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Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae: Epidemiology, Risk Factors, and Duration of Carriage

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To my family

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List of papers

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I Tham, J., Odenholt, I., Walder, M. , Brolund, A., Ahl, J. & Melander, E., 2010. Extended-spectrum beta-lactamase-producing *Escherichia coli* in patients with travellers' diarrhoea. *Scandinavian Journal of Infectious Diseases,* 42: 275–280.

II Stromdahl, H., Tham J., Melander, E., Walder, M., Edquist P.J. & Odenholt I. 2011. Prevalence of faecal ESBL carriage in the community and in a hospital setting in a county of Southern Sweden *Eur J Clin Microbiol Infect Dis*, 30:1159–1162.

III Tham, J., Melander, E., Walder, M., & Odenholt, I. Duration of colonization with extended-spectrum beta-lactamase producing *Escherichia coli* in patients with travellers' diarrhoea. *Scandinavian Journal of Infectious Diseases,* 44(8):573-7.

IV Tham J., Melander E.,Walder M. & Odenholt I. 2012. Prevalence of extended-spectrum beta-lactamase-producing bacteria in food. Accepted in *Infection and Drug Resistance, 2012*

V Tham J, Odenholt I, Walder M & Melander E.. Risk factors for infections with extended-spectrum beta-lactamase-producing *Escherichia coli*. Manuscript (submitted).

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Abbreviations

ARMed	Antibiotic Resistance Surveillance and Control in the Mediterranean Region
CTX-M	An extended-spectrum beta-lactamase with greater activity against Cefotaxime, CTX for cefotaximase and M for Munich
CLSI	Clinical and Laboratory Standards Institute
EAEC	enteroaggregative *Escherichia coli*
EARSS	European Antimicrobial Resistance Surveillance System
ECDC	European Centre for Disease Prevention and Control
ESBL	extended-spectrum beta-lactamase
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ETEC	enterotoxigenic *Escherichia coli*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ICU	intensive care unit
KPC	*Klebsiella pneumoniae* carbapenemase
LOS	length of hospital stay
MIC	minimal inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
PBP	penicillin binding protein
PCR	polymerase chain reaction
PHCU	primary health care unit
rep-PCR	repetitive sequence-based PCR
SHV	sulfhydryl variable
SSYC	*Salmonella*, *Shigella*, *Yersinia*, or *Campylobacter*
SMI	Swedish Institute for Communicable Disease Control (*Smittskyddsinstitutet*)

ST	sequence type
TEM	Temoneira (an ESBL enzyme named after a greek patient)
TEST	Tigecycline Evaluation and Surveillance Trial
TSN	The Surveillance Network
UTI	urinary tract infection
VIM	Verona integron-encoded metallo-β-lactamase

1 Introduction

1.1 The pathogens

Enterobacteriaceae

In human medicine, the most important family of bacteria is Enterobacteriaceae, which includes genera and species that cause well-defined diseases, as well as nosocomial infections. The members of this family are Gram-negative, rod-shaped, non-spore-forming facultative anaerobes that ferment glucose and other sugars, reduce nitrate to nitrite, and produce catalase but seldom oxidase. Most Enterobacteriaceae are components of the gastrointestinal flora of humans and animals, although many are also widespread in the environment. Furthermore, these bacteria can cause many different infections, such as septicaemia, urinary tract infections, pneumonia, cholecystitis, cholangitis, peritonitis, wound infections, meningitis, and gastroenteritis, and they can give rise to sporadic infections or outbreaks. Indeed, the family Enterobacteriaceae might be regarded as the Lionel Messi of infectious diseases: off the pitch being merely a shy boy among his comrades, but on the pitch a rapid and fatal attacker (1).

Escherichia coli

Escherichia coli is the most prevalent facultative anaerobic species in the human gastrointestinal tract (10^9 CFU/g faeces) but it also colonizes the intestines of animals and is thus used as an indicator of faecal contamination of drinking water and food. *E. coli* is usually a harmless microbe, although it is also the most common cause of community-acquired bacteraemia and the fifth most common cause of nosocomial bacteraemia (2). The more virulent pathotypes often have a larger genome compared to the non-pathogenic *E. coli*, and there are also many different virulence factors, which are usually encoded on plasmids, chromosomes, or bacteriophages (3, 4). The serotypes and groups of pathogenic *E. coli* are defined by their lipopolysaccharide (O) and flagellar (H) antigens (5). Geographically widespread epidemic clones with the same chromosomal sequence types (STs) have been identified among *E. coli* strains that cause urinary tract infections. Extended-spectrum beta-lactamase (ESBL)-producing strains are usually community acquired, and only a few hospital outbreaks of such bacteria have been reported (6, 7).

Klebsiella

Klebsiella pneumoniae (including subspecies *K. ozaenae*), *K. oxytoca*, and *K. granulomatis* are the three major species of this genus. Like *E. coli*, *Klebsiella* spp. are usually found in the human gastrointestinal tract (10^4 CFU/g faeces). The major virulence factor of *Klebsiella* is the polysaccharide capsule, which is also responsible for the mucoid colony phenotype. *K. pneumoniae* is the species isolated most often from human infections, and it can cause a wide variety of (nosocomial) infections, including urinary tract infections (UTIs), septicaemia, wound infections, cholecystitis, and pneumonia (Friedländer's disease) (8). Patients with *Klebsiella* infections often have other primary diseases but can nonetheless (like a Trojan horse) be part of a nosocomial outbreak after receiving the bacteria from the hands of hospital personnel (9). It appears that this route is more important in *Klebsiella* spp. than in for *E. coli*, since the former species has been involved in many such outbreaks, and its spread is more epidemic than endemic (10).

Proteus

Enterobacteriaceae of the genus *Proteus* frequently give rise to UTIs, occasionally in healthy individuals and very often in patients with indwelling catheters or anatomic or functional abnormalities of the urinary tract. Compared to infections caused by *E. coli*, those attributable to *Proteus* spp. tend to be more severe and they are associated with a larger proportion of pyelonephritis (8).

Shigella

Over two thousand years ago, Hippocrates coined the word dysentery to describe a patient who was suffering from painful diarrhoea containing blood and mucus. Much later, in 1906 Kiyoshi Shiga found that a particular kind of bacteria of the genus *Shigella* was one of the two causes of dysentery (the other was an amoeba). Historically, this condition has had a great impact on military campaigns in that it has often led to larger death tolls than actual war injuries. The species of *Shigella* are divided into four major groups as follows: Group A (*S. dysenteriae),* Group B *(S. flexneri),* Group C *(S. boydii),* and Group D *(S. sonnei)*. A very low dose of the bacteria (100 viable cells) can produce disease, and thus bacillary dysentery is one of the most common of the communicable diarrhoeas, probably because the bacteria can tolerate the low pH of gastric juice (11).

Salmonella

The bacterial genus *Salmonella* was named after the pathologist Daniel Salmon in the late 1800s, and it can be divided into 2,500 serotypes that can cause a wide variety of diseases ranging from aortitis to enteritis. Humans constitute the sole host of *S. typhi*

and *S. paratyphi*, and infections with these bacteria occur through foodborne or waterborne contamination and are estimated to cause 200,000–600,000 deaths annually. The incidence of non-typhoidal salmonellae has increased in recent years, and these pathogens can be found in multiple animal reservoirs. A large number of the bacteria (10^6) is usually required to induce disease in humans, although in some subspecies as a few as 200 bacteria can be sufficient to cause enteritis. Also, taking medication that raises the pH of the gastric content markedly increases the susceptibility to the diseases caused by these microbes. Non-typhoidal gastrointestinal salmonella is usually self-limited, and antibiotics do not decrease the duration of illness, but rather increase the frequency of relapse and prolong the duration of positive cultures. Treatment should be focused on rehydration and compensation of electrolytic losses, and pre-emptive therapy should only be given to a few groups of patients with special risk factors (e.g., neonates and individuals who are immunocompromised or have cardiac, endovascular, or joint diseases) (12).

1.2 Antibiotics

"One sometimes finds what one is not looking for" -Alexander Fleming

Most antibiotics probably evolved millions of years ago as the result of competition for survival between different microorganisms in soil, plants, and the oceans. Thus, these substances most likely represent part of the evolution and the competition that allows a species to dominate within an ecological niche (13, 14).

In 1909, Paul Erlich and colleagues developed the first synthetic antibacterial compound, arsphenamine (Salvarsan), but it had many adverse effects (15). The first commercially available antibiotic was sulfonamide (Prontosil), which was discovered by Gerhard Domagk in 1932 (16). Also, as early as 1928, Alexander Fleming (17) found that the fungus *Penicillium* had an antibacterial effect, but it was not until Howard Florey and Ernst Chain (18) developed penicillin in 1940 and after World War II that the first ß-lactam antibiotic became available on the market. During the 1950s and 1960s, a massive investigation of soil samples from all over the world was launched to identify active compounds. Actinomycetes (especially subspecies of the genus *Streptomyces*) were found to be some of the most valuable microorganisms for producing antibiotic agents, and a typical pharmaceutical company at that time performed research on as many as 100 000 different actinomycetes in single a year (15).

In 1943, Albert Schatz (19) discovered the first aminoglycoside, streptomycin, which also proved to be the first anti-infective agent that could provide protection against tuberculosis. The polymyxines were detected in and derived from soil bacteria in 1947 (20), and erythromycin was discovered in soil samples from the Philippines in 1949 (21). Azithromycin, clarithromycin, and the ketolides were obtained through further development of erythromycin. Also, nitro groups were introduced into furans

that had been used in the 1940s, and this led to nitrofurantoin, which was put on the market in the 1950s (15). In the late 1940s, Benjamin Minge Duggar discovered chlortetracycline, and Burkholder and colleagues found chloramphenicol in one out of 7000 samples collected in Caracas, Venezuela (22, 23). In the mid 1950s, vancomycin was isolated from an organism found in soil samples in Borneo, and it was introduced on the market in 1958 (24). Rifamycin was discovered in 1957 and named after a French movie (Rififi), and metronidazole was presented in 1959 (25, 26).

The sodium salt of fusidic acid (brand name Fucidin) was developed by Godtfredsen at Leo Laboratories in Denmark, and it was introduced in clinical practice in 1962 (27). The same year, lincomycin was found in a soil organism in Nebraska in the United States, and Lesher identified the first quinolone nalidixicacid among the by-products of chloroquine (28). In the late 1960s, Bushby and Hitchings synthesized a sulfonamide potentiator called trimethoprim, and, when it was combined with sulpha, its antibiotic effect became bactericidal (co-trimoxazole) (29). In 1969, Hendlin et al. discovered a new cell-wall-active antibiotic produced by several *Streptomyces* species, and this agent was first called phosphonmycin but later renamed fosfomycin (30). Walter Gregory and co-workers at Dupont synthesized oxazolidinones that were registered in 1978, but it took an additional 25 years of investigation before they had a useful drug on the market (31). Since then, only a few classes of antibiotics have become commercially available, among them the glycylcycline (tetracycline analogue) tigecycline, which was introduced in 2005 and launched the same year, and in 2012, the microcyclic antibiotic fidaxomicin, which was obtained from Actinomycetes. Fidaxomicin has a bactericidal effect on *Clostridium difficile* infections (32, 33). The discovery of antibiotics is considered to be one of the most valuable findings related to human health.

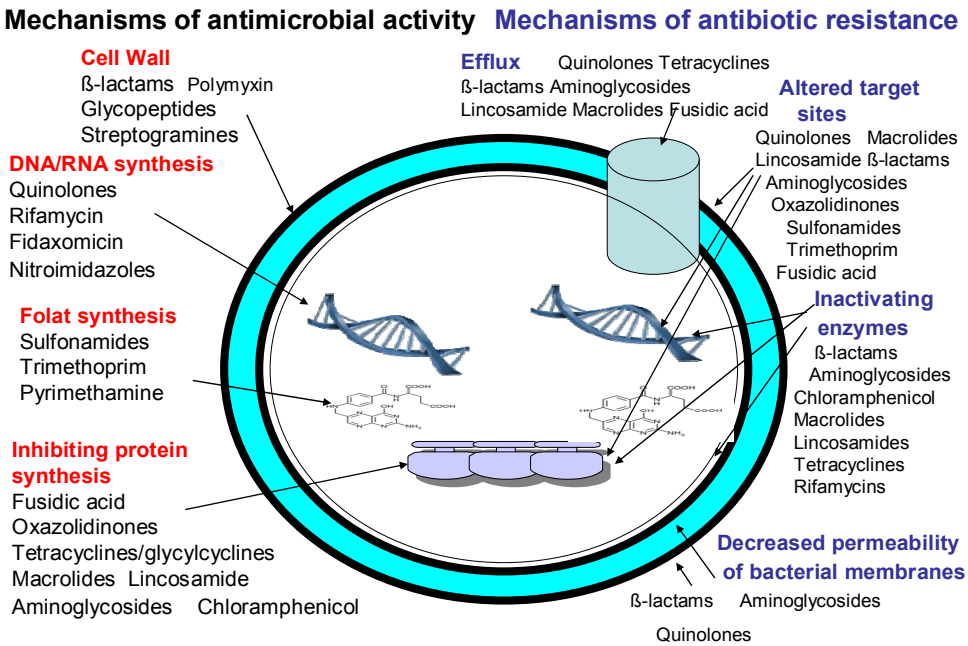

Figure 1: Mechanisms of antimicrobial activity and of antibiotic resistance

1.3 ß-lactam antibiotics

The bactericidal effect of ß-lactam antibiotics involves inhibition of cell wall synthesis, and this effect occurs through covalent attachment to penicillin-binding protein (PBP), which is a peptidoglycan transpeptidase enzyme that catalyzes the final steps in cell wall formation. Damage of the bacterial cell by hydroxyl radicals also plays a role in this process, but the exact mechanism is still somewhat unclear. Several PBPs have been identified, and they are unique to bacteria. Furthermore, the spectrum and effects of the different ß-lactams are determined by the PBPs to which these antibiotics bind (34-36).

The first successful clinical treatment with penicillin was achieved in 1930 by Cecil George Paine at the Sheffield Royal Infirmary, when he used Fleming droplets to treat gonococcal ophthalmia neonatorum (conjunctivitis in newborns). Paine did not publish his results, but many years later (i.e., in 1983) when his discovery was made public, he said "I was a poor fool who didn't see the obvious when placed in front of me". American companies started to produce penicillin G, whereas the British produced penicillin F (37). In Austria, Brandl and Margreiter (38) found the more acid-stable penicillin V, which represented the first active penicillin for oral

administration. Ampicillin and amoxicillin (α -aminopenicillins), two penicillin derivates with greater acid stability and a better Gram-negative effect, were developed by Beecham. Beecham also prepared methicillin in 1959 and nafcillin in 1960, two additional penicillin derivates that were much more stable against the ß-lactamases, and these were soon followed by the compounds flucloxacillin and dicloxacillin, which displayed even greater acid stability.

Carbenicillin and ticarcillin were introduced in 1967 and 1973, respectively, and they became the first antibiotics to be useful in treating *Pseudomonas aeruginosa* infections, because they were more stable against class C ß-lactamases. Temocillin was subsequently developed from ticarcillin, and it was also stable towards class A ß-lactamases but showed no activity against *P. aeruginosa* (37).

In 1977, Toyama developed the ureidopenicillin called piperacillin, which could more easily penetrate the cell envelope of *Pseudomonas spp* (39). In 1972, Leo Laboratories synthesized the penicillin analogue mecillinam; this agent had good Gram-negative activity but exhibited some what inferior bioavailability, and hence it was also necessary to develop the orally active prodrug pivmecillinam to enhance the bioavailability (40, 41).

In 1945 in Cagliari, Italy, Brotzu isolated the fungus *Cephalosporium acremonium* from seawater close to a sewage outlet while studying an outbreak of typhoid fever. Further work on extracting and producing the active substance from *C. acremonium* was done by Abrahams at Florey's laboratory in Oxford, England, and it became apparent that the side chain in the ß-lactam molecule influenced the antibacterial effect. It was possible to extract penicillin N from the fungus, and it had a stronger Gram-negative effect than penicillin. Among the degradation products of penicillin N, Abraham and Newton found cephalosporin C, which proved to be more stable than the penicillins against the bacterial ß-lactamases (42). This discovery ultimately led to the semi-synthetic production of four generations of cephalosporins.

The first semi-synthetic cephalosporins (cephaloridine and cephalothin) were introduced in the mid 1960s (43, 44), and they showed a somewhat limited Gram-negative effect but good activity against penicillinase-producing S*taphylococcus aureus*. Compared to penicillins, it proved easier to modulate the cephalosporins in order to alter their antimicrobial activity, especially to improve their effect on Gram-negative bacteria, and thus many such agents have appeared on the market since the introduction of the first cephalosporin. The system used to produce cephalosporins is based on differences in microbial activity. Therefore, when synthesis of the second generation of cephalosporins was started at the beginning of the 1970s, efforts were made to expand coverage to include an impact on Gram-negative bacteria in addition to the Gram-positive effect. Cefuroxime was introduced in 1984, and it had an enhanced ability to penetrate the blood and brain barrier, which meant that it could be used instead of benzylpenicillin or ampicillin as the initial treatment in cases of meningitis (45).

The third generation of cephalosporins (also known as oxyimino-ß-lactams) included compounds such as cefotaxime (1979), ceftazidime (1980), and ceftriaxone (1981), which offered extended coverage of Gram-negative bacteria and even better ß-lactamase stability. In part, these cephalosporins were developed because of the discovery of narrow-spectrum ß-lactamases (e.g., TEM-1), and some of them also had good oral bioavailability, as exemplified by ceftibuten (1989), which could be used for oral treatment of pyelonephritis. The Gram-negative effect was extended even further in the fourth generation of cephalosporins. Thereafter, a better Gram-positive effect was gained in the fifth generation, and this even applied to methicillin-resistant *S. aureus* (MRSA), and hence these agents are also called MRSA-active cephalosporins (46).

The cephamycins are another group of antibiotics that were developed in the 1970s. These agents proved to have the same antimicrobial effect as the second-generation cephalosporins but were stable against class A ESBLs (47).

At the end of the 1960s, Beecham and Merck used *Streptomyces* to develop the carbapenems, which were found to be highly resistant to enzymatic hydrolysis. In all, more the 50 carbapenems have been found, but many of them are too unstable to use (37). The first carbapenem on the market was imipenem, which was discovered in 1979 and introduced in 1984, and it had to be combined with cilastatin to protect it against renal dehydropeptidase. The second generation of carbapenems included meropenem (1996), ertapenem (2001), and doripenem (2007), all three of which are resistant to renal dehydropeptidase. Moreover, meropenem can penetrate the blood and brain barrier, and it has a better Gram-negative effect but is considered less effective against Gram-positive bacteria such as enterococci. The carbapenems are also known to be the only antibiotics that have some degree of post-antibiotic effect on infections with Gram-negative bacteria (41, 48, 49).

The first monocyclic bacterially produced ß-lactam antibiotics were described in 1979 and were later named monobactams (e.g., aztreonam). Monobactams have a good Gram-negative effect but no useful Gram-positive effect, and they are stable towards several ß-lactamases (41).

In 1977, nature once again became useful when scientists identified the ß-lactamase inhibitor clavulanic acid in *Streptomyces*, and this compound is used primarily in combination with amoxicillin. Other agents in this group include sulbactam, which has good activity against class A ß-lactamases, and tazobactam, which is active against some class C ß-lactamases. Tazobactam is available in combination with piperacillin, which makes it useful as a broad-spectrum antibiotic with good effects on both Gram-negative and Gram-positive bacteria (8, 15, 37).

1.4 Antibiotic resistance

Since the majority of antibiotics have evolved in different microorganisms over millions of years, it can be assumed that in most cases the resistance to antibiotics is no doubt just as old (50, 51). In the battle that microbes have to fight in this context, the ability to adapt to the environment is a crucial aspect, and the same applies to other competitor organisms. Antibiotics and antibiotic resistance are a part of the eternal contest between different microorganisms. There are several mechanisms of resistance, which can be divided into subgroups as follows, according to the actions involved (52): (i) decreased permeability of bacterial membranes; (ii) antibiotic efflux; (iii) altered target sites; (iv) inactivating enzymes (Figure 1).

Antibiotic	Discovery	Antibiotic resistance identified	Bacteria
Salvarsan	1909	1917	*Treponema pallidum*
Sulfonamide	1932	1939	*S.pneumoniae*
Penicillin	1928	1940	*E.coli*
		1942	*S.aureus*
		1965	*S.pneumoniae*
Methicillin		1961	*S.aureus*
Cephalosporin 3:d generation	1978	1983	*K.ozaneae*
Streptomycin	1943	1946	*E.coli*
Tetracycline	1948	1959	*S.dysenteriae*
Vancomycin	1956	1987	*E.Facecium*
Erytromycin	1952	1959	*S.aureus*
Quinolones	1962	First time In Scandinavia 1990	*E.coli*

Figure 2: Antibiotic discovery and the identification of resistance.

1.5 ß-lactamases

The ß-lactamases are the collective name of enzymes that open the ß-lactam ring by adding a water molecule to the common ß-lactam bond, and this inactivates the ß-lactam antibiotic from penicillin to carbapenems. This hydrolyzation was first observed in 1940 by Abraham and Chain (penicillinase) in a strain of *E. coli* (53). However, the clinical effect of such hydrolyzation was not noted until the beginning of the 1950s, when the first ß-lactam-resistant *S. aureus* isolates appeared in hospitals (54, 55).

The ß-lactamases in *S. aureus* are found in the chromosomes and are often inducible, whereas the first plasmid-mediated ß-lactamase was detected in Gram-negative bacteria in Greece in the 1960s and was designated TEM after the name of the patient (Temoneira) who carried the pathogen (56). TEM-1 is the most common β-lactamase in Gram-negative bacteria, and it can hydrolyze penicillins (ampicillin). The ß-lactamases also quickly spread to other bacteria, and soon, after changes in only one or a few amino acids, these enzymes were able to hydrolyze narrow-spectrum cephalosporins and were found in Enterobacteriaceae, *Neisseria gonorrhoeae*, and *Haemophilus influenzae (57)*. Compared to the TEMs, the sulphydrylvariable (SHV) ß-lactamases are similar in biochemical structure but are more common in *Klebsiella* spp. The third-generation cephalosporins were stable against hydrolysis by the original TEMs and SHVs.

1.6 ESBLs

In 1983, Knothe (58) found a single nucleotide mutation in an SHV that represented the first plasmid-encoded ß-lactamase that could hydrolyze the extended-spectrum cephalosporins in an isolate of *K. ozaenae*, and this type was named SHV-2. Outbreaks of primarily *Klebsiella spp* with mutated TEM and SHV enzyme derivates were reported from French hospitals at the end of the 1980s, and, to distinguish these enzymes from broad-spectrum ß-lactamases (mainly TEM-1, TEM-2, and SHV-1), the term extended-spectrum ß-lactamase (ESBL) was coined by Philippon in 1989 (59, 60). ESBLs are defined as ß-lactamases that have the following characteristics: they are transferable; they can hydrolyze penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins); they can be blocked *in vitro* by ß-lactamase inhibitors such as clavulanic acid. The ß-lactamases are usually categorized according to the Bush-Jacoby-Medeiros functional classification system (ESBL = 2be) or the Ambler structural classification (ESBL = class A) (61, 62). Most ESBLs can be divided into three groups, which are designated the TEM (approx. 200 variants), SHV (over 140 variants), and CTX-M (approx. 130

variants) enzymes (http://www.lahey.org/studies/). In the beginning of the ESBL era, the clinical isolates consisted of the TEMs and SHVs (mainly SHV-2 and SHV-5), which were also found predominantly in *Klebsiella spp*. in hospital outbreaks.

Members of the CTX-M group are now the most common ESBLs worldwide. The first CTX-M was described in Japan in 1986 by Matsumoto et al. (63), who found this enzyme in a laboratory dog used for pharmacokinetic studies of ß-lactam antibiotics. In Germany in 1989, Bauernfeind et al. obtained an *E. coli* isolate that was resistant to cefotaxime and produced a non-TEM–non-SHV enzyme, which they named CTX-M-1 due to its elevated activity against cefotaxime (64). The CTX-M enzymes are natural ß-lactamases that are produced by *Kluyvera spp*., and they are found in the chromosomes of those bacteria and have also been transferred to a plasmid that carries these enzymes (65). The CTX-M enzymes can be classified into five major groups, which are designated CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. Each of these includes several plasmid-mediated enzymes. For example, the CTX-M-1 group comprises CTX-M-15 and several other types (66). A new classification scheme with better clinical availability was recently proposed and is now in use in Sweden. This system stipulates the following:

$ESBL_A$	ß-lactamases of the established classical class A (and class 2be) ESBLs
$ESBL_M$	miscellaneous ESBLs (plasmid-mediated AmpC and OXA-ESBLs)
$ESBL_{CARBA}$	ESBLs with hydrolytic activity against carbapenems

This classification system was not in use at the time the present studies were conducted, and therefore any isolates producing ß-lactamases other than those in the $ESBL_A$ class were not included. Whenever the acronym ESBL is used in this thesis, it signifies the $ESBL_A$ enzymes. This wider definition has not yet become internationally established (67).

AmpC β-lactamases are very common, and they are usually chromosomally encoded and are inducible in many Gram-negative bacteria, such as *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *P. aeruginosa*, and *Hafnei alvei*. In *E. coli*, AmpC β-lactamases show poor antibiotic resistance if they are expressed by the chromosomal genes, whereas they can be just as resistant as ESBLs if they are carried on plasmids. AmpC β-lactamases are not inhibited by clavulanic acid, but by oxacillin (13). The OXA ß-lactamases have been found in *Pseudomonas aeruginosa* (and also in *E. coli*); they are not as common as the other ß-lactamases, but they can hydrolyze oxacillin or oxacillin-related antibiotics. Other ESBLs that have been described are PER-1 and -2, VEB-1 and -2, the GES/IBC family, TLA-1, BES-1, and SFO-1 (68).

The carbapenems have the broadest spectrum of all the β-lactams and they are considered to be the last line of therapy because they have remained stable, even against ESBLs. Some bacteria (e.g., *Stenotrophomonas maltophilia*) have inducible chromosomally encoded carbapenemases, although the plasmid-mediated carbapenemases now constitute the "rising star" of the ß-lactamases. Many different carbapenemases have been identified. The IMiPenem (IMP) carbapenemases were first detected in *Serratia marcescens* and *P. aeruginosa* in Japan at the beginning of the 1990s (69, 70). The *Klebsiella pneumoniae* carbapenemases (KPCs) were found in the United States in 1996 (71) and the Verona integron-encoded metallo-β-lactamase (VIM) carbapenemases in Italy in 1999 (72). The carbapenemase that is most widespread today was detected in 2008 in a Swedish patient who had recently travelled to India, and this enzyme was named the New Delhi metallo β-lactamase (NDM-1) (73, 74).

1.7 The plasmids and transfer of genes

Plasmids are linear but usually circular replicons of extrachromosomal DNA in bacterial cells. In general, these structures can be regarded as "hitchhikers" in *E. coli* and other bacteria, and they play an important role in the evolution of these microbes. Plasmids are mobile genetic mosaics that spread multiple traits (e.g., drug resistance and virulence) that are beneficial to the bacteria and are indeed necessary for rapid adaptation to any changes in their environment.

Plasmids are found in most bacteria, and they can vary in size and are often self-transferable. ESBLs are spread by plasmids (this is part of the definition of these enzymes). Horizontal transfer of genes in a microbial ecosystem can occur through three different mechanisms (75-77):

Transformation	direct uptake of naked DNA
Conjugation	transfer of DNA from a donor cell to a recipient cell after plasmid-mediated contact (e.g., through a sex pilus)
Transduction	bacteriophage-mediated transfer of DNA between bacteria

1.8 The global epidemiology of ESBLs

The epidemiology of ESBLs is quite complex. First, there are several different levels to consider: the wider geographical area, the country, the hospital, the community, and the host (in most cases a single patient or a healthy carrier). Furthermore, there are the bacteria (*E. coli* is more endemic, and *K. pneumoniae* is more epidemic) and

their mobile genetic elements, usually plasmids. In addition, there are numerous reservoirs, including the environment (e.g., soil and water), wild animals, farm animals, and pets. The final component entails transmission from food and water, and via direct or indirect contact (person to person) (78-81).

The first ESBL to be identified was found in Germany in 1983, but it was in France in 1985 and in the United States at the end of the 1980s and the beginning of the 1990s that the initial nosocomial outbreaks occurred (82). Soon thereafter, it was discovered that many of the *K. pneumoniae* strains that caused nosocomial infections in France in the early 1990s were ESBL producers (60).

It appears that the spread of ESBL-producing bacteria is greater in developing countries than in nations with more substantial economic resources. Some plausible reasons for this difference include the following conditions that are prevalent in low-income countries: crowded hospitals, more extensive self-treatment and use of non-prescription antimicrobials, poorer hygiene in general and particularly in hospitals, as well as less effective infection control (83-88). Furthermore, only very limited data have been collected regarding the prevalence of these microbes over time in the developing countries and some other parts of the world, because very few investigations have examined this aspect. Nonetheless, a number of studies have been published in recent years that have given a somewhat clearer picture of the situation.

The specific uropathogenic *E. coli* clone ST131, which has been associated with carriage of the ESBL CTX-M-15 and quinolone resistance, has probably contributed to the successful spread of the ESBL-expressing bacteria around the world (6, 89).

Sweden

From an international perspective, the use of antibiotics, especially broad-spectrum agents, is limited in Sweden (90). Furthermore, compared to other European countries, the prevalence of ESBLs in bacteria isolates from blood has long remained relatively low in Sweden, found in less than 3 % of *E. coli* and *K. pneumoniae* strains, although this proportion is increasing (86). Since February 2007, clinical laboratories are required to report all cases involving ESBL-producing Enterobacteriaceae strains to the Swedish Institute for Communicable Disease Control, and the number of such cases increased by 100% from 2008 to 2011 (91). In recent years, there have also been larger nosocomial outbreaks of clonal ESBL strains: one at a neonatal care unit with ESBL-related mortalities, a large outbreak in Uppsala involving *K. pneumoniae* with CTX-M-15, and in Kristianstad caused by a multiresistant CTX-M-15-producing *E. coli* strain (7, 92, 93).

According to data from the European Antimicrobial Resistance Surveillance System (EARSS), 2.6% of *E. coli* and 1.7% of *K. pneumoniae* strains in Sweden were resistant to third-generation cephalosporins in 2010 (86). As in most parts of Europe, the following enzyme types were found: CTX-M group 1 predominated (67%)

followed by CTX-M group 9 (27%), although other types have also been found, for example CTX-M group 2 and TEM- and SHV enzymes (94-96).

Europe

Until the late 1990s, most ESBLs were found in *K. pneumoniae* in nosocomial outbreak situations, mainly in intensive care units (ICUs), and they were primarily SHV and TEM enzymes. New TEM and the SHV enzymes are still evolving in Europe, and specific epidemic clones have been found, for example *Salmonella* isolates with TEM-52 in Spain (97) and *E. coli* and *K. pneumoniae* isolates with SHV-12 in Italy (98). There are marked differences in the prevalence of ESBL-producing Enterobacteriaceae in Europe. According to EARSS data for 2010 concerning only invasive isolates (86), the resistance to third-generation cephalosporins in *E. coli* isolates at that time varied from the above-mentioned 2.6% in Sweden to 25% in Bulgaria, and the corresponding rates for *K. pneumoniae* ranged from the 1.7% noted in Sweden to the incredibly high levels of 75.6% and 74.6% in Bulgaria and Greece, respectively (Fig 3). Isolates with the CTX-M-9 group are common in Spain and strains with the CTX-M-3 enzymes have been described chiefly in Eastern Europe, although clones producing CTX-M group 1 (including the CTX-M-15 type) are the most widespread throughout Europe (66, 89, 99, 100).

Today, *E. coli* and the CTX-M enzymes are not uncommon in outpatients. Furthermore, the resistance shown by *K. pneumoniae* has reached a higher level with emergence of carbapenemases such as OXA-48, which was first found in Turkey (101).

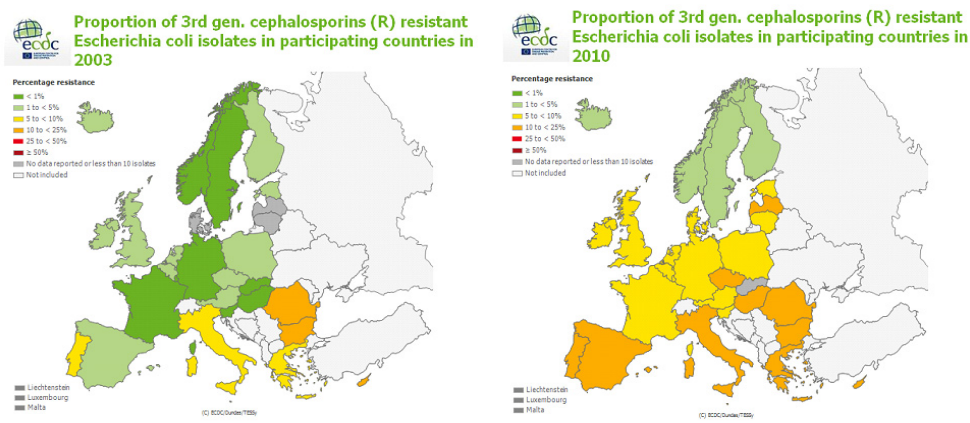

Figure 3: Resistant *E.coli* to third generation cephalosporins from 2003 and 2010 with permission by ECDC. Some of the data should be interpreted carefully since not all of the data from each country is reported.

Africa

As in Europe, reports in the literature have described outbreaks of ESBL-producing *K. pneumoniae* in South Africa (102, 103). Unfortunately, few investigations have been conducted in sub-Saharan Africa, and they have provided very little data (104-107). The first study of ESBLs in Tanzania was performed in 2001–2002 and analysed blood isolates from neonates, and it was found that 25% of the *E. coli* and 17% of the *K. pneumoniae* produced ESBLs, mainly the CTX-M-15 and TEM-63 types (108). In a more recent investigation conducted at a tertiary hospital in Mwanza, Tanzania, the overall prevalence of ESBLs in all Gram-negative bacteria (377 clinical isolates) was 29%. The ESBL prevalence was 64% in *K. pneumoniae* but 24% in *E. coli* (109). Dramatic figures were also obtained in a small study at an orphanage in Mali, where 63% of the adults and 100% of the children were found to carry ESBL-producing Enterobacteriaceae that showed extensive co-resistance to other antibiotics (110). Furthermore, in Madagascar, Herindrainy et al. (88) observed that 10% of non-hospitalized patients carried ESBLs, in the majority of the cases CTX-M-15, and these investigators also found that poverty was a significant risk factor for carriage.

The Middle East

The overall data on ESBL-producing Enterobacteriaceae in the countries of the Middle East are extremely worrisome, and this region might indeed be one of the major epicentres of the global ESBL pandemic. Most of the available data concern isolates from hospital inpatients, and only a limited amount originates from the community. In a study of *E. coli* isolates collected at five hospitals in Egypt in 1999–2000, it was found that 38% were resistant to third-generation cephalosporins (111). In addition, another investigation conducted in that country in 2001 showed that 61% of *E. coli* produced ESBLs of the CTX-M-14, CTX-M 15, and CTX-M 27 types, and all of strains harboured the TEM enzyme (112). No data have been published regarding the community prevalence of ESBLs in Egypt.

In a study of inpatients in Saudi Arabia in 2008, Tawfik and colleagues (113) found that 26% of *K. pneumoniae* isolates produced ESBLs, the majority of which were SHV-12 and TEM-1 enzymes, and 36% were CTX-M-15. Another investigation conducted in the same country in 2004–2005 showed that 10% of clinical urinary *E. coli* isolates from inpatients and 4% of such isolates from outpatients were ESBL producers (114). Moubareck and colleagues (115) analysed faecal samples in Lebanon in 2003 and noted that ESBL carriage differed somewhat between patients (16%), healthcare workers (3%), and healthy subjects (2%), and also that there was a predominance of the CTX-M-15 enzyme (83%). Other researchers in Lebanon (114) observed that the proportion of ESBL-producing isolates was significantly larger among inpatients (15.4%) than in outpatients (4.5%). Moreover, data collected over three years in Kuwait showed that the levels of ESBLs were lower in community

isolates of *K. pneumoniae* (17%) and *E. coli* (12%) than in the corresponding hospital isolates (28% and 26%, respectively) (116).

Six-year surveillance data (2001–2006) on bacteraemic patients in Israel indicated that the prevalence of ESBL-producers changed by around 3–9% among *E. coli* isolates and increased among *K. pneumoniae* isolates, especially those obtained from hospital-acquired infections (31%)(117). In addition, there have been large outbreaks of *K. pneumoniae* caused by strains producing KPC in Israel (118) and OXA-48 in Morocco (119), which perhaps gives us a glimpse of what can be expected in the future.

Asia

Only lately have we begun to understand the extent of the ecological disaster related to ESBL-producing Enterobacteriaceae in parts of Asia and the Indian subcontinent, and the number of reports of very high frequency of such bacteria in those regions continues to rise. It is likely that some of the successful ESBL-producing clones originate from Asia. Deficient sewage routines (the "Delhi belly") and poor quality of drinking water, in combination with a lack of control over prescription and sales of antibiotics, are probably major factors that have promoted the development of resistance. The United Nations has estimated the population of Asia to be 4.2 billion in 2012, and hence it is a very challenging task to try to stop the growing resistance to antibiotics stemming from this part of the world as exemplified by the rapid spread of the carbapenemase NDM-1 (120). A few articles published as early as the end of the 1980s and the beginning of the 1990s have reported occurrence of the SHV-2 and Toho-1 (CTX-M-44) enzymes in China and Japan (85). According to the SENTRY surveillance program there have been rapid increases in ESBL-producing *K. pneumoniae* (up to 60%) and *E. coli* (13–35%) in different parts of China, with a predominance of the CTX-M-14 and CTX-M-3 enzymes (85, 121).

The first report of CTX-M-producing Enterobacteriaceae in New Delhi was published in 2001 (122). Later, in 2006, Ensor et al. (123) found that 66% of third-generation cephalosporin-resistant *E. coli* and *K. pneumoniae* from three medical centres in India harboured the CTX-M-15 type of ESBL, which was also the only CTX-M enzyme found, and an investigation of 10 other centres in that country showed that rates of ESBL-producing Enterobacteriaceae reached 70% (124). In other recent studies, Sankar et al. (125) observed ESBL rates of 46% and 50% in out- and inpatients, respectively, and Nasa and co-workers (126) detected ESBL production in almost 80% of clinical isolates. Investigations from India and Pakistan shows an alarming and rapid increase in the prevalence of Enterobacteriaceae with NDM-1 with prevalence rate from 6.9% in a hospital in Varanasi, India, to 18.5% in Rawalpindi, Pakistan (73, 127) and perhaps the spread of these enzyme could be even more rapid than the spread of the CTX-M enzymes.

As early as the mid 1990s, it was noted that 25% of the Enterobacteriaceae in Thailand were producing ESBLs, mainly different SHV enzymes (85). Luvsansharav et al. (128) analysed stool samples from healthy volunteers in Thailand in 2009, and the results showed that 30–50% of these subjects in three different regions were ESBL carriers (CTX-M types). Reports from other parts of Asia have indicated a variety of ESBLs, such as VEB enzymes (the Vietnamese ESBLs), and have also shown a significant increase in ESBL-carrying Enterobacteriaceae in both out- and inpatients, as well as in stool samples from healthy volunteers (85).

Latin America

Although there are some differences between countries, the highest prevalence of ESBL-producing *K. pneumoniae* in the world is seen primarily in Latin America, where approximately 50% of the bacterial isolates harbour ESBLs, and the rate varies from 8% to 18% for *E. coli* (129, 130). Non-typhoidal *Salmonella* with the CTX-M-2 enzyme spread from Argentina to neighbouring countries from 1989 onwards. Furthermore, the CTX-M-2 enzymes have been found in *Klebsiella* isolates in Paraguay and in *E. coli* and other Enterobacteriaceae in Argentina, and in 2002 75% of the ESBLs in the latter country were from the CTX-M-2 group. As in other parts of the world, the SHV-5 (1982) and SHV-12 ESBLs have been found in South America, and the TEM-10 enzyme has been observed in Argentina (84). Data from 33 centres in Latin America collected over the period 2004–2007 within the Tigecycline Evaluation and Surveillance Trial (TEST) showed ESBLs in 36.7% of *K. pneumoniae* isolates and in 20.8% of 932 *E. coli* isolates (131).

North America

Some of the earliest outbreaks of ESBL-producing bacteria were published in 1989 and concerned *K. pneumoniae* with TEM-10 (132), and new outbreaks involving other TEM enzymes (TEM-12, and TEM-26) were soon to follow (133). A large variety of different types of SHV have also been described. One extensive outbreak of Enterobacteriaceae producing the CTX-M-14 enzyme occurred in Calgary, Canada (134). In a large study performed in 2001, it was demonstrated that about 5.3% of the *E. coli* in the United States harboured ESBLs (129), and an investigation conducted in 2009 showed that 9% of *E. coli* isolates at a cancer centre in Texas were ESBL producers (135). Sanchez et al. (136) investigated data obtained from The Surveillance Network (TSN) concerning *in vitro* antimicrobial resistance in US outpatients between 2000 and 2010, and their results showed that resistance to ceftriaxone rose from 0.2% to 2.3% and resistance to cefuroxime increased from 1.5% to 5%, but the bacterial isolates in focus were not tested for ESBLs.

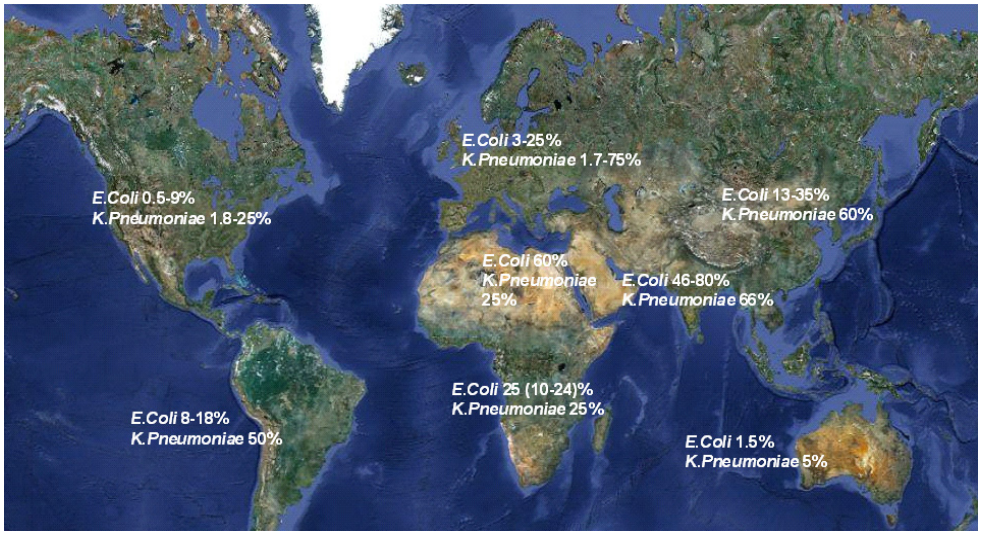

Figure 4: ESBL prevalence in *E.coli* and *K.pneumoniae* from different studies and from EARSS

1.9 Travellers' diarrhoea

What is known as travellers' diarrhoea (i.e., watery stools at least three times a day) affects 20–60% of the more than 900 million people travelling to different parts of the world each year (according to the UNWTO). This type of diarrhoea is most prevalent in the underdeveloped countries, and the course of the condition is usually mild and of short duration. Travellers' diarrhoea is caused mainly by faecally contaminated food and water, although the microorganism involved depends on the traveller's destination. Various enteropathogenic *E. coli* strains (e.g., ETEC and EAEC) are often identified, and *Campylobacter* is a more frequent cause in Asia according to some studies (137, 138). Therefore, traditional rules such as "cook it, boil it, or forget it" are of great importance. Drug prophylaxis or antibiotic treatment can almost never be recommended (139). In trials of pivmecillinam conducted at the beginning of the 1980s, Stenderup et al. (140) showed that the *E. coli* flora changed completely in tourists during travel, and a highly multiresistant *E. coli* flora was acquired in Mexico, Egypt, and the Far East both in the placebo group and in the pivmecillinam group (140-142). Among those who visited India, 47 out of 65 (73%) had diarrhoea during their stay in that country (143). There is no published evidence that ESBL-producing *E. coli* causes diarrhoea.

1.10 Risk factors for colonization and infections with ESBL-producing Enterobacteriaceae

Numerous studies have identified risk factors for acquiring and being infected with ESBL-producing Enterobacteriaceae and most of them have focused on risk factors in health care but there are some important risk factors (ex. household contacts) in the community as well (144). Transmission of such bacteria usually occurs via the faecal-oral route, either directly or indirectly through hand contact with healthcare workers, and it is facilitated by overcrowding. Historically, *K. pneumoniae* and treatment in ICUs are associated with many of the risk factors, which include various medical devices such as central and arterial access lines, and nasogastric and endotracheal tubes. Other risk factors mentioned in the literature are prolonged hospital stays, living in nursing homes or long-term care facilities, underlying medical conditions, recent surgery, haemodialysis, and also prior use of antibiotics, particularly quinolones and third-generation cephalosporins, but also co-trimoxazole, aminoglycoside, and metronidazole (68, 92, 145, 146). However, the positive predictive value of these risk factors is low (147). Plasmids encoding quinolone-resistant ESBLs and other ESBLs in general have been identified around the world, and the association between the use of quinolones and development of ESBLs is often referred to as "collateral damage" that is an adverse ecological effect of antibiotics (148, 149).

Compared to *E. coli*, it is often assumed that *Klebsiella* is more successful in disseminating, and the latter bacteria are now the most abundant. In a study by Harris et al. (150, 151), it seemed that, despite a larger reservoir of *E. coli* at ICU admission, there was more extensive transmission of *K. pneumoniae*. Over the past few years our research group (152) and other investigators (153) have found evidence that international travel to highly endemic areas (i.e., Asia, the Middle East, and Africa) represents one of the most important risk factors for ESBL carriage especially in the community.

1.11 Infection control

"Abstinence is the best medicine" -Indian Proverb.

Measures to limit the emergence of ESBL-producing Enterobacteriacae in the hospital and the community can be divided in actions for limiting the spread of the ESBL-producing Enterobacteriacae and actions to reduce the selection pressure trough use of antibiotics. Unfortunately, no controlled prospective studies have focused on infection control of antibiotic-resistant Gram-negative bacteria (154), although the literature does contain retrospective investigations and descriptions of outbreaks of these species (155-160). The most important measure to prevent spread

is adherence to good hand hygiene. In the 1840s, Semmelweis demonstrated this. Much later, Casewell and Phillips (9) described an outbreak of an aminoglycoside-resistant *K. pneumoniae* strain that occurred in 1977 and their results allowed them to clarify how gastrointestinal colonization and cross infections can be mediated via hand contact between patients. Kaier et al. (161) conducted a study in Germany in 2005–2007 on ESBLs and they were able to confirm that hand disinfection is the key to preventing nosocomial infections with ESBL-producing bacteria, and they also found that more extensive use of alcohol-based hand rub was associated with a lower incidence of such strains.

The risk of cross transmission is higher in *K. pneumoniae* than in *E. coli*, and the reservoir of *K. pneumoniae* can in some cases be environmental (151, 162). Hence ways for limiting the chance of spread through the environment are: implementation of barrier precautions, control of environmental causes and various types of equipment (e.g., bronchoscopes, gels, and cloths), putting patients with ESBL-producing Enterobacteriacae in single rooms and/or cohort isolation, and to avoid overcrowding (65, 159). Knowledge of whether a patient is a carrier of an ESBL-producing Enterobacteriacae or not enables the health care personal to take action and hence screening programmes and journal alert systems are of importance. It has been reported that outbreaks are more often caused by ESBL-producing *K. pneumoniae* than by ESBL-producing *E. coli* (7), and this indicates that health care personal has to show extra attention around patients with ESBL-producing *K. pneumoniae* in the hospital setting.

Several studies have shown that use of antibiotics produces a selection pressure giving the resistant bacteria an advantage in relation to the sensitive bacteria. Many different antibiotics have been blamed for "benefitting" the ESBL-producing Enterobacteriaceae. For example Kaier et al. (161) found that the use of cephalosporins and quinolones "was a driving factor in the emergence of the spread of ESBLs". Some studies have shown that restrictions on the use of cephalosporins and fluoroquinolones helps to decrease the selective pressure, but also that substitution of antibiotics might lead to development of other forms of resistance, as exemplified by imipenem-resistant *P. aeruginosa (163)*. In the United Kingdom, there has been a dip in the prevalence of ESBLs in *E. coli* and *K. pneumoniae*, and a plausible explanation for this might be the massive changes in prescribing of antibiotics that have been made in order to reduce *Clostridium difficile* infections (http://www.bsacsurv.org) (164).

Finally, when indicated in cases involving resistance, antibiotic prophylaxis should be directed to the patients' actual intestinal flora (165, 166).

1.12 Duration of carriage of ESBL-producing bacteria

A systematic search of the literature will reveal only a few studies concerning the duration of faecal carriage of ESBL-producing bacteria, particularly when considering periods longer than 6 months. Tangden et al. (153) investigated a group of healthy volunteers who were potentially exposed to ESBL-producing bacteria during travel outside Northern Europe, and the results showed that five of 21 (24%) were colonized with such bacteria at 6-month follow-up. By comparison, eight of 24 subjects (33%) in a study carried out in Thailand remained colonized after 6 months (167), and this some what higher incidence might have been due to broad dissemination of CTX-M-producing Enterobacteriaceae in the healthy population (128). In France, Tandé et al. (168) observed that the mean duration of carriage of ESBL-producing *E. coli* was 9 months (range 1–15) in a group of 22 adopted children from Mali. However, in Australia, In contrast, Kennedy et al. (169) studied travellers who were predominantly asymptomatic after being abroad for 3 months and found that only 4% of were colonized with *E. coli* resistant to third-generation cephalosporins, whereas at least 18% were colonized with other antibiotic-resistant *E. coli* strains.

As part of a surveillance program in Germany, Buehlmann and colleagues (170) investigated a group of 123 patients who were colonized or infected with ESBL-producing bacteria, and noted that the clearance of these microbes was only 6.8% after three years. Similarly, Asterlund et al. (171) investigated an outbreak of ESBL-expressing *E. coli* that occurred in Kristianstad, Sweden, in 2005–2006, and their results showed that in some cases the duration of carriage was longer than 30 months. Data on these patients recorded in September 2010 showed the following: five of the 42 patients still carried the bacteria after a median of 58 months (range 41–59 months); 18 had repeatedly had negative cultures after shedding bacteria for a median of 7.5 months (range 0–39 months); 16 had died after having shed the bacteria for a median of nine months (range 0–38 months). In France, Zahar et al. (172) studied the risk factors associated with persistence of faecal carriage of ESBL-producing Enterobacteriaceae at the time of readmission in a group of 62 patients previously identified as carriers of these bacteria. Thirty-one (50%) of the 62 were found to be positive at readmission. Subsequent screening at different time points demonstrated that the numbers of patients positive for ESBLs were as follows: 13 out of 21 (62%) within the first three months; 10 out of 19 (53%) within three to six months; seven out of 14 (50%) within six months to one year; one out of eight (13%) after one year. Furthermore, the mean duration of faecal carriage was 179 +/– 166 days, and it was also found that closer time to readmission and transfer from a step-down unit were independent risk factors for persistent faecal carriage.

1.13 Treatment of patients infected with Enterobacteriaceae producing ESBLs (ESBLA)

"Frapper fort et frapper vite" -Paul Ehrlich, address to the 17th International Congress of Medicine, 1913

In low-endemic countries such as Sweden, treatment of infections with ESBL-producing bacteria is often delayed due to the use of empirical antibiotics with a "narrower" spectrum. Another problem when treating patients with these infections is that the plasmids carrying the ESBL gene often have additional mechanisms that give rise to co-resistance to many other antibiotics (173). The clinical efficacy of the treatment does not always reflect the *in vitro* susceptibility to antibiotics. Until 2009, an approach related to the resistance mechanism was applied, and ESBL-producing bacteria have been reported to be resistant to all cephalosporins. Both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the American Clinical Laboratory Standards Institute (CLSI) have subsequently revised the minimal inhibitory concentration (MIC) breakpoints, and the clinical results obtained using these new breakpoints show better correlation with susceptibility than with the resistance mechanism.

Carbapenems are considered the first choice for treatment of patients infected with ESBL-producing Enterobacteriaceae, especially in cases involving severe septicaemia or septic shock (174). The available data are too limited to support therapy with tigecycline. Treatment with a ß-lactam/ß-lactamase inhibitor (e.g., piperacillin-tazobactam) might be used as a carbapenem-sparing regimen when the susceptibility results are known. However, the emergence of CTX-M-15-producing bacteria also frequently leads to production of OXA-1- ß-lactamase, which is worrisome and renders the ß-lactam/ß-lactamase inhibitor ineffective (175). Pivmecillinam, fosfomycin, and nitrofurantoin often show sensitivity in vitro but has some what uncertain effect in vivo and these antibiotics might be suitable alternatives in patients with mild infections (176-180), and polymyxins and temocillin might be considered in special cases (181).

1.14 ESBLs in relation to mortality, length of hospital stay (LOS), and the economic burden

"The drugs don't work" The Verve

It is essential that the correct empirical antimicrobial therapy be given to patients with severe infections, especially if they are hypotensive (182). In Essex, England, Meltzer and Jacobsen (183) performed a prospective study of patients with *E. coli* bacteremia, and, after adjustment for other risk factors, they found a significantly higher mortality

rate in the patients whose *E. coli* were ESBL producers. Delay in appropriate antibiotic therapy was associated with a higher mortality rate, and it was more common in the ESBL-carrying cohort.

In 2007, Schwaber and Carmeli (184) conducted a meta-analysis of 16 studies focused on comparing ESBL-producing and non-ESBL-producing Enterobacteriaceae, and the results revealed an almost twofold increase in mortality and five times more often a delay in appropriate antibiotic therapy in the ESBL-positive patients. However, some of the studies included in the meta-analysis did not show any such difference, but the mortality rates were too low to provide statistical significance (i.e., 20% and 34% for the patients carrying non-ESBL producers and ESBL producers, respectively). Nearly the same results have been obtained in many other investigations, for example, a multicentre case-control study published by Qureshi et al. (185) demonstrated that inadequate antibiotic therapy and mortality were higher in the patients infected with ESBL-producing strains of both *K. pneumoniae* and *E. coli.*

Considering that the above-mentioned studies showed that inadequate empirical therapy led to longer LOS, and LOS has the greatest impact on the costs of bacteraemic episodes, it is not surprising that the presence of ESBLs raises the economic burden in this context (186, 187). In addition, the clinicians have to use more expensive antibiotic therapies, and, due to the high rate of co-resistance, it is not unusual that patients require parenteral therapy during the entire antibiotic regimen. Lautenbach (188) found that infections with ESBL-producing *E. coli* and *K. pneumoniae* entailed a 2.9 times higher cost compared to infections with non-ESBL-producing strains, and other studies have indicated 1.5–1.7 times higher costs (189, 190). In 2007, the costs related to resistant bacteria were estimated to be an incredible €900 million in the European Union, and since then the rates have risen even further, and bacteria exhibiting even more problematic resistance have been found.

1.15 ESBL-producing Enterobacteriaceae in food

Antibiotics are used for various purposes in agriculture and livestock production, for instance to boost growth or as therapeutic treatment and disease prophylaxis. *E. coli* and some other Enterobacteriaceae colonize the intestinal tract of both animals and humans, and the number of studies describing the prevalence of ESBL-producing Enterobacteriaceae has increased rapidly around the world. A recent investigation in the Netherlands identified the same CTX-M type in chicken meat as found in humans (80), and ESBL-producing Enterobacteriaceae have been detected in cattle, chickens, pigs, raw milk, and lettuce. (191-193). Most of the studies on this subject have been conducted in developed countries, but the major epicentres of ESBL-expressing bacteria are located in Asia, Africa, and the Middle East, and only a few studies have

provided support for the theory that food from these regions constitutes a reservoir of ESBLs.

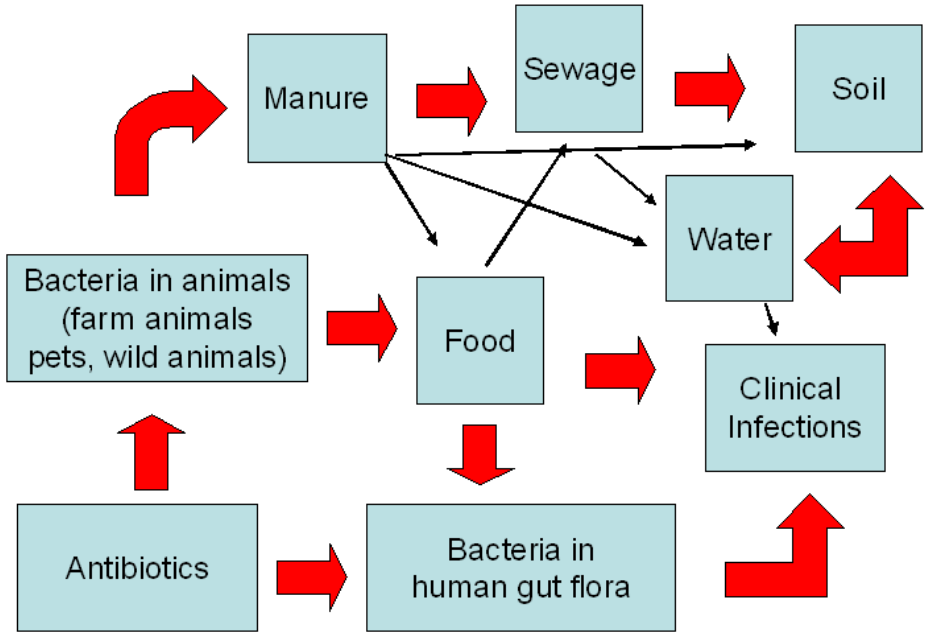

Figure 5: Antibiotic resistance flow chart in bacteria and the environment

2 Aims of the studies

The aims of the present studies were as follows:

To assess the prevalence of ESBL-producing Enterobacteriaceae in patients with travellers' diarrhoea and to identify the ESBL enzymes they harboured and to study the duration of colonization with ESBL-producing *E. coli* in these patients.

To investigate the prevalence of ESBL-producing bacteria in patients treated in different types of hospital wards and in a group of healthy volunteers, and to determine whether the ESBL prevalence changed over time.

To identify different risk factors for developing an infection with ESBL-producing *E. coli* in a low-endemic country.

To ascertain whether certain food products imported to Sweden from other countries (particularly in the Mediterranean area) could constitute a possible reservoir of ESBL-producing bacteria.

To analyse Swedish chicken and beef products to determine whether any ESBL-producing bacteria could be detected in locally produced meat.

3 Materials and Methods

3.1 Overview of the present studies

Paper I: A descriptive study of the prevalence of ESBL-producing *E. coli* in patients with travellers' diarrhoea.

Paper II: A cross-sectional comparative study of faecal ESBL carriage in the community and in a hospital setting.

Paper III: A prospective cohort study of the duration of colonization with ESBL-producing *E.coli* in patients with travellers ' diarrhoea.

Paper IV: A cross-sectional study of the prevalence of ESBL-producing Enterobacteriaceae in food.

Paper V: A case control study of risk factors for infections with ESBL- producing *E.coli.*

3.2 Papers I and III

All of the bacterial isolates in these two studies (Papers I and III) were analysed at the Department of Medical Microbiology Laboratory at Skåne University Hospital in Malmö, which serves a population of about 470 000 people. The first study (Paper I) was performed between October 2007 and January 2008, and the follow-up investigation (Paper III) was carried out in October 2010. The patients who were included in the initial study had travellers' diarrhoea and, during the stipulated study period, delivered stool samples to the Clinical Microbiology Laboratory in Malmö for diagnosis of *Salmonella*, *Shigella*, *Yersinia*, or *Campylobacter* (SSYC). The stool samples were examined for ESBL-producing Enterobacteriaceae. Information on foreign travel was obtained from the referrals.

Fifty-eight of the 242 of the patients had ESBL-producing *E. coli* in their stool samples, and these 58 were included in the follow-up study. During the follow-up, the patients were asked to specify whether they had taken any antibiotics or if they had been abroad. All faecal samples were collected and submitted by the patients themselves. None of the patients received any compensation for participation.

3.3 Paper II

The results reported in Paper II were obtained in a two-part cross-sectional comparison study performed at Skåne University Hospital in Malmö and three primary health care units (PHCUs) in March to April 2008 and April to June 2010. The hospital wards that were included were chosen on the basis of local hospital pharmacy data. Wards at departments with high proportions of patients treated with antibiotics were selected, and these departments included the areas of infectious diseases (three wards), surgery (three wards), urology (two wards), oncology, and haematology, as well as the intensive care unit (ICU). The haematology department and the ICU were not included in the follow-up carried out in 2010, because the former facility had been moved to another hospital, and the number of patients treated at the ICU was considered to be too low during the follow-up period. Considering the PHCUs, one was located in the city of Malmö and two were in smaller communities a few miles outside Malmö; only one of the latter two PHCUs was included in the follow-up. All participants were required to be at least 18 years of age, and they had to give written consent after receiving standard verbal and written information about the study. No patients were included more than once.

Nurses at the hospital wards were supplied with the written information and the consent forms to give to the patients. At each of the hospital wards, the time allowed for enrolling patients in the study was one or two weeks, depending on the number of co-operating nurses available. The nurses ensured that all patients who were already being treated at or were newly admitted to their wards during the study period were given information about the investigation and were asked to participate. For any patients who were unable to give written consent or receive information (e.g., those with dementia and some critical cases at the ICU), this aspect was handled by a relative.

For participants at the PHCUs, the exclusion criteria were admission to a hospital during the last 3 months, recent antibiotic use, and consultations regarding infections. Patients in doctors' and laboratory waiting rooms at the PHCUs were asked to participate in the study, as were any relatives of the patients who were present and staff members. Each person who was interested in taking part was given the standard verbal and written information about the investigation and the sampling procedure by one of the authors. At each PHCU, enrolment of participants was done on two days in 2008 and two days in 2010.

3.4 Paper IV

During the winter of 2007–2008, a total of 419 swab samples were collected from a variety of retail foods: 385 from imported products and 34 from products of domestic origin. The foods were obtained from six different local supermarkets in the Malmö

area. Each food specimen was swabbed with a sterile medical gauze pad, which was then put in peptone water. If possible, the sampling pads were sent directly to the Department of Clinical Microbiology Laboratory in Malmö, but, if transport was delayed, they were refrigerated overnight.

We also examined 99 *E. coli* strains collected from Swedish meat and 94 *E. coli* isolates from unspecified foods provided by a Swedish food-testing laboratory. These isolates were identified in accordance with methods described by the Nordic Committee on Food Analysis (NMKL, 144).

The samples collected from food and the *E. coli* isolates were inoculated on 32-agar plates (selective medium for Gram-negative rods) on which the samples could be analysed qualitatively without pour plating. We used a standard identification procedure to differentiate Enterobacteriaceae. The specimens were also inoculated on plates with medium selective for cephalosporin resistance (ChromID ESBL, BioMerieux^{TM}), and any growth on these plates was further examined for ESBL production by synergy testing with discs containing ceftazidime, cefotaxime, and amoxicillin/clavulanic acid. The 419 swab samples from food were also examined for Gram-negative environmental bacteria such as species of *Pseudomonas*, *Stenotrophomonas*, and *Acinetobacter*.

3.5 Paper V

We performed a case-control survey on material from the Department of Medical Microbiology Laboratory of Skåne University Hospital in Malmö, Sweden, which serves a population of approximately 470 000. We used a computerized database to identify patients diagnosed between January and October 2008 with infections of ESBL-producing *E. coli* in urine or blood, and also an equal number of age- and sex-matched controls infected with non-ESBL-producing *E. coli*. We also developed the survey instrument that was used in the study, which asked both patient groups the same questions regarding different risk factors, such as the following: stomach problems, urinary catheter use, endoscopic procedures, recurrent urinary infections, stomach ulcer medications, hospital stays, antibiotic consumption, comorbid conditions, and trips abroad.

3.6 Microbiological methods

Detection of Enterobacteriaceae and ESBL enzymes

The stool samples in paper I, II and III were inoculated on agar plates containing medium selective for cephalosporine resistant bacteria (ChromID ESBL, Bio-Mérieux TM). Any growth on these plates was further examined for ESBL production by synergy testing with disks containing ceftazidime, cefotaxime, and

amoxicillin/clavulanic acid. The ESBL-producing strains were characterised to the species level by phenotypic tests carried out according to national guidelines (194).

The detection of Enterobacteriacae in paper IV is described above. The urinary and blood samples in paper V were examined for Enterobacteriacae with phenotypic tests according to national guidelines (194). The identified E.coli strains in paper V and the identified Enterobacteriacae in paper IV were screened for cephalosporine resistance with a cephadroxil disk. E. coli strains resistant to cephadroxil were analysed for ESBL production by inoculation on agar plates with medium selective for cephalosporin resistance (ChromID ESBL, BioMerieux^{TM}) and synergy testing with discs containing ceftazidime and cefotaxime and amoxicillin/clavulanic acid (191).

Typing of the ESBL enzymes

A multiplex, real-time TaqMan PCR method was used to detect and characterize the CTX-M genogroups (195, 196). Conventional PCR was conducted using general primers for SHV and TEM enzymes (196).

Typing of the *E. coli* strains

Repetitive sequence-based polymerase chain reaction (rep-PCR) analysis was performed for bacterial strain typing (197) in paper I, II, III, and a difference between the strains was defined as dissimilarities in more than three bands in the lane. The extracted DNA was amplified using the appropriate DiversiLab DNA Fingerprinting kit (Spectral Genomics, Inc., Houston, TX, USA) in accordance with the manufacturer's instructions.

An allele-specific *pabB* PCR was performed to detect O25b-ST131 as described by Clermont et al (198) in paper II.

3.7 Statistics

The statistical methods used in the five studies included analysis of the contingency table (Fisher's exact test) and calculation of 95% confidence intervals (CIs) for the parameters computed by the method devised by Clopper and Pearson. The prevalence of carriage was calculated as the percentage of carriers among the patients in each group. All analyses were performed using GraphPad software. When required, ethical approval for the studies was obtained from the Research Ethics Committee of the University of Lund. In the study reported in Paper V, odds ratios were computed by the Clopper-Pearson method for binomial data and by the Mann-Whitney-Wilcoxon test for continuous data (age). Using statistical power calculations, we concluded that a study population comprising slightly over 100 patients (including those who did not answer) would suffice to obtain significant results. Baseline characteristics of subjects

were summarized using median, range, and frequencies. The prevalence of risk factors was calculated as the percentage of risk factors among patients in each group. Odds ratios (ORs) and 95% CIs were calculated to evaluate the strength of any association that emerged. An unadjusted p-value < 0.05 was considered statistically significant. These analyses were performed using GraphPad software, and the responses in the returned questionnaires were compiled in Excel.

4 Results

4.1 Paper I

During the study period, 242 Swedish patients (124 female, 118 male) who had contracted diarrhoea abroad were included in the investigation, and they had a median age of 40 years (range 7 months to 83 years). The prevalence of faecal carriage of ESBL-producing *E. coli* was 24% (58/242), and there was a high degree of co-resistance to other antibiotics (Table I).

ESBL-producing *E. coli* were carried by 3% (2/63) of the patients who had travelled in Europe but by 36% (50/138) of those who had travelled outside Europe. ESBL-producing *E. coli* strains were especially common in patients who had visited Egypt (50%, 19/38) and Thailand (22%, 8/38). Furthermore, although only 14 persons had travelled to India, 11 of them carried ESBL-producing bacteria (Table II).

Among the ESBL-producing isolates, 90% expressed an enzyme belonging to the CTX-M family. Overall, the CTX-M 1 group dominated, being found in 68% of the isolates, and was the only CTX-M group detected in the isolates from India. The next largest group was CTX-M 9 at 24%. The isolates from Egypt and Thailand produced both CTX-M groups 1 and 9. CTX-M 9 enzymes were also found in the isolates from China, and the CTX-M 1 group was identified in the only ESBL-positive isolate from Spain. The few CTX-M-negative isolates instead harboured SHV or TEM enzymes, and one sample contained both the SHV and TEM enzymes. Furthermore, we found that 15% (7/47) of the patients who were positive for *Salmonella*, *Shigella*, and *Campylobacter* were ESBL positive, and 26% (51/195) of the patients who were negative for those bacteria were also ESBL positive. None of the isolates of *Salmonella* or *Shigella* presented any ESBL phenotype that was detected in the travellers with ESBL-producing *E. coli*. The rep-PCR fingerprint pattern showed that the strains from the same geographical region displayed no genetic similarity, and they also differed from previously studied Swedish *E. coli* isolates.

Table I. Antibiotic resistance (%) among ESBL-producing *E. coli* from stool samples

Antibiotic	Antibiotic resistance in ESBL-producing *E. coli* isolates (%)
Tobramycin	54
Ciprofloxacin	68
Piperacillin-Tazobactam	8
Mecillinam	0
Trimethoprim	91
Trimethoprim-sulfametoxazole	75
Nitrofurantoin	5

Table II. Regions and countries included in the study

Region*	ESBLpositive (n)	ESBL negative (n)	Total (n)	Proportion positive	95% CI	Compared to Europe (P-value)
World	58	184	242	58/242 = 0.24	0.19 to 0.30	
World, excluding Europe and unspecified	50	88	138	50/138 = 0.36	0.29 to 0.45	< 0.0001
Europe, excluding Sweden	2	61	63	2/63 = 0.03	0.004 to 0.11	
Egypt	19	19	38	19/38 = 0.50	0.33 to 0.67	< 0.0001
Thailand	8	28	36	8/36 = 0.22	0.10 to 0.39	0.0042
India	11	3	14	11/14 = 0.79	0.49 to 0.95	< 0.0001
Middle east	4	6	10	4/10 = 0.40	0.12 to 0.74	0.0025
Southeast Asia, including Australia	5	8	13	5/13 = 0.38	0.14 to 0.68	0.0012
Africa, excluding Egypt	2	15	17	2/17 = 0.12	0.015 to 0.36	0.1965 (NS)
America	1	9	10	1/10 = 0.10	0.0025 to 0.44	0.3615 (NS)
Unspecified areas	6	35	41	6/42 = 0.15	0.06 to 0.29	0.0550 (NS)

*Europe (Bosnia, Bulgaria, Denmark, England, France, Germany, Greece, Hungary, Ireland, Italy, Kosovo, Romania, Spain, Turkey, and Ukraine), the Middle East (Kurdistan, Lebanon, Morocco, Iraq, Oman, Saudi-Arabia, Syria, and Tunisia), Africa (Gambia, Ghana, Guinea, Kenya, Tanzania, unspecified countries), Southeast Asia (Afghanistan, Australia, Bangladesh, Cambodia, China, Pakistan, Papua, Philippines, Singapore, and Tahiti), Latin and North America (Argentina, Bolivia, Caribbean, Chile, Mexico, unspecified countries).

4.2 Paper II

A total of 427 participants were enrolled at a university hospital and at primary health care units, PHUCs in Sweden in 2008 and 2010. As expected, there were disparities in the demographic data on the hospital patients and the PHCU patients. The mean and median ages were higher in the hospital patients than in the outpatients. Also, at the PHCUs, younger staff members and relatives were often enrolled in the study, and this also partly explained the greater percentage of female participants at those facilities (Table III). Four (1.9%) of the subjects investigated in 2008 tested positive for faecal carriage of ESBL-producing bacteria; two of those individuals were hospital patients (representing 1.8% of all participating inpatients) and two were PHCU patients (2.1% of all PHCU subjects), and none of them were previously known to be carriers of ESBL. In the follow-up conducted in 2010, 11 subjects (5.0%) were found to be ESBL carriers; eight of them were hospital patients (6.8% of all inpatients) and three were PHCU patients (3% of all PHCU subjects). Moreover, three of those 11 ESBL-positive participants were previously known to be ESBL carriers. The total prevalence of faecal carriage of ESBL-producing bacteria in our study population rose from 1.9% in 2008 to 5.0% in 2010, and the increase was from 1.8% to 6.8% in the hospital patients, and from 2.1% to 3.0% among the PHCU patients. The results of this study show a clear, although not statistically significant, tendency towards ESBL-producing bacteria becoming a growing problem in the community, especially in the hospital setting.

Phylotyping of the ESBL enzymes that were found showed that CTX-M-1 was the predominant phylogroup in our study population, detected in 11 out of the 16 bacterial strains that harboured ESBLs. Four of the 16 strains carried the gene coding for the CTX-M-9 phylogroup, and one strain produced the SHV enzyme, but no TEM enzyme was found.

Epidemiological typing of the ESBL-producing bacteria using the DiversiLab kit showed that none of the bacterial strains were genetically identical. Five (31%) of the 16 ESBL-producing isolates were positive for O25b-ST131 in allele-specific pabB PCR, and all of five of those isolates carried the gene coding for the CTX-M-1 phylogroup.

There was marked variability in the patterns of resistance to tobramycin (seven isolates were resistant) and to trimethoprim–sulfametoxazole and ciprofloxacin (ten isolates were classified as resistant to both, and intermediate resistance was noted in one and three isolates, respectively). Considering piperacillin–tazobactam, 11 of 16 strains were susceptible, four were showed intermediate resistance, and one was resistant. All of the isolates were susceptible to imipenem.

Table III. Demographic data and questionnaire response rates for the study participants in 2008 and 2010

	2008			2010		
	Hospital wards	PHCUs*	Total	Hospital wards	PHCUs*	Total
Participants						
Female *n (%)*	57 (50)	64 (67)	121 (58)	48 (41)	64 (64)	112 (51)
Male *n (%)*	56 (50)	32 (33)	88 (42)	70 (59)	36 (36)	106 (49)
Total *n (%)*	113 (54)	96 (46)	209 (100)	118 (54)	100 (46)	218 (100)
Age in years						
Median *(Range)*	71 (21–98)	46 (21–87)	60 (21–98)	68 (19–94)	55 (20–90)	64 (19–94)
Response rate *% (n)*	59 (113/192)	81 (96/118)	67 (209/310)	61 (118/195)	89 (100/112)	71 (218/307)

*PHCU = primary health care unit

4.3 Paper III

The study presented in Paper III included 58 patients (30 female and 28 male) with a median age of 38 years (range 1–83 years) and faecal carriage of ESBL-producing *E. coli*. Forty-one of the patients submitted stool samples at both of the follow-ups. Seventeen did not complete the study, and three of those patients did not submit any follow-up samples, despite several reminders.

During the first follow-up period (3–8 months)10 of 41 patients (24%) carried ESBL-producing *E. coli*. Six of the patients had the same *E. coli* strain as found at the start of the study, whereas four had *E. coli* isolates with a new rep-PCR fingerprint pattern. In two of the subjects who were a married couple, the husband had become colonized with the same resistant *E. coli* strain as his wife had had from the start of the study.

At the 3-year follow-up, four patients (10%) still carried ESBL-producing *E. coli*. The rep-PCR fingerprint pattern showed that the strains from two of those patients were identical to the strains that those subjects were found to carry at the start of the study, and both of the strains expressed an enzyme belonging to the CTX-M-1 group. One patient had two different new *E. coli* strains producing CTX-M-1 enzymes. Unfortunately, the initial ESBL-producing strain from the fourth patient was lost, and hence it was not possible to determine whether that patient was still colonized with the same strain. It is also regrettable that less than 50% of the patients answered the questionnaire items concerning use of antibiotics and travel abroad.

4.4 Paper IV

Upon culture of the 385 swab samples collected from imported food, 60 (16%) showed no growth of Gram-negative rods, 316 exhibited growth of Enterobacteriaceae, and most of the samples (311) displayed growth of both Gram-negative environmental bacteria and Enterobacteriaceae. None of the Enterobacteriaceae harboured any ESBLs. No significant differences were found between various Mediterranean countries with regard to the amount of food containing Enterobacteriaceae.

Considering food produced in Sweden, we found Enterobacteriaceae mixed with Gram-negative environmental bacteria in 33 of 34 swab samples from chicken, but none of these bacteria harboured ESBLs. Furthermore, no ESBLs were found in 99 *E. coli* isolates collected from beef or in 94 *E. coli* isolates obtained from a Swedish food-testing laboratory.

The number of Enterobacteriaceae isolates was significantly lower ($p < 0.0001$) in air-dried and cured meat products such as ham, sausage, and beef (3/42) than in vegetables (142/157), fresh herbs (24/27), and lettuce (130/134). The air-dried/cured products also contained significantly fewer Gram-negative environmental bacteria (4/42) compared to lettuce (133/134), fresh herbs (27/27), and vegetables (141/147) ($p < 0.0001$).

4.5 Paper V

This study covered a period of 10 months and included 109 patients (median age 65 years, range 2–95 years) suffering from urinary tract infections or bacteraemia caused by ESBL-producing *E. coli*, and 109 controls who were patients (median age 65 years, range 2–95 years) with non-ESBL-producing *E. coli* in urine or blood. Eleven of these 218 subjects were excluded for the following reasons: six moved away and five were deceased. The response rates to the questionnaire did not differ significantly between the two patient groups: 53% (58/109) in the case (ESBL-positive) group and 49% (53/109) in the control (ESBL-negative) group. Also, the participants in the two groups who responded to the survey were well matched with respect to age and sex (Table IV).

The results showed that the patients with ESBL-producing *E. coli* had travelled to Asia (including Turkey) and the Middle East (including Egypt) to a significantly greater extent (14/58; $p < 0.05$) compared to the ESBL-negative group (4/53). In addition, hospitalization during the past year ($p < 0.04$) and especially a hospital stay longer than one month ($p = 0.01$) were found to be risk factors for infection with ESBL-producing *E. coli*, which occurred in eight of 58 of such patients; notably,

three of those eight had been treated with quinolones. None of the patients in the ESBL-negative group had been hospitalized for up to one month. Other risk factors included staying at a surgical department ($p < 0.01$) and receiving treatment during the previous year with antibiotics in general ($p = 0.09$) and quinolones in particular ($p = 0.06$).

We did not find any differences between the ESBL-positive and ESBL-negative groups with regard to civil status, accommodations, diseases (including urinary tract problems), stomach tubes, endoscopies, catheterization, medications, or relatives who had been abroad or had travellers' diarrhoea. It should also be mentioned that 34 of 58 (39%) in the ESBL-positive group were not satisfied with the information they had received from their physicians.

Table IV: Results of the survey of different risk factors for developing an infection with ESBL-producing *Escherichia coli*

Covariate	All = n	ESBL positive, number	ESBL negative, number	OR[1] (95%CI[2])	*p*-value[3]
Response rate (%)	111/218 (51)	58/109 (53)	53/109(49)		
Median age in years (range)	65 (2–95)	65 (2–95)	65 (2–95)		
Female sex (%)	85 (77)	45(77)	40 (75)		
Work or daily setting					
Healthcare	14	7	7		> 0.3
Restaurant	3	2	1		> 0.3
Office	13	6	7		> 0.3
Daycare centre	5	4	1		
Kindergarten/School	10	8	2	4 (0.8–20.2)	0.0970
Co-morbidity					
Gastrointestinal	12	6	6		> 0.3
ENT (ear, nose, or throat)	5	3	2		> 0.3
Urinary tract (including renal diseases)	31	18	13		> 0.3
Heart	22	13	9		> 0.3
Liver	2	2	0		> 0.3
Lung	11	7	4		> 0.3
Diabetes	10	6	4		> 0.3
Cancer	7	4	3		> 0.3
Miscellaneous	15	8	7		> 0.3
Related to endoscopy	49	29	20	1.65 (0.8–3.5)	0.25
Related to urinary tract catheter (including chronic infections)	29	16	13		> 0.3
Recurrent UTIs	41	23	18		> 0.3
Related to anti-ulcer medication (e.g., omeprazol)	42	24	18		> 0.3
Hospitalized during past year	49	31	18	2.2 (1.0–4.8)	0.04

Medical department	20	12	8		> 0.3
Surgical department	17	14	3	5.3 (1.4–19.7)	0.01
Department of infectious diseases	11	7	4		> 0.3
ICU	2	0	2		> 0.3
Length of hospital stay (LOS)					
< 1 w	26	18	8	2.5 (1.0–6.4)	0.07
> 1 w < 4 w	16	6	10		> 0.3
> 4 w	8	8	0		0.01
Antibiotic treatment during past year	62	37	25	2.0 (0.9–4.2)	0.09
Treatment with quinolones	5	5	0		0.06
Foreign travel					
High-risk areas (Middle East, Asia)	18	14	4	3.9 (1.2–12.7)	0.02
Europe	32	16	16		> 0.3
European and other countries	9	6	3		> 0.3
Miscellaneous	4	1	3		> 0.3
Travellers' diarrhoea	10	7	3	2.3 (0.56–9.3)	> 0.3
Adequate information (%)			22/56 (39)		

[1]Odds ratio

[2]95% confidence interval
[3]Result comparing ESBL-positive with ESBL-negative.

5 Discussion

”Vi är precis så blinda som vi vill vara” -Maya Angelou

A great deal has happened since 2007, when we started our research aimed at determining the cause of the rapid increase in ESBL-producing Enterobacteriaceae in patients at our hospital. One of our ideas was that imported food might be a source of these bacteria. Another hypothesis concerned the possibility that multi-resistant bacteria in parts of the world with a known high incidence of such microbes might be transported to other low-incidence countries. At the time we began our studies, almost no data had been accumulated regarding the source of ESBL-producing bacteria. Surprisingly, our initial results clearly demonstrated that an incredible number of ESBL-positive faecal samples were collected from patients who contract travellers' diarrhoea after visiting high-risk areas, but this situation was essentially unknown and unpublished at that time.

In our study of patients with travellers' diarrhoea (Paper I), we found that 24% of the subjects were colonized with ESBL-producing Enterobacteriaceae after trips to foreign countries in 2007–2008, which was a remarkable discovery at that time. Strains of ESBL-producing *E. coli* were especially common in patients who visited Egypt (50%) and India (11/14, 78.5%). None of the *Salmonella* or *Shigella* isolates presented any ESBL phenotype in the travellers carrying ESBL-producing *E. coli*. Antibiotic resistance surveillance data showed a low frequency of ESBL-producing bacteria in Sweden, whereas the incidence was higher (10–70%) in countries in the southern and eastern parts of Europe, the Middle East, and Asia (particularly India) (85, 86, 111, 112, 124, 199). Nonetheless, it was nearly inconceivable that travelling could have such an immense impact on the faecal carriage, especially when considering that global travel is still increasing at the rate of almost a billion passengers a year.

Unfortunately, there is still a lack of well-evaluated surveillance studies of antibiotic resistance and prevalence of ESBL-producing Enterobacteriaceae in the developing countries, although the accumulating data are creating a more comprehensive picture of the situation.

In our study, enzymes of CTX-M type were found in 90% of the ESBL-producing isolates, which was in agreement with contemporary findings from around the world. CTX-M group 1 was the only CTX-M group found in people who had visited India, which was expected considering that CTX-M-15 belongs to the CTX-M-1 group, and studies have shown that this enzyme is the most common ESBL in India (85). In travellers who had been to Egypt and Thailand, both CTX-M groups 1

and 9 were represented among the ESBL-positive samples. CTX-M types 9, 14, and 27 are all part of the CTX-M-9 group and have been found in studies conducted in Egypt and Thailand, along with CTX-M type 15 (which belongs to CTX-M group 1), and this is in accordance with our results. Even though the PCR protocol we used to detect *bla* SHV and *bla* TEM does not discriminate between ESBL variants and narrower-spectrum variants of these enzymes, the identified numbers agree with findings from other contemporary studies in which ESBL enzymes of SHV and TEM type have been represented.

The results reported in Papers I and V showed that Swedish patients who had visited countries outside Europe were more often colonized with ESBL-producing bacteria compared to those who had travelled within Europe. A possible explanation for this difference is that there is a higher frequency of ESBL-producing bacteria in non-European countries than in countries in the southern and eastern parts of Europe. Another plausible reason is that adherence to hand and food hygiene might be lower in the non-European countries, and, since ESBL-producing bacteria are transmitted by the faecal–oral route that would mean an increased risk of being colonized. Follow-up of the same cohort (Paper III) regarding the duration of colonization with ESBL-producing *E. coli* showed that 10 of the 41 patients were positive for such bacteria after 3–8 months, although four of the 10 carried a different strain than the one they had from the start of the study. After 3 years, four of 41 patients still harboured ESBL-producing *E. coli,* although one of those four patients carried two new strains (i.e., different from the initially identified strain).

Our studies of patients with travellers' diarrhoea had some limitations, the most important of which being that these patients were not cultured for ESBL-producing bacteria before going abroad, which would have enabled us to determine whether or not they were colonized during travel. However, other researchers have conducted an investigation in which healthy individuals consulting a vaccination agency in Uppsala, Sweden, were cultured for ESBL-producing bacteria before and after foreign travel, and their results showed that 30% of the subjects had acquired ESBL-producing bacteria during their trip abroad (153). In the cited study, frequencies of ESBL-producing bacteria were particularly high in patients with gastroenteritis and in those who had travelled to India. In our paper II we cultured a group of relatively healthy volunteers (registered at PHCUs) without risk factors for ESBL carriage in 2008 (Paper II) and found that only 2.1% were ESBL positive indicating that the prevalence of ESBL colonization is essentially lower in the general population. This strongly indicates that some of the travellers in our earlier studies (Papers I and III) had indeed become colonized with ESBL producing *E.coli* abroad.

Another limitation of the current investigations is that we did not have complete epidemiological data on all of the patients, and thus we could not rule out possible influence of other risk factors. At the follow-ups, the participants were requested to answer questionnaire items about antibiotic consumption and international travel during the study period. However, the response rate for those questions was less than

50%, and hence we could not ascertain whether these well-known risk factors had an impact on carriage of ESBL-producing bacteria in the respective individuals. It should also be mentioned that the sensitivity of the method would probably have been higher if we had analysed more faecal samples from each patient at each follow-up. In short, it is plausible that some of the cultured samples were falsely negative, because the ESBL strains may have remained subdominant in the colonic flora.

The total prevalence of faecal carriage of ESBL-producing bacteria in the study population described in Paper II was 1.9% in 2008 and 5.0% in 2010. The prevalence increased from 1.8% to 6.8% among the hospitalized participants and from 2.1% to 3.0% among the patients in primary care. This study showed, albeit not statistically significantly, that ESBL-producing bacteria are definitely beginning to represent a growing problem in the community, and especially in the hospital setting. This observation also concurs with the results reported in Paper V, which suggested that a hospital stay of longer than one month, particularly in a surgical ward, can constitute a risk for infections with ESBL-producing *E. coli*. Even though we do not have any evidence of patient-to-patient transmission in our hospital, these findings highlight the need for good infection control and when indicated narrow and rational antibiotic therapy to lower the selective pressure.

We have been among the first researchers in Sweden to study possible occurrence of ESBL-producing Enterobacteriaceae in food (Paper V), and that work did not reveal any such bacteria in either domestic or imported foods. However, we did find significantly more Enterobacteriaceae in lettuce than in air-dried/cured meat products, which was not unexpected, because members of this family of bacteria prefer wet environments. At the time we obtained those results, we felt they were very disappointing, but since then other reports in the literature have described findings of ESBL-producing Enterobacteriaceae in beef, chicken, pork, raw milk, and lettuce (191-193). Most of the published studies on this subject have been conducted in the developed world. For example, in the Netherlands, Leverstein-van Hall et al. (80) found that 94% of representative samples of chicken meat were contaminated with ESBL-producing *E. coli*, and 39% of those bacteria belonged to genotypes that were also found in human samples. A majority of the studies have focused on meat products, and such foods are usually cooked before they are eaten, which will kill any Enterobacteriaceae. This is not the case with fresh lettuce, fruits, and other vegetables, in which we also found significantly more Enterobacteriaceae. We did not analyse samples of food imported from South East Asia, and thus any future studies should focus on foods (especially different types of lettuce) obtained from those regions, because the transfer of ESBL-expressing bacteria from food to humans probably occurs during travelling. According to our results, it appears that food imported from the Mediterranean area or originating from Sweden did not constitute an important vehicle for the dissemination of ESBL-producing Enterobacteriaceae in 2007 and 2008, and, if there is an ongoing spread of such bacteria in imported and domestic food, it must have begun after 2008.

The results of paper I, III and V combined with other published data leads to the conclusion that in patients suffering from severe septicaemia associated with certain risk factors, such as prolonged hospitalization or a history of international travel during the past six months, the empirical antibiotic treatment should include a carbapenem (184). When indicated in cases involving resistance, antibiotic prophylaxis should be directed to the patients' actual intestinal flora (165, 166).

Also, to decrease the selective pressure, a de-escalation of the antibiotic therapy should be considered as soon as possible. Clearly, there is an urgent need to develop new antibiotics. However, until that is achieved, it seems that a rational approach might be to alternate between existing antibiotics and import other antibiotics that are effective against ESBL-producing Enterobacteriaceae (e.g., temocillin) in order to lower the use of carbapenems and thereby avoid selective pressure in other bacteria.

Notwithstanding, the most important factor in this context is probably the need for establishing well-functioning sewage systems in large parts of the world (primarily Asia and Africa). As long as people, especially children, are affected by diarrhoeas, the use of antibiotics (particularly non-prescription varieties) will continue, and this may even be lifesaving in some cases but will usually have no effect at all. Many of the problems could be solved by ensuring that people in the developing world have access to fresh food and water without the risk of faecal contamination. Indeed, if the issue of inadequate sewerage is not solved, Enterobacteriaceae will no doubt rapidly develop resistance to any new and effective antibiotic, although that might be difficult to foretell. The rate at which resistance develops differs between bacterial species, and resistance to the natural antibiotics has probably existed nearly as long as the antimicrobial agents themselves. However, in some cases the process can take longer and is not always successful, but no one knows why (e.g., vancomycin resistance is very rare in *S. aureus*). Thus it is not easy to predict the length of time an antibiotic will remain effective. Nevertheless, most of the resistance or resistant genes against the β-lactam antibiotics are pre-existent in nature, and it seems that this resistance spreads rather quickly, especially among the Gram-negative bacteria. The combination of selective pressure, a virulent *E. coli* strain, and a hitchhiking plasmid is all it takes! The rapid increase and spread of Enterobacteriaceae with NDM-1, especially in the Indian subcontinent and in the Middle East, is a perfect example of how fragile our current antibiotic situation really is and how important it is to continue the development of new antibiotics.

From a historical perspective, target-based screening of chemical antibiotics has not been particularly effective, whereas the development of new antibiotics from a proven source such as actinomycetes has been successful (200). Accordingly, it is time to revive this source, and why not start the search for new antimicrobial agents by assessing actinomycetes found in soil in India?

6 Conclusions

The main finding of our studies is that the overall prevalence of ESBL-producing *E. coli* was surprisingly high in patients with travellers' diarrhoea, particularly those who had visited Asia, India, and the Middle East. There was substantial co-resistance to several other antibiotics, and the majority (90%) of the ESBL enzymes that were detected belonged to the CTX-M family, predominantly the CTX-M-1 group (68%) followed by the CTX-M-9 group (24%).

- The prevalence of ESBL faecal carriage in healthy volunteers from PHCUs were at the same level as in patients with UTI caused by *E.coli* in Sweden. However the prevalence in hospitalised patients was at least twice as high which is of great importance for preventive measures and antibiotic therapy policies.

- We found a high prevalence of ESBL faecal carriers, especially in the hospital setting, but also in healthy volunteers from PHCUs.

- Our results showed that it is not uncommon for patients diagnosed with travellers' diarrhoea to continue to be ESBL carriers and to acquire new ESBL-producing strains.

- We did not find any ESBL-producing Enterobacteriaceae in food imported from the Mediterranean area or produced in Sweden in 2007–2008. This indicates that if any spread of ESBL-expressing bacteria is occurring via imported or domestic food today, it must have begun after 2008.

- We found that travelling to high-endemic areas such as Asia, the Middle East, and Turkey was a significant risk factor (with an unadjusted p-value < 0.05) for contracting a urinary tract or bloodstream infection with ESBL-producing *E. coli*. Our findings also suggest that hospitalization for longer than one month, especially in a surgical ward, is a risk factor for infections with these bacteria.

7 Svensk Sammanfattning (Summary in Swedish)

Bland infektionssjukdomarna är Enterobacteriaceae den viktigaste gruppen av bakterier. Den kan orsaka ett flertal olika infektioner så som blodförgiftning, urinvägsinfektion, hjärnhinneinflammation, bukhinneinflammation, gallvägsinfektioner, lunginflammation och diarrésjukdomar. Den vanligaste orsaken till blodförgiftning är bakterier som har kommit in i blodet från urinvägarna (så kallad urosepsis) och de orsakas vanligen av bakterier från gruppen Enterobacteriaceae (t.ex. *E.coli och K.pneumoniae*). Det är viktigt att behandla dessa infektioner med effektiv antibiotika för ett flertal av dessa infektioner är dödliga om de inte behandlas.

Det är mycket troligt att både de flesta antibiotika och de flesta resistensmekanismer har utvecklats under miljontals år som en följd av konkurrensen mellan mikroorganismer i jord, växter och i haven. Antibiotika och olika resistensmekanismer är en del av den ständiga kampen och konkurrensen mellan arter för att kunna dominera en ekologisk nisch.

De mest använda och effektiva antibiotika är sprunget ur Alexander Flemings upptäckt penicillin och kallas för betalaktamantibiotika. Utanför Cagliari i Italien isolerade Brotzu fram en svamp, Cephalosporium acremonium, från havsvatten nära ett avlopp 1945 när han studerade ett utbrott av tyfus. Ur denna svamp kunde sedan Chain och Abraham i England extrahera fram den första cefalosporinantibiotika, cefalosporin C. Cefalosporinerna har visat sig vara mycket effektiva i behandlingen av många olika sorters infektioner allt från livshotande infektioner som hjärnhinneinflammationer och blodförgiftningar till lindriga urinvägsinfektioner och övre luftvägsinfektioner orsakade av gramnegativa eller grampositiva bakterier.

I början av 1980 talet kom den tredje generationens cefalosporiner som sågs som ett genombrott i kampen mot betalaktamaserna (enzymer som bryter ned antibiotika). Redan 1983 kom tyvärr den första rapporten om enzymer som kunde hydrolysera (bryta ned) även dessa antibiotika. Enzymerna var genetiskt mycket snarlika (skiljer 1-4 mutationer) några av de betalaktamaser som hade upptäckts på 1960-talet (SHV-1, TEM-1 och 2) och benämndes Extended-Spectrum beta-lactamases (ESBL). ESBL definieras vanligen som betalaktamaser som kan hydrolysera första, andra och tredje generationens cefalosporiner, penicilliner samt aztreonam, men som kan inhiberas av klavulansyra in vitro.

Frekvensen av Enterobacteriaceae med ESBL har ökat kraftigt under de senaste åren. Från att 2004 varit ett relativt okänt begrepp har patientfallen med ESBL-

producerande Enterobacteriaceae blivit allt fler. Hos *E.coli* och *K.pneumoniae* som förvärvat ESBL finner man framförallt enzymerna ur klassen CTX-M (CefoTaXimase München). Endast i Skåne har vi över tusen vårddygn varje år p.g.a. ESBL producerande-bakterier. Många av ESBL-producerande Enterobacteriaceae är även resistenta mot andra antibiotika såsom kinoloner och aminoglykosider. Detta leder till stora behandlingssvårigheter. Konsekvenserna av den ökade resistensen orsakar att patienter får felaktig antibiotikabehandling, med ökad mortalitet som följd och även ökade kostnader samt behov av fler isoleringsrum. Det enda effektiva empiriska preparatet som har effekt på ESBL-producerande Enterobacteriaceae är karbapenemer. Ökad användning av karbapenemer kommer med all säkerhet att leda till ökad karbapenemresistens hos andra bakterier (exempelvis Pseudomonas aeruginosa). Man har även de senaste åren noterat en spridning sista året av karbapenemresistenta Enterobacteriaceae från Indien.Därför är det viktigt att klargöra vilka riskfaktorer som finns dels för att man bättre skall kunna ge rätt behandling från början, dels för att förstå epidemiologin av betalaktamaser. Därigenom ökar chanserna att kunna förutspå hur nya betalaktamaser (som exempelvis NDM-1) kan komma att spridas globalt.

Antibiotikaförskrivningen i Sverige är låg med internationella mått mätt. Däremot har inte antibiotikaföreskrivning i andra delar av världen (t ex södra delen av Europa och Sydostasien) varit lika välreglerad. Där har man sedan flera år haft en mer uttalad antibiotikaresistens och också ökad förekomst av Enterobacteriaceae med ESBL. Man har kunnat visa i studier att upp till 60 % av *E.coli* och *K.pneumoniae* isolaten har haft ESBL i Indien och Mellanöstern.

I två av våra studier har vi tittat på prevalensen av ESBL-producerande tarmbakterier hos patienter som har sökt med diarréer efter utlandsresor (turistdiarréer). Vi fann överraskande höga siffror hos de patienter som hade vistats utanför Europa (36 %) jämfört de som vistats inom Europa (3%). Värst drabbade blev de som hade åkt till Mellanöstern (50 %) och Indien (11 av 14 patienter). Vi följde den här gruppen och fann att frekvensen med tarmbakterier med ESBL avtog med tiden, till ca 25 % som var positiva efter 3-8 månader och till ca 10 % efter tre år.

I vår andra studie undersökte vi hur vanliga ESBL-producerande bakterier är på olika sjukhusavdelningar jämfört med hur vanliga de är ute i samhället. Hos relativt friska personer från vårdcentraler mellan år 2008 till 2010. Vi tog faecesprover på 427 personer. Vi fann att prevalensen 2008 var 2,1 % (2/96) hos de relativt friska individerna och 1,8 % (2/117) hos sjukhuspatienterna. År 2010 hade antalet personer med ESBL-producerande bakterier ökat till 3 % (3/100) bland de relativt friska invånarna och till 6,8 % (8/118) bland sjukhuspatienterna. Detta var en snabbare ökning än vad vi hade förväntat även om den inte var signifikant.

För att ta reda på om importerade livsmedel från Medelhavsområdet kunde utgöra en reservoar och en spridningskälla för ESBL-producerande bakterier så tog vi 419 prover på olika livsmedelsprodukter varav 385 var från Medelhavsområdet och 34 av inhemskt ursprung. Vi analyserade även 99 *E.coli* stammar tagna från svenskt

nötkött samt 94 *E.coli*-stammar från ett svenskt livsmedelstestlaboratorium. Vi hittade Enterobacteriaceae i 349 av 419 livsmedelsprover. I de flesta av dessa prover växte även gramnegativa miljöbakterier. Vi hittade inga ESBL-producerande bakterier, däremot fann vi som ett bifynd att lufttorkade livsmedel innehöll signifikant färre Enterobacteriacae än sallader och grönsaker. Denna studie stödjer inte teorin att importerat livsmedel bidrar till spridningen av ESBL-producerande bakterier.

I vår sista studie tog vi två åldersmatchade grupper där ena gruppen hade konstaterad ESBL-producerande *E.coli* och den andra gruppen hade *E.coli* utan ESBL-produktion från odlingar av urin eller blod. Vi fann 109 patienter i vardera grupp under januari till oktober 2008. Ungefär lika många svarade på enkäten i båda grupperna (kring 50 %). De med ESBL-producerande *E.coli* hade signifikant oftare ($p < 0.05$) rest i riskområden (Asien och Mellanöstern). Vi fann också att de som hade *E.coli* med ESBL-produktion signifikant oftare ($p<0,04$) hade vistats på sjukhus senaste året och framför allt vistats på sjukhus under längre tid, > 1 månad ($p=0.01$), jämfört med kontrollgruppen.

För att tackla de problem som den ökade förekomsten av ESBL-producerande tarmbakterier innebär måste man säkra god hygien på sjukhus och vårdinrättningar. Dessutom måste man bara förskriva antibiotika till dem som behöver det. Vidare måste vid misstanke om att en svårt sjuk patient är infekterad av en ESBL-producerande tarmbakterie ge antibiotika täckning för denna bakterie så att patienten inte löper ökad risk att dö. De patienter som bör få bredare antibiotikabehandling är de patienter med känt ESBL-bärarskap, en resehistorik (åtminstone sista halvåret) till Asien, Afrika eller till Mellanöstern och de som har vårdats på sjukhus i mer än en månads tid, särskilt om de har fått flera antibiotika behandlingar.

Då karbapenemer är vår sista effektiva antibiotika mot dessa resistenta bakterier är den mycket snabba spridningen och ökningen av karbapenemresistenta tarmbakterier (Enterobacteriaceae med NDM-1) från de befolkningstäta områdena på den Indiska subkontinenten djupt oroväckande. Konsekvensen av spridningen kommer att leda till en betydande mortalitetsökning i svåra infektioner!

Lösningen på de resistenta bakterierna är inte bara att framställa nya antibiotika. Man måste också komma åt roten till det onda annars kommer vi att förlora nästa antibiotikageneration också. Därför måste man ordna så att utvecklingsländer och länder i Asien och Afrika får tillgång till friskt vatten och livsmedel samt välfungerande avloppssystem.

8 Summary in English

”Så ska det stå på registreringsskylten Toys…… leksak…. på engelska”

-Zlatan Ibrahimovic, Blådårar 2

In human medicine, the most important family of bacteria is Enterobacteriaceae, which includes genera and species that cause well-defined diseases and also nosocomial infections. These bacteria can give rise to numerous infections such as septicaemia and urinary tract infections, as well as pneumonia, cholecystitis, cholangitis, peritonitis, wound infections, meningitis, and gastroenteritis. It is important to combat these infections with effective antibiotics, because many of them are associated with high mortality rates if left untreated.

As early as 1928, the first ß-lactam antibiotic was found when Alexander Fleming (17)realized that the fungus *Penicillium* had an antibacterial effect. In 1945 in Cagliari, Italy, Brotzu isolated the fungus *Cephalosporium acremonium* from seawater close to a sewage outlet during an outbreak of typhoid fever. Further work on extracting and producing the active substance cephalosporin C was done by Abrahams at Florey’s laboratory in Oxford, England. This discovery ultimately led to the semi-synthetic production of four generations of cephalosporins, which have become the global cornerstone of treatment of both mild and life-threatening Gram-negative and Gram-positive infections. The majority of antibiotics have evolved in different microorganisms over millions of years as the result of competition for survival in soil, plants, and the oceans, and thus most resistance to antibiotics is presumably just as old. The collective name ß-lactamases denotes the enzymes that inactivate ß-lactam antibiotics ranging from penicillin to carbapenems. Extended-spectrum beta-lactamases (ESBLs) are defined as ß-lactamases that are transferable, can hydrolyze penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not cephamycins), and can be blocked *in vitro* by ß-lactamase inhibitors (e.g., clavulanic acid). The most common ESBLs today are members of the CTX-M group, which have created a worldwide epidemic of high co-resistance against other antibiotics.

In an international perspective, Sweden has limited use of antibiotics, especially broad-spectrum agents, and the prevalence of ESBLs in bacteria isolates from blood is low (<3% of *E. coli* and *K. pneumoniae* strains), although rapidly increasing. In infections with ESBL-producing bacteria in low-endemic countries, the initial empirical antibiotic treatment will probably be ineffective and thus lead to higher

mortality if the infection is severe. Therefore, it is important to identify patients that are at risk of such infections and also to avoid spreading the bacteria.

The present research aimed to determine the cause of the rapid increase in ESBL-producing Enterobacteriaceae in patients in our hospital and our community. We hypothesized that imported food could be a source of these bacteria and also that multi-resistant bacteria might be transported from high-incidence to low-incidence countries. Our initial results clearly demonstrated that huge numbers of ESBL-positive faecal samples were collected from patients who contracted travellers' diarrhoea after visiting high-risk areas. Twenty-four percent of patients with travellers' diarrhoea were colonized with ESBL-producing Enterobacteriaceae after trips to foreign countries in 2007–2008 (Paper I), a remarkable discovery at that time. Strains of ESBL-producing *E. coli* were especially common in patients who visited Egypt (50%) and India (78.5%). Follow-up of the same cohort (Paper III) regarding duration of colonization with ESBL-producing *E. coli* showed that 10/41 patients were positive for such bacteria after 3–8 months, although four of the 10 carried a different strain than the one initially identified. After three years, 4/41 patients still harboured ESBL-producing *E. coli,* although one of those four carried two new strains. This immense impact of travelling on faecal carriage is noteworthy, especially considering that global travel is still increasing at the rate of almost a billion passengers annually.

The total prevalence of faecal carriage of ESBL-producing bacteria in the study population (Paper II) was 1.9% in 2008 and 5.0% in 2010. The prevalence increased from 1.8% to 6.8% among hospitalized participants and from 2.1% to 3.0% among patients in primary care. These observations distinctly demonstrate that ESBL-producing bacteria represent an emerging problem in the community, particularly in hospitals. The findings also concur with our results suggesting that hospitalization longer than one month, especially in a surgical ward, constitutes a risk for infection with ESBL-producing *E. coli* (Paper V). Despite lack of evidence of patient-to-patient transmission in our hospital, the described findings highlight the need for good infection control and, when indicated, narrow and rational antibiotic therapy to lower the selective pressure. The present data (Papers I, III, and V), together with other published studies, support the use of empirical antibiotic treatment including a carbapenem in cases of severe septicaemia associated with risk factors such as prolonged hospitalization or a history of international travel during the past six months. To decrease the selective pressure, the antibiotic therapy should be de-escalated as soon as possible.

We are among the first to study possible occurrence of ESBL-producing Enterobacteriaceae in food in Sweden (Paper V). We found no such bacteria in either domestic or imported foods. However, significantly more Enterobacteriaceae were detected in lettuce than in air-dried/cured meat products, probably because bacteria of

this family prefer wet environments. Initially, these results seemed very disappointing, but later reports have confirmed ESBL-producing Enterobacteriaceae in beef, chicken, pork, raw milk, and lettuce.

The rapid increase and spread of the carbapenem-resistant NDM-1-producing Enterobacteriaceae, especially in India and the Middle East, plainly illustrates that the current antibiotic situation is very precarious, and that it is essential to continue development of new antibiotics. Novel antibiotics have been successfully derived from proven sources (e.g., actinomycetes), and hence those sources should be revived. However, until that is achieved, it might be advisable to alternate between use of existing antibiotics and import of other antibiotics that are effective against ESBL-producing Enterobacteriaceae in order to lower the use of carbapenems and thereby avoid selective pressure on other bacteria.

The most important factor in this context is probably the need for establishing well-functioning sewage systems in large parts of the world (primarily Asia and Africa). Ensuring that people in developing countries have access to fresh food and water without the risk of faecal contamination would solve many problems. Indeed, if adequate sewerage is not accomplished, it is plausible that Enterobacteriaceae will rapidly develop resistance to any new and effective antibiotics.

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…….(Fyll i ditt namn här)

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