Chapter 1

General introduction and outline of the thesis
ESBL: what does it stand for?

ESBL stands for “extended-spectrum β-lactamase” and is a mechanism of antimicrobial resistance in *Enterobacteriaceae* causing resistance against β-lactam antibiotics: penicillins and cephalosporins. The resistance mechanism is coded by many different genes which all are located on mobile genetic elements called plasmids. This poses the complexity of ESBL; ESBL is not a specific bacterium with a specific resistance gene; but describes a group of bacteria containing at least one of many plasmids with one of many ESBL-genes.

*Enterobacteriaceae* are Gram-negative, non spore-forming rods, which ferment glucose and other sugars, reduce nitrate to nitrite but do not produce oxidase. They grow under aerobic and anaerobic conditions.1 *Enterobacteriaceae* live in the gut of various animals and humans but they are also widely spread in the environment. Some *Enterobacteriaceae*, like *Salmonella* spp., *Shigella* spp. and enterohemorrhagic *Escherichia coli*, are known for their human pathogenicity. Others, like other *E. coli* and *Klebsiella* spp. are colonizers of the human gut.2 Nevertheless, even these colonizers can cause infection. Urinary tract infections are mostly caused by the patient’s own intestinal flora, but these commensals can cause virtually every infection possible.3 The ESBL-phenotype is found in all *Enterobacteriaceae* species, but is of most clinical significance in *E. coli* and *Klebsiella* spp. because they are most frequently causing human infections.

Plasmids are small, extrachromosomal, mobile DNA elements. They contain genes coding for antimicrobial resistance to major classes of antimicrobials. Plasmids also contain virulence factors and elements which promote stability and horizontal transfer of the plasmid.4 One plasmid can contain resistance genes for several antimicrobial classes, and one bacterium can contain more than one plasmid. Plasmids replicate autonomously and can transfer between bacteria. Some families of plasmids are more prevalent than others or show a different distribution in pathogens and commensal strains.

ESBL-genes code for enzymes which decrease susceptibility to narrow- and extended-spectrum cephalosporins. They are inhibited by clavulanic acid, tazobactam and other inhibitors of class A β-lactamases.5 This feature is used for diagnostic tools, but is probably not effective in treatment. Different groups of ESBL-genes are described, which all contain multiple different gene-variants according to their sequences. The main groups are TEM, SHV and CTX-M of which the latter is clustered in 5 different groups: CTX-Mgroup1, CTX-Mgroup2, CTX-Mgroup8, CTX-Mgroup9, and CTX-Mgroup25. All groups, TEM, SHV and the different CTX-M groups, contain up to hundreds of gene variants. The different gene-variants are encoded by numbers. ESBL-genes other than TEM, SHV and CTX-M are described, but they are low in prevalence worldwide.6
Clinical relevance

*Enterobacteriaceae* have become one of the most important causes of nosocomial and community-acquired infections. β-Lactams, fluoroquinolones and trimethoprim-sulfamethoxazole constitute the main therapeutic choices to treat these infections. β-Lactams in particular are used as empirical treatment for a wide variety of infections, including life-threatening sepsis. They are effective against a broad spectrum of bacteria and have little side-effects. By definition, ESBL-producing *Enterobacteriaceae* are resistant to β-lactams, and therefore they escape empirical treatment with β-lactams. Furthermore, plasmids with ESBL-genes often also contain genes coding for resistance against other antimicrobials like quinolones and trimethoprim-sulfamethoxazole. This combination of resistance narrows the treatment options for patients with (severe) infections seriously.

As long as ESBL-producing *Enterobacteriaceae* are colonizing the gut, the resistance is not directly relevant. However, since most infections are caused by the patient’s own microbiota, resistance of commensal flora is an important factor in acquiring infections with resistant microorganisms. Acquisition of a more virulent strain, or changes in host defences increases the risk for infection. Furthermore, these commensal ESBL-genes can be transferred to other patients and can cause outbreaks. Infections with resistant bacteria have adverse effects. Many studies link antimicrobial resistance to increased mortality, length of hospitalization and hospital costs.

Schwaber et al. performed a retrospective cohort study in which they found an increased length of stay with a Odds Ratio (OR) of 1.56 for ESBL-production in multivariate analysis. In a meta-analysis, they found a Relative Risk (RR) of 1.85 for mortality in ESBL-associated bacteraemia (P<0.001) and a RR of 5.56 for delay in effective therapy (P<0.001). It is not clear yet if ESBL directly or indirectly causes these adverse effects. Nasa et al. postulate that ESBL-production may not be associated with a poorer outcome if appropriate antibiotic therapy is instituted early. However, tackling the problem by using last resort antibiotics for empirical treatment is undesirable from the perspective of prudent use of antibiotics, and will lead to increased resistance against these antibiotics as well.

Tumbarello et al. calculated that the healthcare costs of a patient with a blood-stream infection with ESBL is on average €5000,- higher than the costs of patients with susceptible bacteria causing their bacteraemia. Others report an increase of €18.000,- up to €38.000,- for patients with hospital-acquired infections with resistant bacteria. For the US, the annual costs of antimicrobial resistance, including healthcare costs and loss of productivity, are estimated to be 55$bn, which places it in the top 10 of health-issue related costs. Increased length of stay, increased mortality, delay in appropriate therapy and increased healthcare costs are all short term outcome measures. However, it is likely that infections with ESBL have also long-term adverse effects. For instance, patients
with infections caused by resistant bacteria are more often discharged to chronic care facilities.\textsuperscript{11}

History

The first ESBL was detected in Germany in 1983. Three \textit{Klebsiella pneumoniae} isolates and one \textit{Serratia marcescens} isolate of patients in an university clinic, were abnormal resistant to cefotaxime and ceftazidime and this resistance was transferable by conjugation to other \textit{Enterobacteriaceae}.\textsuperscript{18} The resistance was caused by a single amino-acid change in the known SHV-1 gene. SHV-1 coded for ampicilline resistance and occurred mainly in \textit{Klebsiella spp.}\textsuperscript{5}

Not much later, in France, 1984, 89 \textit{Klebsiella pneumoniae} isolates with resistance to cephalosporins were found in several ICU’s of a teaching hospital. These strains were also resistant to several other classes of antibiotics, and this resistance was transferable “en bloc” to \textit{Escherichia coli} strains. At first it was called CTX-M1, but later it appeared that the ESBL resistance was caused by two amino-acid changes of the known TEM-gene and was called TEM-3.\textsuperscript{19} TEM-1 and TEM-2 are genes coding for ampicilllin resistance and occur in all Gram-negative enteric microorganisms.\textsuperscript{5}

In 1989 a new class of ESBL was discovered in both Germany, Argentina an later in France and Italy. It was named CTX-M, and caused resistance to cefotaxim, more than to ceftazidim.\textsuperscript{20} Where SHV and TEM ESBL originated from mutations in existing resistance genes in \textit{Enterobacteriaceae}; CTX-M entered \textit{Enterobacteriaceae} lineages by horizontal transfer of plasmids from the environmental bacterium \textit{Kluyvera spp.}.\textsuperscript{21}

Nowadays more than hundred variants of TEM and SHV genes and over 65 variants of CTX-M genes are described.\textsuperscript{6}

Epidemiology

Until the end of the 1990s, ESBL-genes were mainly recovered in \textit{Klebsiella pneumoniae} isolates obtained from ICU patients during outbreaks where SHV and TEM genes were involved. Since then the situation changed significantly, and nowadays CTX-M \textit{β}-lactamases are the most prevalent. They are recovered often from community-dwelling patients with, for example, an urinary tract infection. Furthermore, these genes are now found in virtually every \textit{Enterobacteriaceae} species and even in \textit{Pseudomonas spp.} and other Gram-negative rods.\textsuperscript{6}

The mechanisms underlying the recent epidemiological evolution and epidemic of CTX-M are not fully understood.\textsuperscript{21} It are specific clones or clonal groups and epidemic plasmids that are now widely disseminated in the community and nosocomial setting. In specific, \textit{Escherichia coli} O25:H4-ST131 is thought to be responsible for the
widespread dissemination of the CTX-M15 enzyme. This clone is now widely present throughout Europe. It is likely that the co-resistance for trimethoprim-sulfamethoxazole or fluoroquinolones helps in the selective pressure favouring ESBL-producing Enterobacteriaceae. Since the discovery, the prevalence of ESBL in clinical isolates has exploded around the world. At the start of European surveillance (earss-net data, ECDC) the prevalence of resistance to 3th generation cephalosporins in Escherichia coli was less than 3% in all European countries, with the exception of some countries in south and eastern Europe. The average prevalence was 1.6% (25 countries included). In 2012 the prevalence has raised to 4.4%-38.1% (average 10.5%, 30 countries included). The increase in prevalence was significant in 23 out of 25 countries included in both surveys (P<0.001 in 21 cases). In the 2 countries where the increase was not significant, numbers were very low but the increase was still substantial.
For *Klebsiella pneumoniae* the situation is even more dramatic. Already in 2005, at the start of European surveillance, the average prevalence was 21.5%, whereas in 2012 the average prevalence was 29.5% (*P*<0.001). Striking is the extreme prevalence in southern and eastern European countries, where >50% of *Klebsiella pneumoniae* isolates are resistant to 3rd generation cephalosporins.

The rise in resistance prevalence is not unique for Europe. In the USA, between 2002 and 2010, the prevalence of *Klebsiella pneumoniae* resistant to 3rd generation cephalosporins has increased significantly from 5.8% to 11.6% (*P*=0.011).\(^2\) In Latin-America 40% of *Escherichia coli* and 30% of *Klebsiella spp.* isolates from patients with intra-abdominal infections were ESBL positive.\(^3\) In south Africa, 7.6% of *Escherichia coli* isolates from intra-abdominal infections were ESBL positive, as were 41.2% of *Klebsiella pneumoniae* isolates.\(^4\)

Worldwide, Asia is front-runner when looking at prevalence of antimicrobial resistance and ESBL. In India ESBL rates for *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were respectively 79.0%, 69.4% and 100%. Also in China (55.0%) and Thailand (50.8%) reported prevalences are high.\(^5\)

Above data all use clinical isolates as basis, and little is known about prevalence of rectal carriage. Woerther et al. reviewed all data available on rectal carriage and found that the prevalence has increased everywhere significantly since 2000 with developing countries being most affected. Furthermore they confirm that CTX-M15 is the dominant type of ESBL and that intercontinental travel may have globalised the problem.\(^2\)
Outline of the thesis

At the start of this research project, ESBL was an upcoming problem in Dutch hospitals. Where before 2008 ESBL-producing bacteria were rarely seen in clinical isolates, over time, cultures with ESBL became more common. Little was known about the epidemiology, and risk factors to identify cases were insufficient. In that time, pharmaceutical company’s developed several screening and typing methods to discover ESBL-production and spread. The aim of this thesis was to improve laboratory techniques and to contribute to the knowledge of epidemiology.

This thesis is divided in 2 parts. Part 1 covers our studies regarding laboratory techniques:

In chapter 2 we describe the evaluation of several new techniques for the detection of ESBL-producing Enterobacteriaceae. In chapter 2.1 we tested 4 techniques for phenotypical detection on a well-described collection of ESBL-producing Enterobacteriaceae. In chapter 2.2 we describe the evaluation of a genotype-based detection method for ESBL-producing Enterobacteriaceae. The tested EbSA agar and Microarray are used in most of our subsequent studies, and are incorporated in our daily routine laboratory procedures.

In chapter 3 we compare several typing methods for Enterobacteriaceae, used on a collection of resistant isolates. In chapter 3.1 we compare DiversiLab to AFLP using a collection of resistant Enterobacteriaceae obtained during a multicentre study. In chapter 3.2 we compare SpectraCell RA to MLST using a collection of ESBL-producing E. coli from human and animal sources. In chapter 5.3 this is repeated for ESBL-producing Klebsiella pneumoniae isolates. We found that DiversiLab is applicable in local settings; but SpectraCell RA was not applicable for population based epidemiology. In our epidemiological studies we used AFLP and MLST for analysis because of its robustness.

Part 2 of this theses covers our epidemiological studies:

In chapter 4, 4.1, we screened faecal samples from patients with gastrointestinal complaints visiting their general practitioner for ESBL and found a high prevalence of ESBL-producing Enterobacteriaceae colonization.

In chapter 5 we explore chicken meat as a possible source for ESBL in the community. In chapter 5.1 we describe a collection of ESBL-producing Escherichia coli obtained from chicken meat, human rectal swabs and human blood cultures. We compared these strains on strain- and ESBL-genotype level. In chapter 5.2 we compare this collection further, using plasmid and virulence factor as discriminatory factors. In chapter 5.3 we compare ESBL-producing Klebsiella pneumoniae isolates from chicken meat and human rectal and blood culture samples.
Chapter 6 describes the effect of phylotype and O25:ST131 status of *Escherichia coli* on rectal carriage and infection. Chapter 6.1 describes a study in which wildtype and ESBL-producing *Escherichia coli* from rectal, urine and blood culture samples were compared regarding phylotype and O25:ST131 status. In chapter 6.2 we describe an outbreak of ESBL in a long-term care facility and the implications of O25:ST131 versus other ESBL on duration of colonization.

In chapter 7 an overall conclusion of this thesis will be depicted. Also, future perspectives and ideas for research will be covered.
Chapter 1

References
