial susceptibility testing

In paper II, all isolates were subjected to susceptibility testing with the disc diffusion method. This was performed according to the guidelines of the Swedish Reference Group of Antibiotics, SRGA, in order to determine the susceptibility against ciprofloxacin, TMP-SMX, meropenem and imipenem.

MIC determinations for beta-lactam and non-beta-lactam antibiotics in paper III, IV, and V were performed using the Etest (bioMérieux, Marcy L'Etoile, France) according to the manufacturer's instructions.
In paper III the antibiotics tested were amoxicillin–clavulanic acid, mecillinam, piperacillin–tazobactam, temocillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftibuten, imipenem, meropenem and ertapenem.
In the subsequent study, paper IV, amikacin, gentamicin, tobramycin, chloramphenicol, ciprofloxacin, nalidixic acid, colistin, fosfomycin, nitrofurantoin, tigecycline, trimethoprim, and TMP-SMX were tested.
Finally, in paper V, imipenem, meropenem, ertapenem, amikacin, gentamicin, tobramycin, ceftazidime, cefotaxime, cefepime, piperacillin/tazobactam, amoxicillin/clavulanic acid, temocillin, mecillinam, fosfomycin, nitrofurantoin, tigecycline, ciprofloxacin and TMP-SMX MICs were tested.
Clinical breakpoints according to EUCAST were used for all tested antibiotics, with the exception of temocillin, to classify isolates as susceptible (S), intermediate (I) or resistant (R). For temocillin, breakpoints according to British Society for Antimicrobial Chemotherapy BSAC were used.[220, 221]

Table 3. MIC and zone diameter breakpoints for Enterobacteriaceae according to EUCAST. For temocillin BSAC breakpoints were used. * At the time the studies in paper III and IV were conducted EUCAST had a higher breakpoint, R>8 mg/L for ceftazidime and cefepime and in paper V the revised breakpoint R>4 mg/L was used for these agents. For nalidixic acid no breakpoints are available

Beta-lactam antibiotics	**MIC breakpoint (mg/L)**		**Other antimicrobial agents**	**MIC breakpoint (mg/L)**	
	S≤	**R>**		**S≤**	**R>**
Temocillin	8	8	Amikacin	8	16
Mecillinam	8	8	Tobramycin	2	4
Piperacillin-tazobactam	8	16	Gentamicin	2	4
Amoxicillin-clavulanic acid	8	8	Tigecycline	1	2
Aztreonam	1	8	Trimethoprim	2	4
Cefotaxime	1	2	TMP-SMX	2	4
Ceftazidime	1	8/4*	Nalidixic acid	-	-
Cefepime	1	8/4*	Ciprofloxacin	0.5	1
Ceftibuten	1	1	Fosfomycin	32	32
Imipenem	2	8	Colistin	2	2
Meropenem	2	8	Nitrofurantoin	64	64
Ertapenem	0.5	1	Chloramphenicol	8	8

Statistics

Linear regression was used to statistically analyse the changes in antibiotic consumption during the period 2001-2009. Binary logistic regression was performed to evaluate changes in the incidence of ESBL-producing bacteria in Östergötland County from 2002 through 2007.
Mann- Whitney, a non-parametric test, was used to analyse differences in antibiotic susceptibility between CTX-M groups 1 and 9.
In the last paper, McNemars test was used for comparison of ESBL colonization rates before and after travel. Furthermore, for risk factor analysis concerning acquisition of ESBL during travel both unadjusted and adjusted logistic regression analysis were performed.
A p-value of <0.05 was considered to represent statistical significance in all above mentioned statistical analysis.

RESULTS AND DISCUSSION

Detection of ESBL genes (paper I-V)

In this thesis characterization of resistance genes in Enterobacteriaceae that were phenotypically ESBL-producers was conducted in different ways.

In the first paper we developed a multiplex PCR assay, which detects and discriminates between ^{bla}TEM, ^{bla}SHV and ^{bla}CTX-M amplicons of 747, 445 and 593 base-pair, respectively. The results obtained were the same as achieved when performing single PCR amplification. Furthermore, the presence of ^{bla}TEM, ^{bla}SHV and ^{bla}CTX-M genes was confirmed by partial DNA sequence analysis of cloned PCR amplicons from a *K. pneumoniae* strain with ^{bla}SHV, an *E. coli* ^{bla}TEM-strain and another *E. coli* strain with ^{bla}CTX-M. As an incidental finding was noted that the primer pair for CTX-M also targeted similar DNA sequences in the *K. oxytoca* K1 enzyme.
In general, the results from the phenotypic ESBL tests and the multiplex PCR showed good agreement. One *E. coli* exhibited an ESBL phenotype but no PCR amplicon was generated and this is explained by the fact that this strain, obtained from SMI, carried a ^{bla}OXA-1 gene (personal communication B. Ohlsson-Liljequist, SMI). An *E. coli* was typed as a ^{bla}TEM-strain and two *K. pneumoniae* as ^{bla}SHV-strains, all of these without having an ESBL phenotype, which may be explained by the fact that these isolates harboured the non-ESBLenzymes TEM-1 and SHV-1, respectively (personal communication B. Ohlsson-Liljequist, SMI). The major limitation of multiplex PCR assay is that it does not allow subtyping of ESBL genes. Woodford et al. described a multiplex PCR assay that was able to detect and distinguish alleles encoding CTX-M enzymes belonging to five phylogenetic groups; 1, 2, 9, 8 and 25.[222] The application of our multiplex PCR is to screen for TEM, SHV and CTX-M genes, and others have consequently used the assay in this way. [223-226]

In paper II, PCR amplification assays were performed for TEM, SHV and CTX-M genes. CTX-M genes were sequenced and divided into the five subgroups. CTX-M genes were detected in 95% of *E. coli* isolates with ESBL phenotype. Among the ESBL-producing *E. coli*, 67% harboured CTX-M genes belonging to group 1, 27% to group 9 and one single isolate harboured a CTX-M group 2 gene. TEM-1 genes were found in 68% of *E. coli*, predominately in combination with CTX-M genes. Half of the *K. pneumoniae* isolates with an ESBL phenotype carried CTX-M genes, all belonging to group 1. The *S. sonnei* and *C. koseri* isolates carried CTX-M group 9 genes. In two isolates of *E. coli* and two isolates (from one patient) of *K. pneumoniae* no CTX-M, TEM or SHV genes were detected.

This dissemination of CTX-M enzymes, especially CTX-M group 1 in Östergötland is in concordance with the global distribution of CTX-M enzymes with a predominance of CTX-M group 1 in Europe.[227]

Table 4. Beta-lactamase encoding genes in *E. coli* and *K. pneumoniae*, [a] Seven patients with *E. coli* had two or more different combination of genes, thus the total number of patients will be >128.

	***E. coli* n (%)**		***K. pneumoniae* n (%)**	
ESBL gene	**isolates** n=208	**patients** n=128[a]	**isolates** n=18	**patients** n=13
CTX-M group 1	140 (67)	86 (41)	9 (50)	8 (62)
CTX-M group 1 + TEM-1	96 (46)	60 (47)	-	-
CTX-M group 1 + TEM-135	1 (<1)	1 (<1)	-	-
CTX-M group 1 + TEM-1 + SHV	1 (<1)	1 (<1)	7 (39)	7 (54)
CTX-M group 1 + SHV	-	-	2 (11)	1 (8)
CTX-M group 2	1 (<1)	1 (<1)	-	-
CTX-M group 9	57 (27)	36 (28)	-	-
CTX-M group 9 + TEM-1	39 (19)	24 (19)	-	-
TEM-1 + SHV	5 (2)	2 (2)	-	-
TEM other	1 (<1)	1 (<1)	-	-
SHV	2 (1)	2 (2)	7 (39)	4 (31)
No CTX-M, TEM or SHV	2 (1)	2 (2)	2 (11)	1 (<1)

In paper V, from returning travellers 116 isolates that demonstrated an ESBL phenotype were examined for the presence of CTX-M genes, which was detected in 74% of the isolates. Those isolates not carrying CTX-M genes were further investigated for the presence of TEM- and SHV-genes and in 5% SHV-genes were found and in one single isolate a TEM-19-gene was detected. Plasmid-mediated AmpC (CIT and DHA-genes) were detected in 12% and 1% of isolates, respectively. In 13 isolates no ESBL genes were detected and it may be due to carriage of other ESBL genes, for instance OXA genes.

Table 5. Number of isolates with ESBL-encoding genes per geographic region from travellers

ESBL gene	**Africa North**	**Africa South**	**Asia**	**Indian subcontinent**	**America South**	**Total**
CTX-M-15-like	7	4	8	16	1	36
CTX-M-14-like	13	1	19	3	0	36
CTX-M-27-like	0	0	5	0	0	5
CTX-M-53-like	0	0	5	0	0	5
CTX-M-1/61- like	0	3	0	0	0	3
CTX-M-2-like	0	0	0	0	2	2
CTX-M-3-like	1	0	0	0	0	1
TEM	0	0	0	1	0	1
SHV	1	1	4	0	0	6
CIT/DHA	0	6	5	3	1	15
No gene detected	0	3	9	0	1	13
Total	**22**	**18**	**55**	**23**	**5**	**123**

The rapid emergence of antibiotic resistant Enterobacteriaceae is worrisome and as a part of the fight against resistance rapid and reliable detection methods are required. For this purpose development of molecular techniques, such as PCR, are crucial.[228]

Antibiotic consumption and ESBL-producing Enterobacteriaceae in a low-prevalence area (paper II)

Paper II presents the first comprehensive description of epidemiology of ESBL-producing Enterobacteriaceae in a county of Sweden. Consecutive clinical isolates with an ESBL phenotype were collected during a six years period, from 2002 until 2007, in the county of Östergötland. *E. coli* with an ESBL phenotype were detected in 224 isolates, originating from 132 patients. A few patients had ESBL-producing *E. coli* during different years. *K. pneumoniae* with an ESBL phenotype were found in 23 isolates, originating from 14 patients. Both ESBL-producing *E. coli* and *K. pneumoniae* were isolated from 4 patients. Furthermore ESBL-production was discovered in one isolate each of *Citrobacter koseri* and *Shigella sonnei*. Isolates of ESBL-producing Enterobacteriaceae was detected in clinical samples from 144 patients with a wide age distribution (from newborn to 90 years). Over the study period the number of patients with ESBL-producing *E. coli* per year increased, from 5 in 2002 to 47 in 2007 ($p<0.001$). However, the patients found with ESBL-producing *K. pneumoniae* remained between 1 and 4 per year. The most common source of ESBL-producing *E. coli* was urine (65%), followed by wounds (14%), hygiene screening (14%) and blood (5%). A higher prevalence of ESBL-producing E. coli in blood isolates than in urine was observed, in concordance with a report from Norway, and the reason for this has not been established.[229]

The conclusion was that the prevalence of ESBL-producing Enterobacteriaceae was still low, less than 1% in Östergötland during the study period but the number of patients with ESBL-producing isolates increased over time. These prevalence data are essentially similar to that reported from other Nordic countries.[229-231]

After the study period, from 2008 and forward, an increased occurrence of ESBL-producing bacteria has been reported to SMI. The total number of patients with ESBL-producing Enterobacteriaceae in clinical specimens from Sweden and Östergötland, respectively, is presented in figure 7. At the national level patients with $ESBL_{CARBA}$-producing bacteria has increased from 23 in 2012 to 42 in 2013, during the same period 3 patients annually were reported from the county of Östergötland of which two isolates had OXA-genes and one isolate with NDM (personal communication Smittskyddsenheten Östergötland).[97]

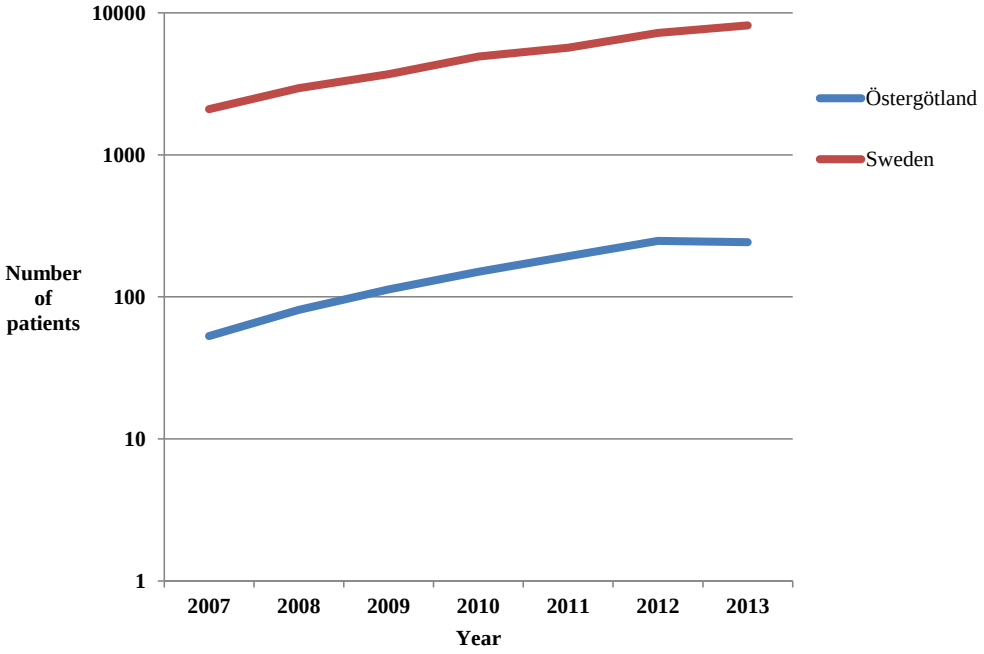

Figure 7. Number of patients with ESBL-producing Enterobacteriaceae reported during 2007-2013 according to notifications to SMI.

Furthermore, paper II is the first Scandinavian study that compares prevalence of ESBL-producing Enterobacteriaceae with antimicrobial consumption data. The total antibiotic consumption in Östergötland, including both primary and hospital care, for a period of nine years remained unchanged around 12.7 DID per year. Ninety percent of the antibiotics were prescribed in primary care. Among the different antibiotic classes, there was observed some differences such as a 50% decreased consumption of cephalosporins and trimethoprim ($p<0.001$ and $p=0.009$, respectively) whereas nitrofurantoin increased three-fold and penicillins combined with beta-lactamase inhibitor increased approximately 60% (both $p<0.001$). A less pronounced, but still significant, increase was noted regarding broad-spectrum penicillins, pivmecillinam, TMP-SMX and tetracyclines during the study period.

The remaining percentage of antibiotics used in the county, approximately 10%, was used in hospital care and a small, but significant ($p=0.003$), increase in consumption was observed and is explained by a pronounced increased consumption of penicillins with ß-lactamase inhibitors ($p<0.001$). A significant but less marked increase of pivmecillinam, carbapenems and TMP-SMX was observed.

Table 6. Antibiotic consumption in primary and hospital care in Östergötland 2001-2013.

Antimicrobial agent	DDD/1000 inhabitants/day and year												
Primary care	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
ß-lactamase -sensitive penicillins	4.327	3.989	3.788	3.490	3.485	3.700	4.091	3.857	3.769	3.639	3.531	3.354	3.142
Broadspectrum penicillins	1.105	1.056	1.082	1.110	1.140	1.141	1.213	1.216	1.189	1.049	1.025	1.191	1.289
Mecillinam	0.482	0.463	0.469	0.485	0.465	0.465	0.498	0.525	0.519	0.502	0.449	0.445	0.474
Cephalosporins	0.464	0.453	0.451	0.395	0.368	0.369	0.355	0.315	0.249	0.256	0.255	0.235	0.234
Penicillins/ ß-lactamase inhibitors	0.225	0.236	0.236	0.218	0.250	0.294	0.312	0.354	0.359	0.394	0.369	0.364	0.350
Tetracyclines	2.138	1.999	2.090	2.168	2.245	2.422	2.548	2.487	2.475	2.706	2.778	2.757	2.534
Quinolones	0.863	0.855	0.894	0.897	0.916	0.945	0.904	0.833	0.848	0.845	0.813	0.739	0.694
Trimethoprim	0.517	0.538	0.553	0.511	0.494	0.513	0.484	0.383	0.277	0.256	0.226	0.190	0.180
Nitrofurantoin	0.119	0.139	0.142	0.172	0.210	0.212	0.239	0.254	0.316	0.326	0.373	0.424	0.464
Trimethoprim-sulfametoxazole	0.113	0.105	0.110	0.116	0.124	0.131	0.133	0.131	0.145	0.160	0.159	0.174	0.164
Total antibiotic consumption	11.580	11.079	11.025	10.707	10.944	11.518	12.131	11.742	11.458	11.453	11.253	11.147	10.772
Hospital care	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
ß-lactamase sensitive penicillins	0.106	0.100	0.095	0.088	0.086	0.099	0.095	0.101	0.097	0.095	0.116	0.126	0.131
Broadspectrum penicillins	0.123	0.131	0.133	0.137	0.151	0.135	0.141	0.124	0.135	0.197	0.224	0.259	0.273
Mecillinam	0.032	0.030	0.028	0.026	0.034	0.037	0.038	0.041	0.042	0.039	0.043	0.044	0.052
Cephalosporins	0.213	0.202	0.207	0.206	0.201	0.202	0.213	0.200	0.198	0.197	0.189	0.199	0.206
Penicillins/ ß-lactamase inhibitors	0.018	0.021	0.024	0.028	0.032	0.042	0.049	0.065	0.066	0.089	0.107	0.097	0.106
Carbapenems	0.050	0.050	0.060	0.058	0.057	0.061	0.068	0.077	0.076	0.091	0.103	0.108	0.112
Tetracyclines	0.171	0.163	0.175	0.194	0.205	0.170	0.224	0.216	0.183	0.174	0.203	0.215	0.174
Quinolones	0.144	0.164	0.164	0.147	0.152	0.154	0.145	0.139	0.132	0.144	0.149	0.134	0.134
Trimethoprim	0.024	0.039	0.022	0.028	0.026	0.033	0.042	0.044	0.024	0.020	0.016	0.012	0.009
Trimethoprim-sulfametoxazole	0.014	0.015	0.015	0.013	0.014	0.015	0.019	0.018	0.024	0.024	0.023	0.025	0.023
Aminoglycosides	0.010	0.010	0.013	0.014	0.012	0.011	0.018	0.015	0.013	0.021	0.024	0.023	0.021
Total antibiotic consumption	1.140	1.166	1.168	1.142	1.200	1.195	1.299	1.279	1.256	1.311	1.418	1.460	1.492

Although the total antibiotic consumption has not declined it seems like a shift towards antibiotics with lower ESBL selection potential has occurred, particularly in primary care. Antibiotic consumption data from 2001 through 2013 is presented in table 6. After the study period, from 2010 and onward the total antibiotic consumption has remained unchanged, with a significantly reduced consumption in primary care and simultaneously an increased consumption in hospital care. Nevertheless, this modified antibiotic prescribing pattern is not enough to avoid a further increase in ESBL-producing bacteria in the county of Östergötland, or indeed at a national level, because this is a multifactorial issue with global spread.[232]

Antibiotic susceptibility patterns of CTX-M-producing *E. coli* (paper III, IV)

Antibiotic resistance is dynamic and changing; whether an antimicrobial agent can be used for empirical therapy when infection caused by ESBL-producing Enterobacteriaceae is suspected will largely depend on local susceptibility patterns. Consequently, it is important to know local susceptibility patterns. In paper III and IV CTX-M-producing *E. coli* from county of Östergötland were investigated regarding antibiotic susceptibility to both beta-lactam and non-beta-lactam agents. The MIC distributions for twelve different beta-lactam antibiotics and non-beta-lactam agents are presented in table 7.

Furthermore, as multidrug resistant gram-negative bacteria is emerging and there are no new drugs in the antibiotic arsenal it is necessary to re-evaluate the antibiotics we have available to determine their optimal use. Regarding in-vitro susceptibility for CTX-M-producing *E. coli*, more than 90% were susceptible to mecillinam, and more than 80 % to temocillin, amoxicillin-clavulanic acid and piperacillin-tazobactam. In general, CTX-M group 9-producing isolates were more susceptible than isolates belonging to CTX-M group 1. Among E. coli isolates in CTX-M group 9 more than 90% were susceptible to temocillin, amoxicillin-clavulanic acid, piperacillin-tazobactam but also to ceftibuten and ceftazidime. CTX-M group 1- producing *E. coli* demonstrated less than 10% susceptibility to all tested cephalosporins. A subsequent study from Stockholm reported rather similar susceptibility results.[233] All isolates were susceptible to imipenem and meropenem but three isolates exhibited reduced susceptibility to ertapenem. Two isolates had MIC values above 0.125, the epidemiological cut-off values (ECOFF) for meropenem. One isolate demonstrated a MIC above 0.5, the ECOFF for imipenem. For ESBL-producing Enterobacteriaceae resistance to ertapenem is more common than resistance to other carbapenems. In-vitro data shows that exposure of an ESBL-producing *E. coli* to ertapenem is associated with selection of porin-deficient subpopulations.[234, 235] Regarding non-beta-lactam agents, more than 95% of isolates were susceptible to colistin, fosfomycin, tigecycline, nitrofurantoin and amikacin. Lower susceptibility rates were seen for chloramphenicol (77%), gentamicin (60%), tobramycin (55%), TMP-SMX (35%), ciprofloxacin (34%) and trimethoprim (29%). Also in these MIC-determinations were differences between CTX-M groups observed, CTX-M group 9-producing isolates tended to be more susceptible.

Table 7. MIC distributions for ESBL-producing *E. coli* n=198, CTX-M group 1 n=132, CTX-M group 9 n=55. Breakpoints according to EUCAST (S≤/R>) except for temocillin where instead breakpoints from BSAC were used. *Italic* = MIC 50 Underlined=MIC 90

Number of isolates with indicated MIC (mg/L)

	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128	%S	%R	Break-points
Nitrofurantoin																	64/64
All E. coli						2	4	37	*70*	39	29	10	7		96.5	3.5	
CTX-M group 1							4	29	*43*	23	22	6	5		96.2	3.8	
CTX-M group 9						2		7	*22*	13	7	2	2		96.4	3.6	
Colistin																	2/2
All E. coli			24	*83*	82	7		1	1						99.0	1.0	
CTX-M group 1			13	*56*	58	4		1							99.2	0.8	
CTX-M group 9			11	*23*	18	2			1						98.2	1.8	
Fosfomycin																	32/32
All E. coli				1	23	*107*	46	11	1	4	4			1	99.5	0.5	
CTX-M group 1				1	17	*68*	31	8	1	2	3			1	99.2	0.8	
CTX-M group 9					5	*30*	14	3		2	1				100	0	
Ciprofloxacin																	0.5/1
All E. coli	4	1	9	8	5	3			5	1	121				34.3	64.1	
CTX-M group 1	4	1	3	5	3	1					94				28.0	71.2	
CTX-M group 9			6	3	2	2			4	1	24				43.6	52.7	
Tigecycline																	1/2
All E. coli	1	3	24	*105*	53	10		2							99.0	1.0	
CTX-M group 1		1	18	*63*	40	8		2							98.5	1.5	
CTX-M group 9	1	2	4	*36*	10	2									100	0	
TMP-SMX																	2/4
All E. coli	20	17	10	5	5	5	1				129				34.8	65.2	
CTX-M group 1	15	13	5	3	4	5					81				38.6	61.4	
CTX-M group 9	5	3	3	2	1		1				40				27.3	72.7	
Trimethoprim																	2/4
All E. coli		2	8	21	20	3	3				141				28.8	71.2	
CTX-M group 1		2	7	15	12	3	2				91				31.3	69.9	
CTX-M group 9				4	8						42				23.6	76.4	
Gentamicin																	2/4
All E. coli				6	53	*55*	4	1	10	16	32	8	2	11	59.6	39.9	
CTX-M group 1				5	28	*34*	3		6	14	27	6	2	7	53.0	47.0	
CTX-M group 9				1	24	*16*	1			2	5	2		4	76.4	23.6	
Tobramycin																	2/4
All E. coli				2	61	*34*	12	11	27	39	8			4			
CTX-M group 1				2	31	20	5	3	*23*	36	8			4	43.9	53.8	
CTX-M group 9					*28*	11	3	8	3	2					76.4	9.1	
Amikacin																	8/16
All E. coli						11	*118*	47	13	4	1	2	1	1	95.5	2.5	
CTX-M group 1						8	*67*	41	8	4	1	1	1	1	93.9	3.0	
CTX-M group 9						3	*46*	4	2						100	0	
Chloramphenicol																	8/8
All E. coli						2	18	*112*	21	6	6	6	2	25	77.3	22.7	
CTX-M group 1						2	14	*76*	15	4	3	4	1	13	81.1	18.9	
CTX-M group 9							4	*29*	6	1	2	2	1	10	70.9	29.1	

Number of isolates with indicated MIC (mg/L)

	<0.064	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128	%S	%R	Break-points
Temocillin																	8/8
All E. coli						1	15	67	*82*	30	3				83.3	16.7	
CTX-M group 1						1	6	26	*71*	25	3				78.8	21.2	
CTX-M group 9							6	*34*	10	5					91.0	9.0	
Mecillinam																	8/8
All E. coli			8	22	32	*67*	33	12	7	3	4			10	91.4	8.6	
CTX-M group 1			3	14	25	*45*	23	9	6		2			5	94.7	5.3	
CTX-M group 9			5	8	6	*19*	8	2		2				5	87.3	12.7	
Amoxicillin-clavulanic acid																	8/8
All E. coli								47	*120*	31					84.3	15.7	
CTX-M group 1								19	*87*	26					80.3	19.7	
CTX-M group 9								23	*29*	3					94.5	5.5	
Piperacillin-tazobactam																	8/16
All E. coli			2	2	5	30	*72*	30	26	11	5		5	10	84.3	10.1	
CTX-M group 1				2	3	13	39	*26*	25	11	5		4	4	81.8	9.8	
CTX-M group 9			2		2	15	*29*	4	1					2	96.4	3.6	
Aztreonam																	1/8
All E. coli			2	3	1	9	28	29	13	*46*	30	16	9	12	7.6	57.1	
CTX-M group 1							4	7	12	*43*	29	16	9	12	0	82.6	
CTX-M group 9					1	9	*22*	21		1	1				18.2	3.6	
Cefotaxime																	1/2
All E. coli		2				3	3		25	26	31	*33*	22	53	2.5	96.0	
CTX-M group 1									4	7	18	33	*20*	50	0	100	
CTX-M group 9							1		20	*17*	13		1	3	0	98.2	
Ceftazidime																	1/8
All E. coli			4	13	28	22	10	*39*	40	24	8	4	6		33.8	21.2	
CTX-M group 1					2	9	8	32	*40*	24	7	4	6		8.3	31.1	
CTX-M group 9			3	12	*25*	12	1	2							94.5	0	
Ceftibuten																	1/1
All E. coli			2	8	27	37	14	*35*	36	24	6	5	1	3	37.4	62.6	
CTX-M group 1					4	9	12	35	*33*	24	6	5	1	3	9.8	90.2	
CTX-M group 9			1	5	19	27	1		2						94.5	5.5	
Cefepime																	1/8
All E. coli			1	4	2	9	32	*60*	42	22	13	3	2	8	8.1	24.2	
CTX-M group 1					2	2	7	38	*37*	22	13	2	2	7	3.0	34.8	
CTX-M group 9			1			5	*24*	20	3			1		1	10.9	3.6	

	<0.008	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	>16	%S	%R	Breakpoints
Imipenem																	2/8
All E. coli						28	*161*	8	1						100	0	
CTX-M group 1						20	*106*	6							100	0	
CTX-M group 9						5	*47*	2	1						100	0	
Meropenem																	2/8
All E. coli		1	33	*127*	29	6	2								100	0	
CTX-M group 1			11	*87*	28	6									100	0	
CTX-M group 9		1	16	*35*	1		2								100	0	
Ertapenem																	0.5/1
All E. coli		10	52	*52*	46	16	8	11	1	2					98.5	1.0	
CTX-M group 1		4	21	34	*38*	15	8	11	1						99.2	0	
CTX-M group 9		3	*30*	15	5					2					96.4	3.6	

Studies on antimicrobial susceptibility may be difficult to compare because material and methodology differ considerably. Many studies report only SIR data and MIC data is presented differently and different breakpoints are used in time and space. The strength of our studies is that we have performed MIC-determinations for a rather large group of CTX-M–producing *E. coli* isolates with an extensive panel of different antibiotics. Because there are limited treatment options against ESBL-producing Enterobacteriaceae and for severe infections treatment is often carbapenems used, there is of interest to find alternatives. Not only in-vitro data of MICs correlate with the clinical outcome, consideration of the antibiotic exposure to the infecting organism is of major importance. Monte-Carlo simulations based on multiple PK/PD variables take into account the antibiotic, microorganism and dosing regimen.

Cefepime has been proposed as an option for definitive therapy of invasive infections caused by ESBL-producing *E. coli* and Klebsiella spp. when the MIC is ≤ 1 mg/L, although higher doses may be considered for MICs in the 4-8 mg/L ranges. Piperacillin-tazobactam is another alternative for definitive therapy if MIC is ≤ 8mg/L or as prolonged infusion if MIC is ≤ 16 mg/L. When CTX-M-15-producing isolates are prevalent it is likely that cefepime and beta-lactam/beta-lactamase inhibitor combinations will not be effective. [236] The CTX-M-producing *E. coli* in our region exhibits low susceptibility, less than 10%, to cefepime but rather high, >80%, in-vitro susceptibility to piperacillin-tazobactam. However, only Etest has been performed and recent data indicates that this method for MIC determination may not be sufficient to detect resistance to piperacillin-tazobactam compared to broth microdilution.[233]

The higher susceptibility to amikacin than to other aminoglycosides among ESBL-producing *E. coli* is consistent with other studies. In a recent study of ESBL-producing *E. coli* from Norway isolates with reduced susceptibility to gentamicin were 45%, tobramycin 57% and amikacin 8.3%.[237] Amikacin is generally regarded as more stable against aminoglycoside-modifying enzymes produced by Enterobacteriaceae. In 2013, the Swedish Reference Group of Antibiotics published recommendations for the rational use of aminoglycosides and emphasizes that use of amikacin in many situations is preferable to gentamicin/tobramycin.[238]

Despite the very high susceptibility to tigecycline, it is a sparsely used drug for treatment mainly due to the limited urinary secretion and therefore not approved for treating UTIs, the most common site for infections caused by ESBL-producing *E. coli.* Serum concentrations of tigecycline are generally not adequate to treat bloodstream infections and increased mortality in patients with severe infections treated with tigecycline compared to other agents are reported.[239]

Colistin has re-emerged as the last-resort therapy for many infections caused by multidrug-resistant bacteria, e.g. carbapenem-resistant gram-negatives. ESBL-producing Enterobacteriaceae is still very susceptible to polymyxins, as reported by us as well as others. The clinical use is limited by poorer outcome compared to treatment with beta-lactams, associated toxicity and risk for emergence of colistin resistance. Therefore, colistin is reserved for treatment of infections caused by bacteria resistant to other, more potent antibiotics.[86, 214, 240]

Thus, for invasive infections caused by ESBL-producing Enterobacteriaceae carbapenems is still the drug of choice, but in cases of carbapenem-resistance, allergy or other contraindications may amikacin, tigecycline and colistin be alternative treatment options, either alone or as a part of combination therapy.[239, 241]

For treatment of lower urinary tract infection there are still some treatment options for CTX-M-producing *E. coli* such as mecillinam, nitrofurantoin and fosfomycin as in-vitro susceptibility was high among isolates in our region. However, for treatment of ESBL-producing Enterobacteriaceae with these drugs only a few clinical efficacy studies with

small patient numbers have been conducted. Recently, a population-based study from Norway showed a higher rate of mecillinam treatment failure in patients with UTI caused by ESBL-producing *E. coli* compared to non-ESBL producing strains. This could be explained by the fact that the MIC of mecillinam in ESBL-producing strains was higher than in non-ESBL producing strains but also that mecillinam is not stable against ESBL-producing *E. coli*. The authors suggest that mecillinam should only be prescribed in uncomplicated UTIs caused by ESBL-producing *E. coli* if no other per oral options are available and they also suggest that higher doses of pivmecillinam should be used. Furthermore, according to the results obtained in the Norwegian study should the mecillinam MIC breakpoints for ESBL-producing *E. coli* be reconsidered. [242-244] Moreover, the results available on treatment options for infections with ESBL-producing Enterobacteriaceae come from observational studies and in vitro susceptibility since no randomized trials have been conducted.

Multiresistance (paper II, IV and V)

In paper II, 40% of ESBL-producing *E. coli* had reduced susceptibility to gentamicin, 66% to ciprofloxacin, 63% to TMP-SMX and 58% were regarded as multi-resistant, i.e. decreased susceptibility to ≥ 2 classes of antibiotics in addition to the beta-lactams (except carbapenems).

In paper IV, 68% of ESBL-producing *E. coli* was multi-resistant. Of isolates carrying CTX-M group 1 were 66% multi-resistant, compared to 71% carrying CTX-M group 9. The most frequent multi-resistance pattern was the ESBL phenotype combined with decreased susceptibility to trimethoprim, TMP-SMX, ciprofloxacin, gentamicin and tobramycin.
Additional screening for plasmid-mediated quinolone resistance among CTX-M-producing *E. coli* was performed in paper IV. In one multi-resistant isolate we found a *qnrS1* gene together with CTX-M group 14. *Qnr A, B, C* or *D* genes were not detected. It may appear aberrant that only one isolate carried a *qnr*-gene even though 66% of CTX-M-producing *E. coli* demonstrated reduced susceptibility to ciprofloxacin. In other studies from Sweden and Norway 3.8% and 9.1% of ESBL-producing Enterobacteriaceae carried gnr-genes but these isolates were more heterogeneous regarding bacterial species and ESBL enzymes, with less CTX-M-producing isolates, and it is previously described that *qnr*-genes are more prevalent among *Klebsiella spp*. than among *E. coli*.[245-248]
The *aac(6')-I* enzymes are known to convey resistance, in particular to amikacin, and with the modified aminoglycoside-modifying gene *aac(6')-Ib-cr* the resistance also includes fluoroquinolones and this gene was detected in 73 (37%) of isolates almost all belonging to CTX-M group 1. All these isolates were all resistant to tobramycin. However, 95% of isolates carrying the *aac(6')-Ib-cr*-gene were susceptible to amikacin when performing MIC-determination. Nine isolates were resistant to amikacin and they all carried the gene. Among the CTX-M-producing *E. coli* with the identified *aac(6')-Ib-cr*-gene were only 23% and 14% susceptible to gentamicin and ciprofloxacin, respectively. Furthermore 60% were resistant to all three of tobramycin, gentamicin and ciprofloxacin. The prevalence of the *aac(6')-Ib-cr*-gene is essentially at the same level as reported in studies from Scandinavia as well as China.[237, 245, 247]

Among travellers colonized with ESBL-producing Enterobacteriaceae 71% harboured multi-resistant isolates and these demonstrated non-susceptibility to anything between three and eight different classes of antibiotics, just above half of those multi-resistant isolates exhibited reduced susceptibility to third-generation cephalosporins, trimethoprim/

TMP-SMX and aminoglycosides. Of 116 isolates were all except two susceptible to carbapenems. There were differences between the different aminoglycosides, almost half of all ESBL-producing Enterobacteriaceae acquired abroad were resistant to tobramycin and gentamicin while only 2% were resistant to amikacin. These results may have implications for the choice of empirical therapy to a patient with suspected gram-negative sepsis if the patient has recently returned from travel to high-risk area.

The high frequency of multi-resistance, from clinical specimens as well as in faecal samples from travellers, detected in these studies is alarming and worrisome. These findings further underscore the importance to identify and implement strategies to limit the emergence and spread of these multi-resistant bacteria, both locally and globally.

International travel and ESBL acquisition (paper V)

In paper V, the relationship between international travel and colonization with ESBL-producing Enterobacteriaceae as well as associated risk factors were investigated.

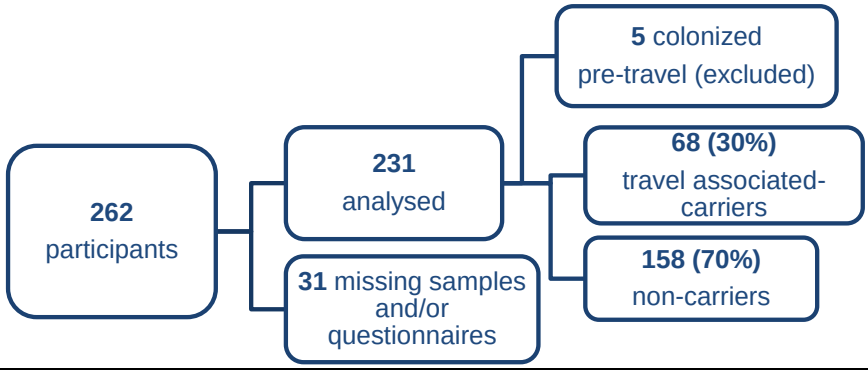

Figure 8. The study population in paper V.

The colonization rate before travel was 2.4%, consistent with previous findings in Swedish outpatients.[113]Risk factor analysis indicated that the geographical area visited plays the most important role for acquiring ESBL-producing Enterobacteriaceae during travel abroad, with the Indian subcontinent showing the highest risk, followed by Asia and Africa north of the equator. Figure 9 visualizes the frequency of travel-associated carriers from different destinations. As illustrated in figure 4 the colonization rate in the community varies considerable between geographic areas and to some extent these regional differences agree with colonization rates among travellers in our study. For instance, 43% of visitors to Africa north of the equator, the most popular destination were Egypt, acquired ESBL-producing Enterobacteriaceae. Among Asia travellers, excluded Indian subcontinent, 45% were colonized and a majority had visited Thailand. The considerable risk of ESBL colonization when travelling to India confirms the previous finding by Tängdén et al.[125] Interestingly, the duration of the journey did not affect the risk of becoming colonized, which suggests that colonization might occur quite early during travel abroad.

Another significant risk factor was diarrhoea or other gastrointestinal symptoms during travel, consistent with what Tängdén et al. reported.[125] By contrast, fever during travel was shown to reduce the risk for ESBL colonization. No association was seen with the use of antibiotics during the trip. Finally, age seems to affect the risk with the highest risks among travellers 65 years and older.

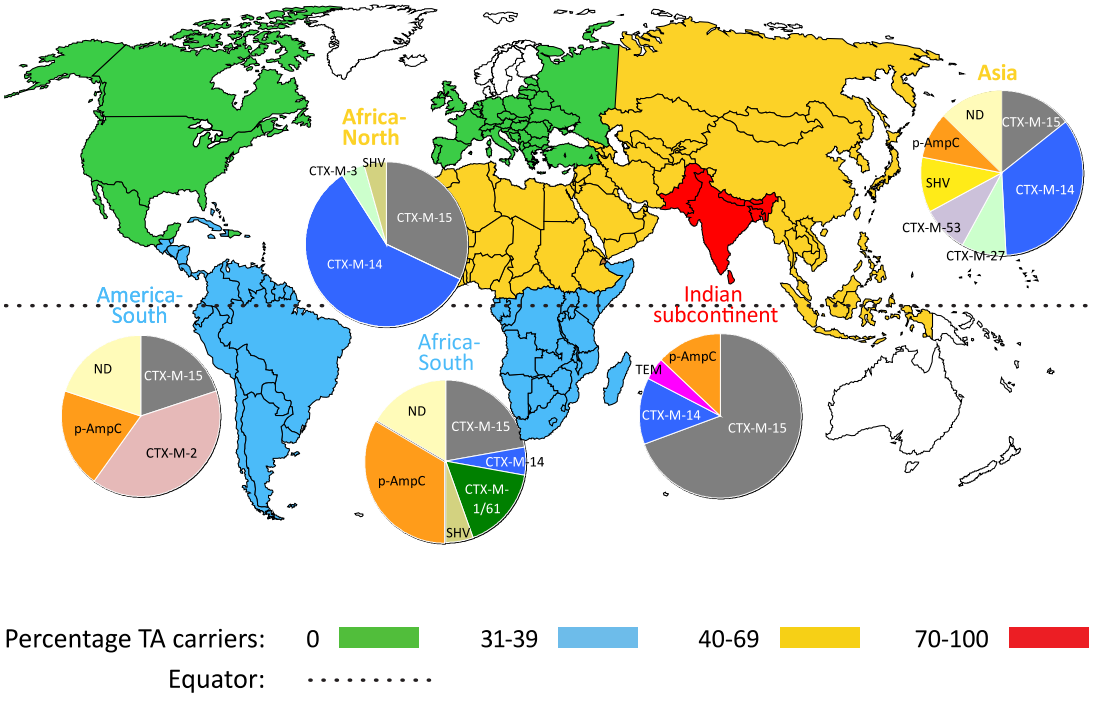

Figure 9. Frequency of travel-associated carriers with respect to geographical area visited and the global distribution of ESBL-encoding genes in isolates from travel-associated carriers.

Thus, travel abroad entails a risk for acquisition of multidrug-resistant ESBL-producing Enterobacteriaceae, which may affect both the individual and the society. The individual patient is at risk of having an ineffective initial antibiotic treatment unless travel history is requested. In case of infection, it is also important that adequate cultures are taken. At the community level, the emergence and dissemination of ESBL-producing Enterobacteriaceae is worrisome and it is obvious that global action must be taken against antimicrobial resistance.

FUTURE PERSPECTIVES

The emergence of multi-resistant ESBL-producing Enterobacteriaceae is worrisome and the causes are multifactorial which requires actions at multiple levels.

Meta level / Society
Antibiotics and resistance needs to be seen from an ecological and environmental perspective.

- At the global level, clean water and sanitation is crucial.
- Interventions to stop unnecessary antibiotic use in humans and animals. Banish the use of antibiotics as growth promoters in agriculture.
- Sustained and coordinated surveillance of antibiotic use, antimicrobial resistance and burden of disease attributable to antimicrobial resistance, both on a national and global level are desirable.
- Development of new antimicrobial agents with gram-negative effect is urgently needed.
- Improved infection control measures and a better compliance to hygiene routines in society, long-term care facilities and hospitals.
- Public education and awareness regarding antibiotics to avert inappropriate use.
- Regulate the sale of antibiotics and prohibit over-the counter sales worldwide.

Macro level / Hospital and Patient

- Investigate the field of antibiotic stewardship, may improved antibiotic prescribing affect the emergence of ESBL-producing Enterobacteriaceae and what are the clinical outcomes?
- Education, training and feedback to healthcare providers about antimicrobial resistance, rational antibiotic use and infection control measures.
- Improve antibiotic treatment of patients with infections caused by ESBL-producing Enterobacteriaceae, both empirical and definitive therapy.
- Studies on “the search, destroy and restore concept” would be interesting to conduct. Thus, screening and identifying patients carrying ESBL-producing Enterobacteriaceae, decolonisation therapy and finally re-colonisation either with faecal transplants, probiotics or a specific *E. coli* strain.

Micro level / Pathogen

- To type, map and track the ESBL-producing Enterobacteriaceae, their reservoirs, virulence and transmissibility.
- Further develop microbiological methods for fast detection of resistant bacteria. Rapid diagnostic methods in the microbiology laboratory may help to reduce antibiotic use or narrow the antimicrobial spectrum, thus reducing the antibiotic pressure.

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APPENDIXES

Paper I-V

Papers

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