UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 840

Multidrug-Resistant *Escherichia coli* and *Klebsiella pneumoniae*

Treatment, Selection and International Spread

THOMAS TÄNGDÉN

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2012

ISSN 1651-6206
ISBN 978-91-554-8537-5
urn:nbn:se:uu:diva-182897

Dissertation presented at Uppsala University to be publicly examined in Gustavianum, Auditorium minus, Akademigatan 3, Uppsala, Saturday, December 15, 2012 at 09:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract
Tängdén, T. 2012. Multidrug-Resistant *Escherichia coli* and *Klebsiella pneumoniae*: Treatment, Selection and International Spread. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 840. 72 pp. Uppsala. ISBN 978-91-554-8537-5.

The prevalence of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases is increasing worldwide. Therapeutic options for infections with these bacteria are limited not only by the production of ESBLs and carbapenemases, which confer resistance to cephalosporins and carbapenems, but also by frequent co-resistance to other antibiotics. The overall aim of this thesis was to obtain a better understanding of multidrug-resistant *E. coli* and *K. pneumoniae* in relation to epidemiology, selection and susceptibility to antibiotic therapy.

In a prospective study ESBL-producing *E. coli* was found to spread easily through international travel. Twenty-four of 100 Swedes travelling outside Northern Europe acquired ESBL-producing *E. coli* in the intestinal flora. The risk was highest for travelers visiting India and those suffering from gastroenteritis during travel.

To minimize selection of ESBL-producing *K. pneumoniae* during a hospital outbreak with these bacteria, an educational antibiotic intervention was performed at Uppsala University Hospital in 2006. The primary aim of the intervention was to reduce the consumption of parenteral cephalosporins. An immediate and radical reduction of cephalosporins was demonstrated with interrupted time series analysis. The outbreak declined during 2007 and no increased resistance to replacement antibiotics was detected.

The impact of ESBL production on the antibacterial activity of ertapenem was studied in time-kill experiments. It was shown that porin-deficient subpopulations with reduced susceptibility to ertapenem frequently emerged in ESBL-producing *E. coli* during exposure to ertapenem at concentrations simulating human pharmacokinetics.

Further, the antibacterial effects of antibiotic combinations against four strains of *K. pneumoniae* producing carbapenemases of the metallo-beta-lactamase type were studied in time-kill experiments. Double and triple combinations of aztreonam, fosfomycin, meropenem, rifampin and colistin at clinically relevant static concentrations were effective despite that the bacteria were frequently resistant to the individual drugs. These results indicate that there is a largely unexplored potential of antibiotic combination therapy for multidrug-resistant *K. pneumoniae*.

Keywords: Escherichia coli, Klebsiella pneumoniae, extended-spectrum beta-lactamases, carbapenemases, metallo-beta-lactamases, synergy, antibiotic interventions

Thomas Tängdén, Uppsala University, Department of Medical Sciences, Infectious Diseases, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-554-8537-5
urn:nbn:se:uu:diva-182897 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-182897)

To my family

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Tängdén T, Cars O, Melhus Å, Löwdin E. (2010) Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicr Agents Chemother,* 54(9):3564-8.

II Tängdén T, Eriksson BM, Melhus Å, Svennblad B, Cars O. (2011) Radical reduction of cephalosporin use at a tertiary hospital after educational antibiotic intervention during an outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*. *J Antimicr Chemother* 66(5):1161-7.

III Tängdén T, Adler M, Cars O, Sandegren L, Löwdin E. (2012) Frequent emergence of porin-deficient subpopulations with reduced carbapenem susceptibility in extended-spectrum beta-lactamase-producing *Escherichia coli* during exposure to ertapenem in an in vitro pharmacokinetic model. Submitted.

IV Tängdén T, Hickman R A, Forsberg P, Giske C G, Lagerbäck P, Cars O. (2012) Evaluation of Double and Triple Antibiotic Combinations for VIM- and NDM-producing *Klebsiella pneumoniae* by in vitro time-kill experiments. Manuscript.

Contents

Abbreviations

AUC	Area under the concentration–time curve
C_{max}	Maximum concentration of drug in serum
C_{min}	Minimum concentration of drug in serum
cfu	Colony-forming unit(s)
DDD	Defined daily dose
ESBL	Extended-spectrum beta-lactamase
ICU	Intensive care unit
IMP	Imipenemase
ITS	Interrupted time series
KPC	*Klebsiella pneumoniae* carbapenemase
MBL	Metallo-beta-lactamase
MDR	Multidrug resistant
MIC	Minimum inhibitory concentration
NDM	New Delhi metallo-beta-lactamase
Omp	Outer membrane porin
OXA	Oxacillinase
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
SHV	Sulfhydryl variable
T > MIC	Time of drug concentration in serum above the MIC
TEM	Temoniera (a Greek name)
UTI	Urinary tract infection
VIM	Verona integron-encoded metallo-beta-lactamase

Introduction

The challenges of Gram-negative resistance

Antibiotic resistance in Gram-negative bacteria is a major threat to public health.[1-4] Patients with non-severe infections caused by multidrug-resistant bacteria are subject to in-hospital intravenous therapy because there are no effective oral drugs available. Resistance to empirical antibiotic therapy results in delayed appropriate antibiotic treatment for severe infections, which is associated with increased mortality, prolonged hospital stay and higher costs.[5-7] Further, resistance challenges the achievements of modern medicine, including advanced surgery and immunosuppressive treatment, which are dependent on effective antibiotics.

New antibiotics will not resolve the problem within the near future. Many of the large pharmaceutical companies have abandoned the search for antibiotics. Scientific difficulties in the search for new compounds, short therapies and the restrictive use of new antibiotics (needed to prolong their lifespan), make the development of antibiotics less profitable for the pharmaceutical industry than that of drugs used for chronic medical conditions.[8] Since 1981, only one new antibiotic subgroup targeting Gram-negative bacteria has been introduced. New antibiotics and beta-lactam/beta-lactamase inhibitor combinations are under clinical trials, but with the exception of combinations that include avibactam and a cephalosporin, are not expected to be in clinical use within the next 5-10 years.[9]

In the current situation of increased resistance it is essential to identify risk factors for infection and colonization with resistant bacteria in order to tailor empirical therapy and hygienic measures. Knowledge is needed on how to change antibiotic prescription patterns to reduce selection and spread of resistant pathogens. Moreover, because there will be no new antibiotics in the near future, a better understanding is needed on the how to optimize the use of existing antibiotics, alone and in combination with other drugs.

Evolution, selection and spread of antibiotic resistance

Antibiotic resistance can be acquired through mutations in the bacterial DNA that result in:

- changes in the antibiotic target site, which reduce the affinity for antibiotics
- production of enzymes that hydrolyze the active site of the antibiotics and prevent binding to the target site
- porin loss or alterations of outer membrane proteins that reduce antibiotic permeability through the bacterial outer membrane
- increased exportation of antibiotics through efflux pumps

Once a DNA sequence encoding antibiotic resistance has evolved, it can be transferred to mobile genetic elements and spread to other bacteria of the same or other bacterial species.

Evolution and spread of antibiotic resistance have occurred in the environment for millions of years but have accelerated during the past 70 years because of the massive use of antibiotics in humans and animals. Repeatedly, resistance rates with clinical implications have appeared within 10-20 years after the introduction of a new antibiotic.[10,11] On an individual level, emergence of resistance during antibiotic treatment typically takes place by selection of pre-existing resistant subpopulations at the site of infection or in the normal flora. In the presence of antibiotics susceptible strains die, whereas resistant isolates increase. Selection of resistant subpopulations during antibiotic treatment can result in treatment failure and future infections with resistant organisms.[12-14] On the population level, a high antibiotic consumption is strongly correlated to high resistance rates.[15]

The beta-lactamases

Broad-spectrum beta-lactamases

Resistance to beta-lactam antibiotics in *E. coli* and *K. pneumoniae* is typically mediated by beta-lactamases.[16] These enzymes, located in the periplasma, hydrolyze the beta-lactam ring and thus prevent antibiotic binding to the penicillin binding proteins (PBPs) on the bacterial cytoplasmic membrane. In the 1960s and 1970s, TEM- and SHV-type beta-lactamases, which are active against penicillin and first-generation cephalosporins, emerged in *E. coli* and soon spread to other bacterial species.[17] These enzymes were referred to as “broad-spectrum beta-lactamases”. In the late 1970s and 1980s, new cephalosporins containing an oxyimino-beta-lactam ring, which could not be hydrolyzed by the existing beta-lactamases, were introduced. These

antibiotics (including cefuroxime, cefotaxime and ceftriaxone) were referred to as second- and third-generation cephalosporins and have been used extensively because of high efficacy, low toxicity and little resistance.[18,19]

Extended-spectrum beta-lactamases (ESBLs) and carbapenemases

Beta-lactamases with activity against oxyimino-beta-lactam antibiotics were first reported in 1982, only six months after the introduction of third-generation cephalosporins.[20] They were referred to as extended-spectrum beta-lactamases (ESBLs) and defined by their ability to hydrolyze oxyimino-cephalosporins at a rate >10% of that for benzylpenicillin and that they could be inhibited in vitro by clavulanic acid.[16] The first described ESBLs were point-mutated derivatives of the broad-spectrum TEM- or SHV-type beta-lactamases that had acquired affinity for the oxyimino-beta-lactam ring through replacement of just one or very few amino acids in their active site. These enzymes were classified as Ambler molecular class A and Bush/Jacoby functional class 2be beta-lactamases.[18,21] The functional classification system, based on substrate affinity and susceptibility to beta-lactamase-inhibitors, has largely been abandoned and therefore will not be used in this thesis.[16,22]

Later, the definition of an ESBL has been altered so that all transferrable beta-lactamases that hydrolyze oxyimino-cephalosporins are now referred to as ESBLs, although some of them cannot be inhibited by clavulanic acid. For example, plasmid-borne AmpC beta-lactamases are classified as ESBLs of molecular class C. Hundreds of ESBLs have been characterized and sorted to one of the Ambler molecular classes A to D (Table 1). Beta-lactamases of molecular classes A, C and D are referred to as serine beta-lactamases, whereas the zinc-ion dependent enzymes belonging to class B, which probably have another origin than the other ESBLs, constitute the metallo-beta-lactamases (MBLs). Usually, the term ESBL is accompanied by the origin of the enzyme: for instance, “CTX-M-type ESBL” or “TEM-type ESBL”.[17-19]

An increasing number of beta-lactamases, including the serine KPC-type and OXA-type ESBLs and the MBLs, are also active against the carbapenems. They are commonly referred to as carbapenemases rather than ESBLs and will be addressed separately in this work. However, most of the carbapenemases are also highly active against oxyimino-cephalosporins.[18] Serine carbapenemases, especially of the OXA-type, have a relatively weak hydrolytic activity against the carbapenems and thus might require additional resistance mechanisms to confer phenotypic resistance to these antibiotics. [23] In contrast, the MBLs are typically highly active against all beta-lactam antibiotics except for the monobactam aztreonam.[24]

	ESBLs	Carbapenemases
Class A	**TEM**, **SHV**, **CTX-M**, VEB, PER, TLA, SFO	**KPC**, IMI, NMC, SME
Class B		**NDM**, **IMP**, **VIM**, SPM, GIM
Class C	**AmpC** (CMY, FOX and others)	
Class D	**OXA**	**OXA**

Table 1. Examples of beta-lactamases as sorted by Ambler molecular classes A-D. ESBLs and carbapenemases are listed in separate columns. OXA-type beta-lactamases occur in both columns because some (but not all) confer resistance to carbapenems. Enzymes prevalent in clinical isolates, in the literature and in this work are highlighted with bold typeface.

ESBL epidemiology

During the late 1980s and the 1990s, hospital outbreaks of ESBL-producing bacteria, predominantly *K. pneumoniae* and *Enterobacter* spp. producing SHV- or TEM-type ESBLs, were described from all continents. Resistance rates of 20-30% or more to third-generation cephalosporins were reported from some intensive care units (ICUs).[17] So far, ESBL-producing bacteria were isolated mainly from hospitalized patients. Risk factors for infection with ESBL-producing bacteria were severe underlying disease, extended hospital stay, ICU stay, intravenous or urinary catheters and previous colonization with ESBL-producing bacteria.[25,26] Retrospective studies identified recent antibiotic use as a risk factor for acquisition of ESBLs. Most studies identified oxyimino-cephalosporins or fluoroquinolones as independent risk factors,[26-29] whereas use of carbapenems, other beta-lactam antibiotics, aminoglycosides, vancomycin or metronidazole was a significant risk factor in some studies.[29-31]

By year 2000, ESBL-producing bacteria occurred sporadically as causative agents for community-acquired infections, typically urinary tract infections (UTIs) caused by *E. coli* producing CTX-M-type ESBLs.[32,33] Risk factors for community-acquired infections with these bacteria included recent antibiotic use, recent hospitalization, urinary tract catheters, diabetes, severe underlying disease, older age and male sex.[12,26,34] One study demonstrated that household members to patients infected with ESBL-producing bacteria had a higher risk for fecal carriage with ESBL producers than non-household relatives and unrelated patients, suggesting person-to-person transmission in the community.[35] ESBL-producing Enterobacteriaceae have increased dra-

matically in the community setting and are no longer a predominantly in-hospital complication. Further, it has been noted that risk factors for community-acquired ESBL infection that were found in the early reports have sometimes been missing in patients with these infections.[34,36] International travel to countries with a higher prevalence of ESBLs has been established as an additional risk factor for infection or colonization with ESBL-producing bacteria and will be addressed separately in this work.

There are marked geographical differences in the proportion of ESBL production among clinical isolates. The highest rates so far have been reported in India (>55% of *K. pneumoniae* and >70% of *E. coli* isolates).[37,38] A high prevalence has also been reported in South America (>40% and 5-10%) and Asia (20-30% and 15-20%).[39-41] Significantly lower prevalence has been reported in North America (5-10% and <5%).[41-43] In Europe, the proportion of isolates resistant to third-generation cephalosporins varies significantly between countries, with the highest rates reported in Southern and Eastern European countries.[44] During 2010, resistance rates in European blood isolates ranged from 1.7 to 75% (mean 27.5%) for *K. pneumoniae* and from 2.7 to 25% (mean 8.5%) for *E. coli*. In Greek ICUs, the proportion of ESBLs in clinical isolates of *K. pneumoniae* increased from <30% in 2005 to 70% in 2010.[2]

The rate of ESBL production in *K. pneumoniae* and *E. coli* isolates is still low in Sweden (<3%).[44] Since 2008, all detected ESBL-producing Enterobacteriaceae are reported to the Swedish Institute for Communicable Disease Control. In 2011, 5666 cases were reported, corresponding to an incidence of 60/100,000 inhabitants per year.[45] *E. coli* was the predominant pathogen (87%) followed by *K. pneumoniae* (7%). The prevalence was higher in isolates collected from female patients, probably reflecting the higher incidence of UTIs in females, and older patients.

Starting in May 2005, Uppsala University Hospital experienced the first major hospital outbreak of ESBL-producing Enterobacteriaceae in Scandinavia.[46] A CTX-M-15-producing *K. pneumoniae* clone infected 247 patients and fecal screening revealed that at least twice as many were colonized with the outbreak strain. The outbreak abated in 2007 following intensive hygienic and structural measures as well as reduced consumption of cephalosporins (Paper II).[47,48] Outbreaks have been reported from other Swedish hospitals as well.[49-51]

Data on fecal colonization with ESBLs in healthy individuals are lacking for most countries but probably co-varies with the prevalence of ESBL production in clinical isolates. The colonization rate has been estimated to be 10% or higher in Asia and the Middle East.[52,53] In contrast, only 1 of the 105 healthy travelers included in Paper I was colonized with ESBL-producing *E. coli* prior to travel.[54] In another Swedish study the prevalence of ESBL carriage was 2-3% in relatively healthy primary care patients.[55]

Carbapenemase epidemiology

Carbapenemases have been prevalent in non-fermentative Gram-negative bacteria since the early 1990s and contribute to carbapenem resistance rates exceeding 50% for *Acinetobacter baumanni* in many reports, and somewhat lower for *Pseudomonas aeruginosa*.[2,44,56-58] In contrast, carbapenemase resistance was rare in Enterobacteriaceae throughout the 1990s. Hospital outbreaks with carbapenemase-producing *K. pneumonaie* were first reported in the early 2000s. Since then, carbapenem resistance rates in Enterobacteriaceae have increased, particularly in *K. pneumoniae*. Few European countries reported >5% carbapenem resistant *K. pneumoniae* in 2009. However, higher rates were detected in isolates from Cyprus (10-25%) and Greece (>50%).[2,44] In the New York area carbapenem resistance rates of >10% were reported in 2007 due to hospital outbreaks and endemic spread of KPC-producing *K. pneumoniae,* but with lower rates following the decline of the outbreaks.[58,59] The American TRUST study reported increased meropenem resistance rates in *K. pneumoniae* isolates from 0.04% in 2007 to 3.5% in 2009.[58] Hitherto, resistance to imipenem and meropenem in *E. coli* is rare worldwide.[3,44,57,58]

The clinically most important carbapenemases in *K. pneumoniae* and *E. coli* include the serine KPC- and OXA-type carbapenemases (molecular classes A and D) and the VIM-, IMP- and NDM-type MBLs (molecular class B).

Serine carbapenemases

KPC-type carbapenemases have been described almost exclusively in Enterobacteriaceae. The first KPC-producing *K. pneumoniae* isolates were detected in the USA.[60] Hospital outbreaks and inter-hospital spread of KPC-2- and KPC-3-producing *K. pneumoniae* occurred in the New York area during the early 2000s and resulted in endemic spread.[59,61-63] In Europe, KPC-2 and KPC-3 were first detected in *K. pneumonaie* and *Enterobacter cloacae* isolates collected from patients that had been transferred from New York hospitals in 2005.[64,65] An outbreak of a KPC-2-producing *K. pneumoniae* strain occurred at a Greek hospital in 2007-2008 and seemed to replace VIM-1-producing *K. pneumoniae*, which was endemic at this institution.[66] During the past five years KPC-producing *K. pneumonaie* has been increasingly reported from European countries.[67-70]

OXA-type carbapenemases, which are prevalent in *A. baumannii,* generally have a weak hydrolytic activity against cephalosporins, carbapenems and aztreonam. OXA-48-producing *K. pneumoniae* was first discovered in Turkey in 2001 and has since been reported in several other countries in Europe and the Middle East and sometimes been detected in other enterobacterial species.[71,72] Although OXA-48 is more active against imipenem than

most OXA-type enzymes, other contributing resistance mechanisms (such as porin loss or efflux) are necessary to achieve phenotypic resistance to carbapenems. Therefore, the presence of OXA-48 might be overlooked at the laboratory and the prevalence of this enzyme underestimated.

Metallo-beta-lactamases (MBLs)

VIM production in *K. pneumoniae* isolates was first detected in Greece.[73] The first hospital outbreak of VIM-producing *K. pneumoniae*, which occurred in France in 2003-2004, originated from a patient transferred from a Greek hospital.[74] It was followed by a hospital outbreak in Italy with a VIM-1-producing *K. pneumoniae* strain that was also believed to originate from Greece because of genetic similarity to Greek VIM-1 enzymes.[75] IMP-type and, to a lesser extent, VIM-2-type MBLs are predominant in Asia.[23,56,76]

NDM-1 was first detected in a clinical isolate of *K. pneumoniae* derived from a patient transferred from an Indian hospital to Sweden in 2008.[77] Since then, NDM-1-producing Enterobacteriaceae have been detected worldwide in patients that typically have a history of recent hospitalization in the Indian subcontinent, recent travel to South Asia, or originate from these regions.[78,79] MBLs inactivate all beta-lactam antibiotics except for aztreonam and strains harboring these enzymes are considered a major clinical threat because co-resistance to multiple antibiotic subclasses is frequent and severely limits therapeutic options.[38,77,79] Further, NDM-1 has frequently been detected in *E. coli* isolates, in contrast to other carbapenemases that are mostly restricted to the less virulent *K. pneumoniae*. In a recent Pakistan study fecal colonization with NDM-1-producing Enterobacteriaceae was detected in 13.8% of outpatients and 27.1% of inpatients, indicating a large reservoir of resistance within the community in this area.[80]

Thus far, carbapenemase-producing Enterobacteriaceae have been rare in Sweden.[45] In total, approximately 50 clinical isolates have been reported, predominantly *K. pneumoniae.* Half of these isolates produced MBLs, predominantly NDM-1, and the vast majority of patients were thought to be infected abroad.

In summary, carbapenem resistance is still rare in Enterobacteriaceae but has increased in recent years, reaching endemic levels for *K. pneumoniae* in some areas. Interestingly, carbapenemase-producing Enterobacteriaceae emerged almost simultaneously in different continents during the early 2000s but with marked geographic differences in the epidemiology. KPC-type serine carbapenemases are predominant in the USA. VIM-1 has been more prevalent in Europe, although KPC-type carbapenemases have increased in Europe during the past years. IMP-type, and to a lesser extent, VIM-2 type MBLs are predominant in Asia. However, NDM-1 is increasing worldwide and will probably represent a key clinical challenge in the near future.

International spread of ESBLs and carbapenemases

Based on retrospective studies and case reports, foreign travel has been suggested as a risk factor for acquisition of resistant *E. coli*. Ten patients in New Zeeland with community-acquired infections caused by ESBL-producing *E. coli* lacked traditional risk factors for ESBLs but all had a history of travel to the Indian subcontinent.[81] A retrospective study performed in the USA revealed that overseas travel within the preceding 12 months increased the risk for colonization with trimethoprim-sulfamethoxazole-resistant *E. coli*.[82] Overseas travel, particularly to India, was a significant risk factor for community-onset infections caused by ESBL-producing *E. coli* in a population-based surveillance study in Canada.[83] Further, in a Swedish study with 242 patients suffering from travelers' diarrhea, fecal colonization with ESBL-producing Enterobacteriaceae was more frequent in patients returning from countries outside Europe compared with those who had visited a European country (36 vs. 3%).[84]

The first published prospective study on foreign travel as a risk factor for fecal colonization demonstrated that 24 of 100 Swedish healthy volunteers travelling outside northern Europe acquired *E. coli* producing CTX-M-type ESBLs and, consistent with previous reports, most frequently after visits to India, other Asian countries or the Middle East (Paper I).[54] These results were later confirmed by an Australian study with a similar prospective design.[85]

Several outbreaks with carbapenemase-producing *K. pneumoniae* have originated from patients recently transferred from hospital with ongoing outbreaks or endemic spread with these bacteria.[65,74] A recent Scandinavian study on VIM-1-producing *K. pneumoniae* revealed that 7 of 8 patients had a history of recent hospitalization in Greece or Turkey.[86] In addition, the pandemic spread of NDM-1 has been linked to recent hospitalization or travel to the Indian subcontinent.[77-79]

Today, the importance of country-to-country transfer of patients for the global spread of resistant Gram-negative bacteria is well established and therefore screening for colonization with these bacteria has been recommended for patients recently hospitalized abroad.[79,87] However, the clinical implications of higher risk for colonization with ESBL- or carbapenemase-producing Enterobacteriaceae in previously healthy patients with a history of recent international travel have not yet been settled.

Carbapenem resistance caused by combination of ESBL- or AmpC-production and impermeability

ESBLs or AmpC enzymes with low hydrolytic activity against carbapenems alone can sometimes contribute to carbapenem resistance in Enterobacteri-

aceae.[88] However, other mechanisms that reduce antibiotic concentrations in the periplasmic space (e.g., loss of outer membrane porins OmpK35 and OmpK36, which has been frequent in ertapenem resistant *K. pneumoniae* isolates)[89,90] are needed in addition to ESBL production to achieve phenotypic resistance. Several studies have reported emerging imipenem resistance during treatment for ESBL-producing *K. pneumoniae* or *E. cloacae* and suggested the combination of the pre-existing ESBL and emerging porin loss as resistance mechanisms in these isolates.[91-93]

Ertapenem seems to be more affected by ESBLs than the other carbapenems. MICs for ertapenem have been elevated in ESBL-producing *K. pneumoniae* compared with non-ESBL-producing strains, whereas MICs for other carbapenems have been less affected by the presence of these enzymes.[94-97] In a surveillance study on clinical isolates of *K. pneumoniae* originating from intra-abdominal infections only 77.9% and 91.1% of ESBL-producing isolates were susceptible to ertapenem and imipenem, respectively, whereas 95.5% and 97.5% of the non-ESBL-producing isolates were susceptible.[98] These data suggest that ESBL production is a major mechanism of resistance to ertapenem and, to a lesser extent, to other carbapenemases. Further, it has been shown that the vast majority of ertapenem-resistant *K. pneumoniae* isolates produce ESBLs rather than carbapenemases.[89,90,97]

Less is known about ertapenem resistance in *E. coli*, which is still rare.[99] In a recent in vitro study ESBL production was associated with reduced susceptibility to carbapenems and higher frequency of emerging carbapenem-resistant subpopulations in *E. coli*.[100] Reduced expression of OmpF and OmpC, which are *E. coli* homologues of OmpK35 and OmpK36,[101] was frequent in resistant strains. These findings indicate that resistance mechanisms for ertapenem-resistant *E. coli* are similar to those described in *K. pneumoniae*.

Antibiotic interventions intended to reduce selection of cephalosporin-resistant strains

A large number of antibiotic interventions have been performed to reduce selection and prevalence of cephalosporin-resistant Enterobacteriaceae during an ongoing outbreak or high endemic prevalence of resistant strains.[48,102-116] These interventions have typically aimed at reducing prescriptions of parenteral cephalosporins, which are known to select for cephalosporin-resistant strains and recommended a penicillin/beta-lactamase inhibitor combination (piperacillin-tazobactam or ampicillin-sulbactam) as replacement therapy.

The analysis of performed intervention studies typically include (1) changes in antibiotic consumption, (2) changes in the prevalence of the targeted resistant bacteria and (3) collateral damage, i.e. unwanted trends in the local resistance epidemiology during the post-intervention period that may have resulted from changes in antibiotic prescription patterns. The effects of published antibiotic interventions are difficult to evaluate and compare for the following reasons:[117-119]

- The settings vary substantially between studies in pre-intervention antibiotic use, local resistance epidemiology and patient groups. Some studies have included entire hospitals and others just one or a few departments at the hospital.

- Most studies have used before-and-after analysis to evaluate the effects of performed intervention with respect to antibiotic use and resistance epidemiology. However, the duration of pre-intervention, intervention and post-intervention phases has varied widely from a few weeks to several years.

- Many antibiotic interventions, especially those performed during an outbreak situation, have been preceded or accompanied by hygienic measures, which may have affected the outcome. In addition, even when no hygienic intervention has taken place, an antibiotic intervention or an outbreak situation might have improved the awareness and compliance to existing directives on hygienic measures.

- The causative effect of an antibiotic intervention on resistance epidemiology is difficult to assess in that the natural course of an outbreak and natural variation of endemic resistance are unknown. Moreover, data on antibiotic susceptibilities have often been based on very few isolates.

- The analysis of collateral damage, when performed, has often included very few antibiotics and bacterial species during a follow-up period typically no longer than 12 months. Delayed resistance or emerging resistance in other bacterial species than those included in the analysis might have occurred but passed unnoticed.

Most studies to date have used before-and-after analysis. However, the recommended statistical method for intervention studies is interrupted time series (ITS) regression analysis on monthly antibiotic consumption, which is associated with lower risk for bias than other study designs.[117,120] Further, ITS analysis provides information on pre-intervention trends of antibiotic

consumption and immediate vs. sustained effects of the intervention, which cannot be assessed with the other study designs.[120]

The vast majority of published interventions have reported reduced levels of cephalosporin-resistant strains following reduced consumption of cephalosporins.[48,102-105,107-109,111-116,121] Four studies detected increased resistance to antibiotics that were used more frequently in the post-intervention period: imipenem-resistant *P. aeruginosa*,[114] fluoroquinolone-resistant *P. aeruginosa* and *E. coli* and ampicillin/sulbactam-resistant *A. baumannii*,[108] ampicillin/sulbactam-resistant *A. baumannii*[105] and piperacillin/tazobactam-resistant *P. aeruginosa* and *E. coli*.[109] Increased prevalence of penicillinase-producing *K. pneumoniae* was reported in one study.[104] On the other hand, other studies have demonstrated decreased rates of resistance to the replacement antibiotics.[102,104,111,116]

Parenteral therapeutic options for infections with ESBL-producing and carbapenem-resistant isolates

ESBL or carbapenemase production in Enterobacteriaceae is often accompanied by co-resistance to fluoroquinolones, trimethoprim, sulfonamides and aminoglycosides.[44,122] Co-existing resistance genes may be located on the same plasmid as the genes encoding beta-lactamases or in the bacterial chromosomal DNA. Bacteria resistant to at least one drug in three or more relevant antibiotic subclasses are referred to as multidrug resistant (MDR).[123] For severe infections with ESBL-producing *E. coli* and *K. pneumoniae*, antibiotic therapy typically consists of one of the antibiotics described below. Combination therapy is recommended for infections with carbapenem-resistant strains.

Carbapenems

The carbapenems are effective for all severe infections with Enterobacteriaceae susceptible to these antibiotics, including ESBL-producing bacteria.[7,19,124-126] Most clinical data exist for imipenem and meropenem. Ertapenem has been mainly used for the treatment of UTIs caused by ESBL-producing bacteria and proven effective for these infections.[127,128] Ertapenem resistance is more frequently reported than resistance to other carbapenems, especially in ESBL-producing strains.[98,99] Some data suggest that doripenem, which was recently introduced but currently not used in Sweden, is more stable against emerging resistance than the other carbapenems.[129,130]

Cephalosporins

Cephalosporins have been less effective than comparative regimens in treating severe infections with ESBL-producing bacteria.[131,132] However, some studies suggest that third- and fourth-generation cephalosporins might be considered if the MIC of the isolated strain is ≤1 mg/L. Cefepime has proven effective for various infections with susceptible ESBL-producing Enterobacteriaceae, although it is still regarded as inferior to carbapenems for severe infections and sepsis.[133-135] There have been some concerns of the inoculum effect that has been demonstrated in vitro for cephalosporins and other beta-lactam antibiotics, i.e. impaired antibacterial activity when higher bacterial concentrations are used, but the clinical relevance of this finding is uncertain.[136,137]

Piperacillin/tazobactam

Piperacillin/tazobactam has proven effective for UTIs caused by susceptible ESBL-producing bacteria.[138] Susceptibility rates are high in ESBL-producing *E. coli* (62 to 87% in published reports) but lower in ESBL-producing *K. pneumoniae* (26 to 47%).[139,140] For sepsis and severe infections other than UTIs, the use of piperacillin/tazobactam is under current debate.[2,17,137,141,142] One study reported non-inferiority to carbapenems for the treatment of sepsis originating from the urinary or biliary tract caused by susceptible ESBL-producing Enterobacteriaceae.[143] A recent review on sepsis with ESBL-producing Enterobacteriaceae demonstrated no significant difference in mortality rates between piperacillin/tazobactam and carbapenem therapy (16 vs. 10%).[142,143] Higher cure rates were found for piperacillin/tazobactam in patients infected with *E. coli* rather than other Enterobacteriaceae, perhaps reflecting that MIC values are typically lower in this species.

Fluoroquinolones

The fluoroquinolones are effective for the treatment of UTIs caused by susceptible bacteria.[144] However, resistance levels are high in *E. coli* and *K. pneumoniae*, especially in ESBL-producing strains.[44,145] Ciprofloxacin treatment has been associated with worse outcome compared with carbapenems and other comparator regimens for septic patients infected with ESBL-producing *E. coli* or *K. pneumoniae,* possibly because of insufficient ciprofloxacin tissue concentrations achieved at infection sites other than the urinary tract.[141,146]

Aminoglycosides

Monotherapy with an aminoglycosides is equally effective as a beta-lactam antibiotic or a fluoroquinolone for the treatment of UTIs but is associated with higher toxicity.[147] The majority of ESBL-producing *E. coli* and *K. pneumoniae* are resistant to tobramycin and gentamicin. In contrast, amikacin has retained high susceptibility rates, particularly in *E. coli*.[38,145] Therapy with an aminoglycoside alone has been found to be inferior to comparators for the treatment of sepsis and severe infections other than UTIs and is therefore not recommended for these conditions.[147] However, combinations that consist of an aminoglycoside and other active drugs can be effective for MDR and carbapenem-resistant strains.

Tigecycline

Tigecycline susceptibility rates are still close to 100% in ESBL- and carbapenemase-producing *E. coli* and somewhat lower in *K. pneumoniae.*[148-150] However, its use for severe infections has been questioned because of the bacteriostatic effect and reported breakthrough bacteraemia with susceptible VIM-producing *K. pneumoniae* during tigecycline treatment.[2,150] It has significantly lower cure rates than imipenem for hospital-acquired and ventilator-associated pneumonia and is not approved for these conditions.[151] Pooled analysis has revealed higher mortality for tigecycline compared with other regimens, particularly for the treatment of hospital-acquired and ventilator-associated pneumonia. Accordingly, it has been recommended that monotherapy with tigecycline should be considered only when there are no other suitable treatment options.[152] Conventional dosage (50 mg 12-hourly) might fail to achieve sufficient non-protein-bound tigecycline concentrations in serum and higher dosage regimens (100-200 mg 12-hourly) are now being studied in clinical trials.[2,150]

Colistin

Colistin has become the drug of choice for the treatment of carbapenem-resistant Gram-negative bacteria. The vast majority of ESBL-and carbapenemase-producing *E. coli* and *K. pneumoniae* are susceptible to this drug.[57] However, colistin resistance has increased in *K. pneumoniae* and emerged during treatment with this drug.[153-155] Colistin should always be used in combination with another active antibiotic to improve efficacy and reduce the risk for selection of resistant strains.[9,149,156,157] For severe infections with carbapenem-susceptible strains, colistin is associated with higher mortality than imipenem and meropenem, but can be considered as part of a combination therapy for patients unable to tolerate carbapenems.[156,158] The optimal dosing of colistin is still being investigated.[159,160] A prospective

study on severely ill patients has given some guidance but the recommended target steady state concentrations of 2-2.5 mg/L seem unsatisfactory considering that the clinical breakpoint for susceptibility is 2 mg/L for Enterobacteriaceae.[161] Elevated serum creatinine levels have been reported in 15-45% of treated patients, with increased risk when combined with other nephrotoxic drugs or used for patients with pre-existing renal failure, but is usually mild and reversible.[156,162]

Fosfomycin

Fosfomycin susceptibility rates are high in ESBL-producing *E. coli* and *K. pneumonaie* (>80%).[163,164] Parenteral fosfomycin has been used as a last-resort agent in combination with other antibiotics for severe infections with Enterobacteriaceae that are resistant to carbapenems, colistin and tigecycline.[2,165] Because resistance to fosfomycin occurs easily, combination treatment with another effective antibiotic is recommended.[2,166] To date, limited data exist on efficacy and proper dosage regimens for intravenous fosfomycin therapy and, to our knowledge, there are no published prospective clinical studies. Dosage regimens have ranged from 3g x 3 to 8g x 4 in published reports, and have been reduced for patients with impaired renal function.[2,167,168]

Combination therapy

Clinical studies have concluded that combination antibiotic therapy is associated with better outcome than monotherapy for the treatment of severe infections with carbapenemase-producing *K. pneumoniae*, even when the isolated bacteria are susceptible in vitro for the individual drugs.[9,149,157] Prescribed antibiotic combinations for these infections have typically included colistin, which presumably increases the permeability of other drugs through the bacterial outer cell membrane.[169] However, only limited data are available on which antibiotic combinations are the most effective. So far, recommendations have been based on a few retrospective clinical studies and in vitro studies with time-kill or chequerboard techniques.

Published clinical studies have reported a favorable outcome for patients treated with combinations of colistin, a carbapenem, tigecycline, fosfomycin or an aminoglycoside.[9,165,170] In Greece, combination treatment with two active drugs, including an aminoglycoside, colistin, tigecycline or fosfomycin, has been recommended for infections with carbapenemase-producing *K. pneumoniae*.[2] A review on antibiotic therapy for KPC-producing *K. pneumoniae* revealed that the cure rate of combination therapy with meropenem and colistin was highly dependent of the MIC values for meropenem.[170] The lowest mortality rate overall was reported in patients infected with bacteria displaying an MIC $\leq$ 4 mg/L for a carbapenem, who received treatment with

this carbapenem in combination with an aminoglycoside, colistin or tigecycline. Another study reported clinical cure in 11 patients treated with fosfomycin and colistin, gentamicin or piperacillin/tazobactam for severe infections caused by carbapenem-resistant *K. pneumoniae*.[165]

In vitro, double or triple combinations including colistin, carbapenems, rifampin, tigecycline and fosfomycin have proven effective against carbapenemase-producing *K. pneumoniae* and been proposed for clinical use.[130,166,171,172] In a time-kill study the triple combination of colistin, doripenem and rifampicin was bactericidal against KPC-producing *K. pneumoniae* and *E. coli*.[130] Another study demonstrated a bactericidal effect of meropenem and fosfomycin against the majority of KPC-producing *K. pneumoniae* isolates at clinically relevant static antibiotic concentrations.[166] Colistin in combination with fosfomycin or tigecycline demonstrated synergy against only 4 of 28 NDM-producing Enterobacteriaceae strains in a study with chequerboard method, but the authors concluded that the combinations were at least not antagonistic.[172]

Beta-lactam/beta-lactamase inhibitor combinations

The beta-lactamase inhibitors clavulanate, sulbactam and tazobactam, which were introduced in the 1970s and 1980s, are still active against most class A (including SHV-, TEM- and CTX-M-type ESBLs, but not KPC) and class D beta-lactamases.[173] These beta-lactamase inhibitors bind and inactivate the enzymes irreversibly. Clinically used combinations include amoxicillin/clavulanate, ticarcillin/clavulanate, piperacillin/tazobactam, ampicillin/sulbactam and cefoperazone/sulbactam.[173]

The new beta-lactamase inhibitor avibactam is active against class A (including KPC), class C and class D beta-lactamases.[96] In one study, the addition of avibactam to piperacillin, cephalosporins (cefotaxime, ceftazidime or cefepime) and aztreonam resulted in significantly reduced MICs for KPC-producing *K. pneumoniae*.[174] The combination of avibactam/ceftazidime was effective in a murine model for the treatment of KPC-producing *K. pneumoniae*.[175] Another study showed that the ceftazidim/avibactam combination was highly active against 65 carbapenemase-producing Enterobacteriaceae, including isolates with impermeability and AmpC production, but not against MBL-producing strains.[96] In this material the aztreonam/avibactam combination was effective against all isolates. The authors concluded that the aztreonam/avibactam combination might be an effective therapeutic option for infections with MBL-producing Enterobacteriaceae because aztreonam is typically stable against the MBLs produced in these strains but not against the frequently co-produced AmpC or ESBLs that can be inhibited by avibactam.[77]

Several effective MBL-inhibitors, including monobactam derivatives, have been presented and might be useful in combination with broad-

spectrum beta-lactam antibiotics. However, none of these inhibitors has been approved for clinical use.[173] In the absence of new beta-lactamase-inhibitors effective against MBLs the inhibitory properties of existing antibiotics have been considered. Competitive inhibitory activity has been demonstrated for aztreonam against MBLs and for meropenem against class A, C and D beta-lactamases.[24,176,177] However, the clinical use of these findings has yet to be shown.

Aims

The aims of the present thesis were:

- To prospectively study the risk for fecal acquisition of ESBL-producing Enterobacteriaceae during foreign travel and the persistence rate of acquired ESBLs after six months (Paper I).

- To assess potential travel-associated risk factors for acquisition of ESBL-producing bacteria, such as destination, accommodation, gastroenteritis and antibiotic use during travel (Paper I).

- To evaluate the effects of a hospital-wide antibiotic intervention performed at Uppsala University Hospital on antibiotic consumption and local antibiotic resistance epidemiology (Paper II).

- To explore the impact of ESBL production in *E. coli* on the antibacterial activity of ertapenem in vitro at concentrations simulating human pharmacokinetics (Paper III).

- To find antibiotic combinations with synergistic and bactericidal activity against VIM- and NDM-producing *K. pneumoniae* isolates in vitro at clinically relevant static antibiotic concentrations (Paper IV).

Methods

Healthy volunteers (I)

Healthy volunteers travelling outside Northern Europe were included in the study. Written information and necessary materials to participate in the study were available at travel consultation clinics in Uppsala. In total, 105 volunteers provided written consent, questionnaires, and rectal swabs and were enrolled in the study. Four of them did not complete the study, and one participant carried an ESBL-producing *E. coli* strain before leaving Sweden. One hundred travellers (55 women and 45 men) with a median age of 43 years were included in the analysis.

The study was performed with permission from the Regional Ethical Review Board in Uppsala.

Bacterial strains (III, IV)

In Paper III, two previously characterized *E. coli* strains were used: one native non-ESBL-producing strain (DA14781) and one ESBL-producing derivative of this strain (DA14833).[100] Both strains originated from the *E. coli* MG1655 strain. DA14833 was constructed by conjugation of the pUUH239.2 plasmid (encoding CTX-M-15, TEM-1 and OXA-1 beta-lactamases) into DA14781. In the static time-kill experiments, a previously described *ompR*-mutant strain derived from DA14833 (DA16808) was also used.[100] Since expression of outer membrane porins OmpF and OmpC is depending on the transcriptional factor OmpR, the mutant strain has reduced expression of these porins.

In Paper IV, clinical *K. pneumoniae* isolates producing VIM- and NDM-type carbapenemases were obtained from the Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden. The VIM-KP strains T14789 and ÖN-2211 were originally isolated in Scandiavia but derived from patients recently transferred from Greek hospitals and the NMD-1-producing *K. pneumoniae* (NDM-KP) isolates IR8 and IR62E originated from Chennai, India. All strains have been characterized previously.[86,178]

Medium and growth conditions (I, III, IV)

In Paper I, fecal samples were collected with the Copan transport system and inoculated in Luria-Bertani broth (Becton Dickinson and Co., Sparks, MD) supplemented with cefotaxime (2.5 μg/ml) to select for cephalosporin-resistant strains. MacConkey agar (Acumedia Manufacturers, Inc., Lansing, MI) was used during antibiotic susceptibility testing in this study. Broths and plates were incubated overnight at 35°C.

In Paper III, Mueller-Hinton (MH) agar and MH broth (Difco Laboratories, Detroit, MI) was used. For the ESBL-producing strain, the MH broth was supplemented with tetracycline 20 mg/L during pre-culture to select for the plasmid. Cation-adjusted Mueller Hinton II broth agar and broth (Becton Dickinson & Co., Sparks, MD) was used in Paper IV, because the activity of colistin activity is affected by altered concentrations of calcium and magnesium ions. In these studies, bacterial growth was obtained at 37°C.

Determination of minimal inhibitory concentrations (I, II, III, IV)

Minimal inhibitory concentrations (MICs) were determined with the Etest method according to the instructions of the manufacturer (bioMérieux, Marcy l'Etoile, France). All MICs were determined at least in duplicate. In order to increase the bacterial density in endpoint samples from kinetic time-kill experiments in Paper IV, 50 ml of the samples were spun at 3939×g for 15 min and cells were resuspended in 1 mL MH broth before spreading on plates. When the number of colonies was still insufficient for proper reading, 10 individual colonies were isolated from the Etest plate and inolulated in MH broth over night. The MICs of these cultures were then determined separately with Etest.

Viable counts (III, IV)

During time-kill experiments, samples were serially diluted in phosphate-buffered saline (pH 7.4), spread on plates and incubated at 37°C. Colonies were counted after 24 h. Bacterial counts within the range of 10 to 300 per plate were considered most reliable and, if present, were used in the analysis.

Antibiotics and antibiotic concentrations (III, IV)

Antibiotics were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) with the exception of ertapenem, which was kindly provided by

Merck and Co. (NJ, USA). During the kinetic time-kill experiments of Paper III, ertapenem concentrations simulating human pharmacokinetics of non-protein-bound antibiotic substance were used. For experiments simulating conventional dosage (1g once daily), ertapenem was added to an initial concentration of 11 mg/L and during experiments with higher concentrations to an initial concentration of 22 mg/L.[179] The elimination half-life was set to 2 h during the first 4 hours and to 4 h during the rest of the dose interval. During static time-kill experiments in Paper III, ertapenem concentrations ranging from C_{min} to C_{max} with conventional dosage were used: 0.085, 0.68, 1.37, 2.75, 5.5 and 11 mg/L.

In Paper IV, antibiotic concentrations used during time-kill experiments represent steady state concentrations of non-protein-bound drug in humans calculated from literature data (based on the area under the antiobiotic concentration in serum or plasma over 24 hours divided by 24 hours (AUC_{0-24h}/24 h)). For rifampin the mean concentration ((C_{max}+C_{min})/2) was used instead due to the lack of other data. The following concentrations were used: 17 mg/L for aztreonam,[180] 1.0 mg/L for ciprofloxacin,[181] 4.0 mg/L for colistin,[161] 1.7 mg/L for daptomycin,[182] 83 mg/L for fosfomycin,[183] 6.8 mg/L for meropenem,[184] 1.7 mg/L for rifampin,[185] 3.3 mg/L for telavancin,[186] 0.1 mg/L for tigecycline,[187] and 9.1 mg/L for vancomycin.[188]

Determination of ertapenem concentrations (III)

The concentration of ertapenem in samples taken during kinetic time-kill experiments was determined by a microbiological agar diffusion method with *Bacillus stearothermophilus* ATCC 3032 as the test strain.[189] A standardized inoculum of the spore suspension was mixed with tryptone-glucose agar adjusted to pH 7.4. Medium was poured into the plates, and 0.03 mL portions of the samples and standards were applied into the agar wells. The lowest detectable concentration of ertapenem was 0.01 mg/L. All assays were performed in triplicate.

Stability of ertapenem at thermostate temperature (III)

To study the stability at the temperature used during time-kill experiments, ertapenem was diluted in 1 mL MH broth to a concentration of approximately 11 mg/L and was left at 37°C for 24 hours. The ertapenem concentration was determined at 0 and 24 h. This experiment was performed in triplicate.

Kinetic time-kill experiments (III)

A previously described in vitro kinetic model was used to simulate the free drug concentration-time curve achieved in patients during treatment.[190,191] The model consists of a spinner flask (105 mL) which is connected to a pump and a bottle with fresh medium. Antibiotic added to the flask is diluted according to the first-order kinetics $C = C_0 \times e^{-kt}$ where C is the antibiotic concentration at time t, C_0 the initial antibiotic concentration, k the rate of elimination and t the time elapsed since addition of antibiotic. To compensate for instability of ertapenem at thermostate temperature detected in previous experiments (described above), the pump flow was reduced by 2 mL/h throughout kinetic time-kill experiments. The apparatus was placed in a thermostatic room at 37°C during experiments.

Single bacterial colonies were inoculated in 1 ml MH broth for 6 hours. The 1 mL culture was added to 105 mL of pre-warmed MH broth in the flask and further pre-cultured for 5 h to achieve a starting inoculum of approximately 1 x 10^8 cfu/mL. Ertapenem was added at 0 and 24 h to achieve the desired concentrations. Samples were taken for viable counts and determination of antibiotic concentrations at 0, 2, 4, 8, 12, 20, 24, 28, 32, 36, 44 and 48 h. All experiments were performed at least in triplicate.

Static time-kill experiments (III, IV)

Bacteria were grown overnight, diluted in pre-warmed MH (III) or MHII (IV) broth and then further pre-cultured to achieve starting inocula of approximately 5 x 10^6 cfu/mL. Antibiotics were added to the desired concentrations. Cultures were incubated in 37°C rocking water bath and samples were taken for viable counts repeatedly during 24 hours. All experiments were performed at least in duplicate.

Analysis of time-kill experiments (III, IV)

Mean $\log_{10}$ cfu/mL values were used in the analysis and in the presentation of data. Starting inocula were normalized to the median start inoculate of all experiments. Bacterial concentrations <1.0 $\log_{10}$ cfu/mL, the lower limit of detection for an individual bacterial count, were counted as 1.0 $\log_{10}$ cfu/mL.

Definitions of synergy and bactericidal effect (IV)

Synergy was defined as ≥2 $\log_{10}$ decrease in cfu/mL between the combination and its most active constituent after 24 h. Bactericidal effect was defined as ≥3 $\log_{10}$ decrease in cfu/mL after 24 h compared to the start inoculum.

ESBL detection and typing (I)

Colonies growing within the expected zones of cefotaxime and ceftazidime or just cefotaxime (inhibition zones <24 mm) were characterized to the species level by conventional methods or by a VITEK 2 instrument. Phenotypic confirmation of ESBL-production was performed by disc diffusion clavulanic acid synergy test. Isolates with phenotypic ESBL-production were screened for blaCTX-M, blaTEM and blaSHV with polymerase chain reaction (PCR). PCR products from isolates carrying genes encoding ESBLs of the CTX-M type were sequenced after using modified primers.

Detection of porin mutations (IV)

OmpC, ompF and *ompR* genes were PCR-amplified using primers up- and downstream of the coding sequence. PCR products were purified using the Fermentas GeneJet Gel extraction kit, dried in a speedvac with one of the amplification primers and sent to Eurofins MWG Operon, Germany, for sequencing. Primers used for amplification and sequencing were as follows:
E. coli ompC: Fwd2 5'-GACTTGCCGACTGATTAATGA-3', Rev 5'-CTGATGTTGTACGCTGAAAAC-3'.
E. coli ompF: Fwd 5'-AATATCATCACGTTCCTATGG-3', Rev 5'-GTGAGATTGCTCTGGAAG-3'.
E. coli ompR: Fwd 5'-CACATTGGGTATAACGTGATC-3', Rev 5'-CAACATACGCACTTCGTACG-3'.

Antibiotic intervention (II)

The antibiotic intervention was performed with the following targets: (1) to reduce consumption of second- and third-generation cephalosporins and (2) to avoid increased prescription of fluoroquinolones and carbapenems. New guidelines for empirical antibiotic treatment were introduced (Table 2). Penicillin G and piperacillin/tazobactam were suggested rather than cephalosporins for the treatment of abdominal infections, pneumonia and fever of unknown origin. Carbapenems were recommended only for patients in septic

shock and patients with severe sepsis and previous colonization or infection with ESBL-producing *K. pneumoniae*.

The new recommendations were presented at the local intranet and on hard copies distributed to physicians throughout the hospital by the local group of the Strama network (the Swedish strategic programme against antibiotic resistance). The Strama group arranged meetings for physicians at every department of the hospital, where the content and rationale for the new recommendations were communicated, data on the department's antibiotic consumption were presented and specific problems and improvement strategies discussed. No restrictive measures were used in this intervention.

Diagnosis	**Recommended antibiotic treatment**
Abdominal infections	Piperacillin/tazobactam
Community-acquired pneumonia	Non-septic patients, penicillin G Septic patients, penicillin G + moxifloxacin
Hospital-acquired pneumonia	Piperacillin/tazobactam
Febrile urinary tract infection	Piperacillin/tazobactam *or* cefotaxime
Fever of unknown origin	Non-septic patients, penicillin G + gentamicin Septic patients, piperacillin/tazobactam
Septic shock	Imipenem *or* meropenem
Severe sepsis in patients with previous colonization or infection with ESBL-producing *K. pneumoniae*	Imipenem *or* meropenem

Table 2. New recommendations for empirical intravenous antibiotic treatment implemented at Uppsala University Hospital during the antibiotic intervention. Addition of a single dose gentamicin was recommended for all septic patients.

Data on antibiotic use (II)

During the study period, only the state-owned National Corporation of Swedish Pharmacies (Apoteket AB) provided antibiotics in Sweden. Total antibiotic sales data were collected in a national database using the Anatomical

Therapeutic Chemical classification system (ATC) and defined daily doses (DDDs), according to the WHO Collaborating Centre for Drug Statistics Methodology (www.whocc.no). Monthly data on antibiotic consumption at Uppsala University Hospital during 2000 to 2007 were obtained from this database for the antibiotics targeted in the intervention: cephalosporins (cefuroxime, ATC code J01DC02; and cefotaxime, J01DD01); fluoroquinolones (ciprofloxacin, J01MA02; and norfloxacin, J01MA06); carbapenems (imipenem, J01DH51; meropenem, J01DH02; and ertapenem, J01DH03); piperacillin/tazobactam (J01CR05); and penicillin G (J01CE01). Data on the corresponding monthly consumption at all other Swedish hospitals during 2005 to 2007 were also obtained for comparison.

Statistical analysis of travel associated risk factors (I)

McNemar's test, generalized previously by Durkalski et al. for clustered data, was used for comparison of ESBL colonization rates before traveling to colonization rates after traveling.[192] In the analysis of travel associated risk factors, the Pearson test was used for categorical data and the Wilcoxon test for continuous data. For all comparisons, a P value of <0.05 was considered to represent statistical significance. All statistical analyses were performed with R software, version 2.8.0 (available at http://www.r-project.org).

Analysis of changes in antibiotic prescriptions (II)

To adjust for variation in the number of patients treated at the hospital, monthly data on antibiotic consumption at Uppsala University Hospital were divided by the monthly patient bed-days. For national hospital data, DDDs rather than DDDs/1,000 patient bed-days were used instead due to the lack of other data.

ITS regression analysis was used to determine trend shifts in antibiotic consumption in accordance with the recommendations of Wagner et al.[120] The breakpoint in the analysis was set to October 2006 when the new recommendations were introduced. Because the use of carbapenems had increased markedly since the beginning of the outbreak, two breakpoints were used for these antibiotics (May 2005 and October 2006). To control for seasonality a modified model was used.[193] The model

$$y_t = \beta_0 + \beta_1 \cdot time_t + \beta_2 \cdot intervention_t + \beta_3 \cdot time\,after\,intervention_t + \sum_{i=4}^{14} \beta_i \cdot Month_{(i-2)_t} + \varepsilon_t,$$

was fitted using least squares regression. In this model y_t is DDD antibiotic consumption at UUH/1.000 patient bed-days at time t; $time_t$ is time in months at time t from start (January 2000); $intervention_t$ is an indicator for t occurring before the intervention ($intervention_t$=0) or after the intervention

(*intervention*$_t$=1); and *time after intervention*$_t$ is time in months since intervention (this variable is 0 for *t* before breakpoint). The reference month was set to January, with remaining months representing dummy variables (*Month*$_2$=February, *Month*$_3$=March, etc.).

Three points of interest were calculated for each antibiotic subclass: (1) pre-intervention trend (slope) of antibiotic use, (2) post-intervention change of baseline level representing an immediate effect and (3) post-intervention trend (slope) of antibiotic use representing a sustained effect of the intervention.[120] A P value of <0.05 was regarded as statistically significant. If coefficient β_1 was significantly different from 0, the pre-intervention trend of antibiotic use was deemed statistically significant. Similarly, if β_2 or β_3 were significantly different from 0, it was assumed that there had been a significant post-intervention change of baseline level or trend. The regressions were performed with Newey-West standard errors (automatic lag selection) to correct for possible autocorrelation.[194]

For national data, there was no obvious breakpoint and the following model was used instead:

$$y_t = \beta_0 + \beta_1 \cdot time_t + \sum_{i=2}^{12} \beta_i \cdot Month_{i_t} + \varepsilon_t,$$

where y_t is now DDD antibiotic consumption and $time_t$ the time in months from start (January 2005).

Wilcoxon test was used in the before-and-after analysis of mean monthly antibiotic consumption during pre- and post-intervention phases.

Analysis of changes in antibiotic resistance levels (II)

Monthly data on new cases of infection with the ESBL-producing *K. pneumoniae* outbreak strain were obtained from the Department of Clinical Microbiology at Uppsala University Hospital. An individual patient is counted only once in this dataset. Production of ESBLs was detected by the clavulanic acid synergy test.[46] ESBL-producing *K. pneumoniae* isolates were included in the outbreak statistics based on phenotypic antibiotic susceptibility patterns similar to that of the outbreak strain.

As an indicator of trends in the local resistance epidemiology, susceptibility patterns of all *E. coli*, *K. pneumoniae* and *P. aeruginosa* strains isolated from blood cultures performed at the hospital during the period of July 2005 to June 2009 were extracted and analyzed in 6-month periods. Reliable data on susceptibility patterns before June 2005 were not available. When the same bacteria were detected repeatedly from an individual patient, only the first strain isolated was considered. Antibiotic susceptibility testing was performed using disc diffusion technique [46] Strains isolated from urinary or fecal samples are typically not tested for the antibiotics targeted in the intervention and were therefore not analyzed in this study.

Results and discussion

International travel as a risk factor for ESBLs (Paper I)

One hundred healthy volunteers were included in the analysis. The median length of stay abroad was 2 weeks (range 1-26) and the most common reason for travel was vacation (n=89). Fifteen travellers visited more than one country and three of them more than one continent. In total, 35 countries were visited.

Twenty-four of 100 participants with negative pre-travel samples were colonized with ESBL-producing *E. coli* upon return (Tables 3 and 4). Travel to India was associated with significantly higher risk for acquisition of ESBLs than other destinations (88%, $P < 0.001$). A high risk of colonization with ESBL-producing *E. coli* was observed also for travelers to other countries in Asia (32%) and the Middle East (29%). Gastroenteritis during the travel was an additional risk factor ($P = 0.003$). Antibiotic treatment was not a significant risk factor in this study, but notably, all three participants treated for gastroenteritis with ciprofloxacin acquired ESBL-producing strains.

Continent or region	**No. of travellers**	**No. (%) of travelers positive for ESBL-producing *E. coli***
Africa	25	1 (4)
Asia (India excluded)	31	10 (32)
Central America	6	0 (0)
India	8	7 (88)
Middle East	14	4 (29)
North America	2	0 (0)
South America	1	0 (0)
Southern Europe	16	2 (13)

Table 3. Travel destinations and the rate of fecal colonization with ESBL-producing *E. coli* strains upon return.

CTX-M genes were detected in all ESBL-producing strains in our study, which is consistent with the fact that these enzymes dominate the ESBL pandemic (Table 5).[122] Further, the high prevalence of CTX-M-15 genes detected in our material reflects the worldwide spread of these enzymes.[52,195-197] All ESBL-producing strains isolated from travelers to India carried CTX-

M-15 genes, which is the only CTX-M type reported from India so far, and the majority of ESBL-producing isolates from other Asian countries carried CTX-M-14 genes, which is the dominating CTX-M type in this region.[52,198] These results support that the ESBL-producing isolates detected in our study were in fact acquired at the travel destinations.

Parameter	No. of travelers (%)	
	ESBL negative (n = 76)	**ESBL positive (n = 24)**
Male sex	35 (46)	10 (42)
Median age (*yr*)	42	47
Vegetarians	2 (3)	0 (0)
Median length of stay (*wk*)	2.0	2.0
Vacation	67 (88)	22 (92)
Business	10 (13)	2 (8)
Visiting friends or relatives	10 (13)	1 (4)
Staying at a hotel	61 (80)	20 (83)
Backpacking travellers	6 (8)	4 (17)
Staying with friends or relatives	9 (12)	5 (21)
Gastroenteritis during travel	17 (22)	13 (54)
Antibiotic treatment during travel	7 (9)	3 (12)

Table 4. Descriptive statistics on 100 Swedish travelers with negative pretravel rectal swabs for ESBL-producing Enterobacteriaceae. Seventy-six travelers were negative for ESBL-producing strains after their trip, whereas 24 carried ESBL-producing *E. coli*. The only statistically significant difference between the groups was gastroenteritis during travel (P = 0.003).

A high rate of co-resistance to potentially active antibiotics, other than cephalosporins, is a common feature of ESBL-producing *E. coli* strains[122] and was seen also in our material. Overall, nine strains were multi-drug resistant (resistant to three or more relevant antibiotic subclasses), which seriously limits therapeutic options in cases of a clinical infection with these bacteria. Although the level of carbapenem resistance among ESBL-producing *E. coli* and *K. pneumoniae* strains is still very low worldwide, it is worrisome that two isolates in our material displayed reduced susceptibility to these antibiotics. High susceptibility rates were demonstrated for nitrofurantoin and mecillinam.

Five of 21 who completed the follow-up after six months had persistent colonization with ESBLs. None of them reported a clinical infection or antibiotic use during the follow-up period.

Continent or region	No. of isolates				
	Group I			Group IV	
	CTX-M-1	CTX-M-15	CTX-M-9	CTX-M-14	CTX-M-27
Africa		1			
Asia (India excluded)		2	1	5	2
India		7			
Southern Europe	1	1			
Middle East		2	2		
Total	1	13	3	5	2

Table 5. Distribution of CTX-M genes detected in 24 strains of *E. coli* isolated from rectal swabs after foreign travel.

This is the first prospective study on foreign travel as a risk factor for colonization with ESBL-producing Enterobacteriaceae. Our results clearly demonstrate that travel to areas with a higher prevalence of strains producing ESBLs is a risk factor for the acquisition of ESBL-producing bacteria. The acquisition of ESBL-producing *E. coli* during foreign travel may be a significant source for increasing rates of colonization with ESBL-producing bacteria in Sweden and other countries with a low prevalence of ESBLs and a comparably low rate of consumption of antibiotics.

Considering the high rate of acquisition of ESBL-producing isolates and the extent of international travel in many countries, it is obvious that global efforts are needed to meet the emergence and spread of CTX-M enzymes and other antimicrobial resistance.

Antibiotic intervention at Uppsala University Hospital (Paper II)

The monthly use of cephalosporins (cefuroxime and cefotaxime) and piperacillin/tazobactam at Uppsala University Hospital during May 2005 to December 2007 is illustrated in Figure 1. The primary aim of the intervention was to reduce consumption of second- and third-generation cephalosporins. The secondary aim was to prevent increased use of fluoroquinolones and carbapenems, in order to prevent increased resistance to these antibiotics or other collateral damage. In the interrupted time series (ITS) analysis a post-intervention change of baseline level represents an immediate effect and a post-intervention trend (slope) represents a sustained effect of the intervention.

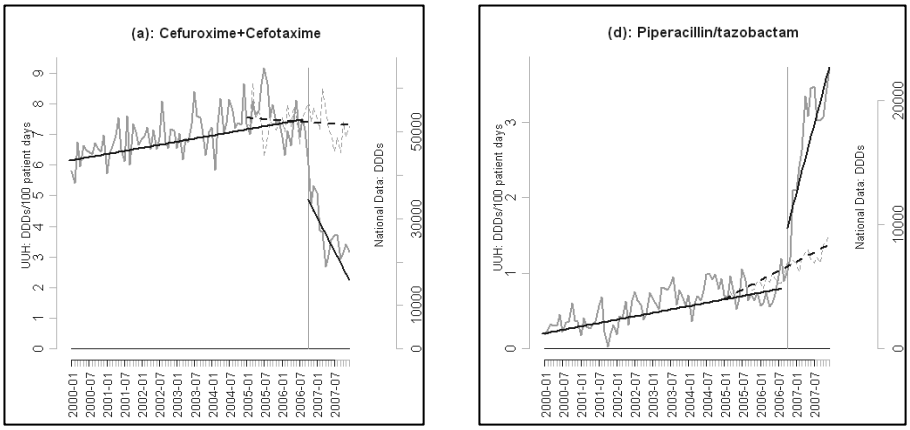


Figure 1. Segmented trend lines (grey) for monthly use of (a) cefuroxime and cefotaxime and (d) piperacillin/tazobactam at Uppsala University Hospital during January 2000 to December 2007. Regression lines (bold) represent mean monthly consumption. New recommendations for antibiotic treatment were introduced in October 2006 (vertical line). The corresponding national hospital antibiotic use during January 2005 to December 2007 is shown on the right y-axis, as well as national trend lines (scattered) and regression lines (scattered and bold).

Antibiotics	**Before-and-after analysis (12-month pre- and post-intervention phases)**			**Interrupted time series analysis**			
	Monthly pre-intervention use (mean)	**Change in mean**	**P**	**Change in level**	**P**	**Change in slope**	**P**
Cefuroxime, cefotaxime	73.7	- 35.7	<0.001	- 26.2	<0.001	- 2.0	<.0001
Ciprofloxacin, norfloxacin	45.8	- 5.8	0.045	- 13.5	<0.001	.77	<0.001
Imipenem, meropenem, ertapenem	24.9	4.5	NS	- 4.0	0.014	.56	<0.001
Piperacillin/ tazobactam	7.8	19.7	<0.001	8.0	0.0076	1.4	<0.001
Penicillin G	8.1	6.7	<0.001	7.3	<0.001	- .20	NS

Table 6. Changes in antibiotic consumption at Uppsala University Hospital after the antibiotic intervention, assessed with before-and-after analysis and interrupted time series analysis.

Following the antibiotic intervention there were dramatic immediate and sustained effects with respect to the primary target, i.e. the reduction of se-

cond- and third-generation cephalosporins (Table 6). Also, there was an immediate decrease in level for fluoroquinolones and carbapenems. However, positive slopes followed the immediate decrease of these antibiotics, indicating that the reduction might not be sustained.

The cephalosporins were largely replaced by piperacillin/tazobactam and to a lesser extent by penicillin G. Before-and-after analysis of mean monthly consumption during 12-month pre- and post-intervention phases demonstrated a 48% reduction in the use of cefuroxime and cefotaxime, a 253% increase of piperacillin/tazobactam and a 77% increase of penicillin G (Table 6). The reduction of cephalosporins, the increase of piperacillin/tazobactam and the immediate effects demonstrated for fluoroquinolones, carbapenems and penicillin G clearly differed from the national data.

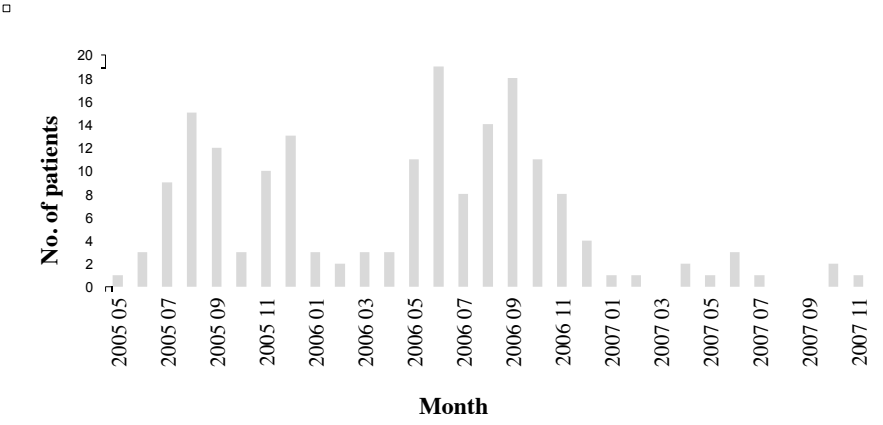


Figure 2. Monthly numbers of patients infected by the ESBL-producing *K. pneumoniae* outbreak strain at Uppsala University Hospital during May 2005 to December 2007. The antibiotic intervention started in October 2006.

Although the outbreak with ESBL-producing *K. pneumoniae* subsided during 2007 (Figure 2), the causal effect of the antibiotic intervention is difficult to assess because of an unknown natural course and other simultaneous actions, including hygienic measures. However, considering that the use of second- and third- generation cephalosporins has been identified repeatedly as a risk factor for ESBL infection,[26-29] and that several intervention studies report reduced prevalence of ESBL-producing *K. pneumoniae* after reduction of third-generation cephalosporin use,[103,111,112,114,199] we believe that the radical change in antibiotic-prescribing policies at our hospital contributed to the containment of the outbreak. No increased resistance to carbapenems, cephalosporins, gentamicin or piperacillin/tazobactam was detected during the follow-up period.

In conclusion, our study clearly shows that an educational approach can have a radical effect on antibiotic prescriptions at a tertiary hospital. We believe that the outbreak situation and the strong support from the hospital management facilitated the prompt and successful implementation of the new antibiotic guidelines. Further, the intervention could be performed efficiently by the pre-existing local Strama group.

Ertapenem for the treatment of ESBL-producing *E. coli* (Paper III)

Antibiotic concentrations during the kinetic time-kill experiments were well fitted to the targeted values. For both strains, there was a >5 $\log_{10}$ decrease in cfu/mL during the first 4 h of the experiments, less bacterial reduction (0.7 to 2.6 $\log_{10}$ cfu/mL) following the second dose of ertapenem and considerable regrowth at the end of the dose intervals (Figure 3). With ertapenem at concentrations simulating conventional dosage, endpoint bacterial concentrations were >2.5 $\log_{10}$ cfu/mL higher at 44 and 48 h in the ESBL-producing strain than in the native strain. Higher dosage of ertapenem against the ESBL-producing strain resulted in approximately 1 $\log_{10}$ lower bacterial concentrations at 44 and 48 h compared with the experiments simulating conventional dosage. Still, bacterial concentrations were approximately 1.5 $\log_{10}$ cfu/mL higher at these time points than during experiments with the native strain.

The ESBL-producing strain had 1.5 to 2-fold higher MIC values for ertapenem, meropenem and doripenem compared with the native strain, whereas the MIC for imipenem did not differ (Table 7). No significant MIC elevations were detected after kinetic time-kill experiments with the native strain. However, for the ESBL-producing strain increased ertapenem MICs were found in five endpoint samples (after three of five experiments simulating conventional dosage and two of four experiments with higher dosage). Ertapenem MICs were increased approximately by 40-fold in these populations to values within the range of 0.75 to 1.5 mg/L, which corresponds to intermediate susceptibility or resistance according to EUCAST definitions. In the endpoint population with ertapenem MIC of 0.75 mg/L MICs for meropenem and doripenem were not affected. In contrast, all four populations with ertapenem MICs ≥1 mg/L had increased MICs for meropenem (0.25-0.38 mg/L) and doripenem (0.094-0.25 mg/L). Still, all meropenem and doripenem MICs were within the range of susceptibility according to EUCAST definitions of clinical breakpoints. The imipenem MIC was not increased in any of the endpoint populations.

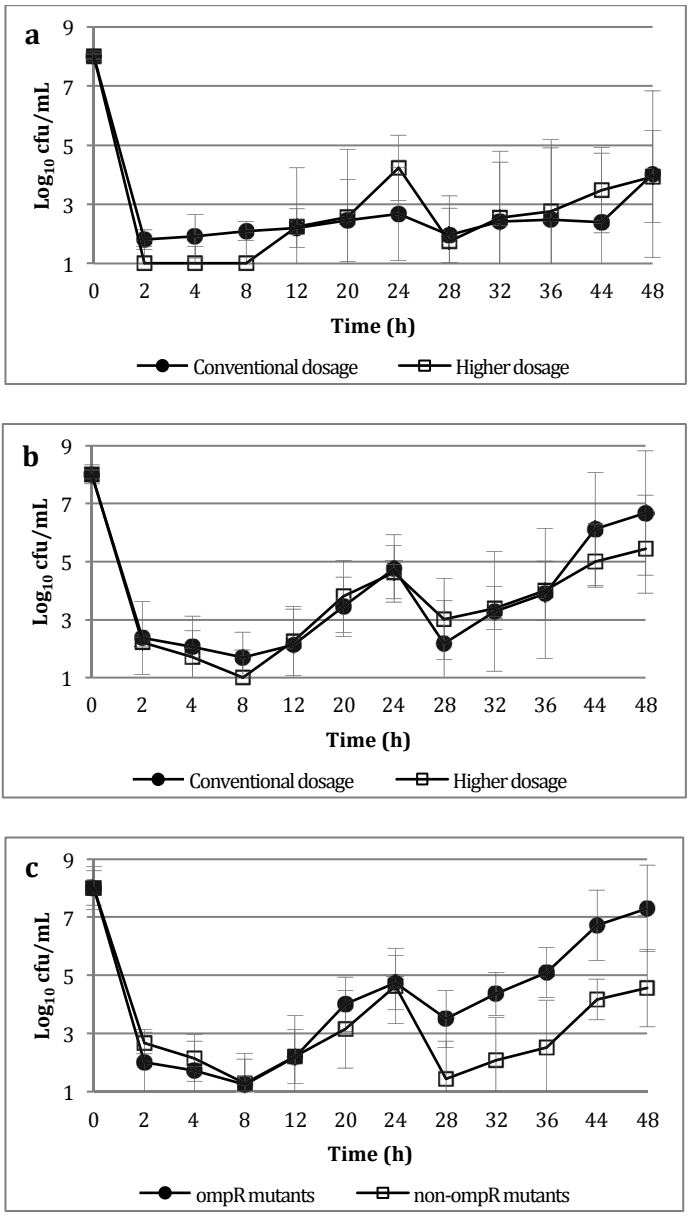


Figure 3. Results from kinetic time-kill experiments with (a) the native *E. coli* strain DA14781, (b) the ESBL-producing strain DA14833 and (c) subgroups of experiments with ESBL-producing strain DA14833: *ompR* mutants vs. non-*ompR* mutants. Each point represents the mean value of 3-5 experiments with ertapenem at concentrations similar to human pharmacokinetics with conventional and higher dosage. The error bars indicate the standard deviation.

Strains			MIC (mg/L)				Muta-tions
			ETP	MEM	IMP	DOR	
Native strain DA14781			0.012	0.023	0.19	0.016	–
ESBL-producing strain DA14833			0.023	0.032	0.19	0.023	–
ompR-mutated ESBL-producing strain DA16808			1	0.125	0.19	0.19	*ompR* R209C
48 h samples	**Dosage**	**Experiment**					
Native strain	Conventional dosage	1	0.006	0.023	0.25	0.023	ND
		2*	–	–	–	–	–
		3	0.004	0.023	0.19	0.016	ND
		4	0.004	0.023	0.25	0.023	ND
	Higher dosage	1	0.008	0.032	0.25	0.023	ND
		2	0.008	0.032	0.25	0.023	ND
		3	0.006	0.023	0.19	0.023	ND
ESBL-producing strain	Conventional dosage	1	0.012	0.023	0.19	0.023	ND
		2	0.016	0.032	0.25	0.032	ND
		3#	0.023/ 0.75	0.047	0.19	0.032	*ompR* R209C
		4	1	0.25	0.19	0.094	*ompR* R45S
		5	1	0.25	0.25	0.25	*ompR* D100E
	Higher dosage	1	0.023	0.064	0.25	0.032	ND
		2	0.064	0.047	0.125	0.032	ND
		3	1.5	0.38	0.25	0.19	*ompR* L112R
		4#	0.016/1	0.064/ 0.38	0.25	0.047/ 0.19	*ompR* Q204Stop

* no bacteria detected, # 2 populations.
ETP, ertapenem; MEM, meropenem, IMP, imipenem, DOR, doripenem; ND, not determined.

Table 7. MIC values and detected mutations for the native *E. coli* strain DA14781, ESBL-producing strain DA14833 and *ompR*-mutated ESBL-producing strain DA16808, prior to experiments and as determined in 48 h samples after kinetic time-kill experiments.

OmpR mutations were detected in all five populations with elevated ertapenem MICs (Table 7). Four of the mutations resulted in amino acid changes and one resulted in a premature stop codon. No *ompF* or *ompC* mutations were detected. Based on these results, we performed a subanalysis of the kinetic time-kill results for the ESBL-producing strain. This analysis revealed that experiments in which *ompR* mutants were detected in endpoint samples showed lower bacterial reduction after the second dose of

ertapenem than experiments with no MIC elevation detected followed by >2 $\log_{10}$ higher bacterial concentrations throughout the experiments (Figure 3c).

Static time-kill experiments with the native strain, the ESBL-producing strain and the *ompR* R209C ESBL-producing mutant were performed at ertapenem concentrations ranging from C_{min} to C_{max}. Rapid initial killing was accomplished against both the native and the ESBL-producing strain at all concentrations used. Bacterial concentrations at 24 h were 2 to 5 $\log_{10}$ cfu/mL lower in the native strain than in the ESBL-producing strain at three of the concentrations used and 0.5 to 1.5 $\log_{10}$ lower at other concentrations. For the ESBL-producing strain, all experiments resulted in less than 3 $\log_{10}$ bacterial reduction after 24 h compared to the starting inoculum. Further, the bactericidal activity of ertapenem was significantly lower against the *ompR*-mutated strain compared to the original ESBL-producing strain. Following significant regrowth, endpoint bacterial concentrations for the *ompR*-mutated ESBL-producing strain were similar or higher than the starting inoculum in all experiments with ertapenem at concentrations ≤5.5 mg/L.

In summary, we have shown that exposure of an ESBL-producing *E. coli* to ertapenem at concentrations simulating human pharmacokinetics is associated with frequent selection of porin-deficient mutants. Experiments with *ompR* mutants detected in endpoint samples showed much lower bacterial reduction after the second dose of ertapenem compared with other experiments with this strain. This finding indicates that pre-existing *ompR* mutants were selected during experiments. Considerable regrowth and less bacterial killing following the second ertapenem dose compared to the initial killing occurred also in experiments with unchanged MICs in endpoint samples. These observations might have been caused by formation of surviving persister cells during the experiments.[200]

The fact that porin-mutated subpopulations were only detected after experiments with the ESBL-producing strains might be explained in part by insufficient target attainment for these isolates at the antibiotic concentrations used. Conventional ertapenem dosage results in concentrations exceeding the MIC (T > MIC) of approximately 40% against an *ompR*-mutated ESBL-producing *E. coli* with MIC 1 mg/L. Reduced expression of OmpF and OmpC in the native strain results in an ertapenem MIC of 0.25 mg/L.[100] Therefore, even with conventional dosage the T > MIC will exceed 70% against *ompR*-mutated native *E. coli,* which might be sufficient to prevent selection of these subpopulations.

The clinical relevance of these findings has yet to be established. We used high bacterial inocula in this study and the experiments were performed in vitro where the effects of the immune system are not taken into account. Although ertapenem resistance is still rare in *E. coli* and the porin-deficient isolates detected in our study were not highly resistant, inappropriate use of ertapenem should be avoided to minimize the risk for selection of ESBL-producing bacteria with reduced susceptibility to carbapenems.

Antibiotic combination therapy for VIM- and NDM-producing *K. pneumoniae* (Paper IV)

All strains were susceptible to colistin according to the EUCAST breakpoints, whereas susceptibilities to the other antibiotics varied substantially (Table 8). Colistin, alone and in any combination with other antibiotics, was associated with a >3 $\log_{10}$ decrease in cfu/mL during the first hour of experiments. However, considerable regrowth occurred for most combinations and during experiments with colistin alone. Synergistic and bactericidal effects after 24 h were found for combinations including aztreonam, meropenem, fosfomycin, rifampin and colistin (Table 9). Illustrative time-kill curves for NDM-KP IR62E are shown in Figure 4.

Antibiotic	**VIM-KP T14789**		**VIM-KP ÖN-2211**		**NDM-KP IR8**		**NDM-KP IR62E**	
	MIC	SIR	MIC	SIR	MIC	SIR	MIC	SIR
Aztreonam	2	I	4	I	>256	R	>256	R
Ciprofloxacin	> 32	R	4	R	>32	R	>32	R
Colistin	0.125	S	0.125	S	0.125	S	0.125	S
Daptomycin	> 256	-	> 256	-	> 256	-	> 256	-
Fosfomycin	32	S	4	S	256	R	48	R
Meropenem	>32	R	2	S	4	I	>32	R
Rifampicin	> 32	-	> 32	-	32	-	>32	-
Telavancin	256	-	256	-	256	-	256	-
Tigecycline	2	I	1	S	2	I	3	R
Vancomycin	> 256	-	> 256	-	> 256	-	> 256	-

SIR, classification according to EUCAST definitions; S, susceptible; I, intermediate susceptible; R, resistant; -, not defined.

Table 8. Antibiotic susceptibilities of VIM- and NDM-producing *K. pneumoniae* strains (mg/L) expressed as MIC values and classification according to the clinical breakpoints defined by EUCAST.

Aztreonam + colistin and aztreonam + meropenem + colistin was bactericidal against the VIM-KP strains, both of which displayed intermediate susceptibility for aztreonam. These combinations resulted in more rapid killing than aztreonam alone and less regrowth than colistin alone, and proved synergistic effect against VIM-KP ÖN-2211. Against the NDM-KP strains, aztreonam + meropenem + colistin demonstrated synergistic effect despite high-

level resistance to aztreonam and non-susceptibility to meropenem in these strains. However, the effect was not bactericidal.

The combination fosfomycin + colistin demonstrated synergy and bactericidal effect against one of the VIM-KP strains and both NDM-KP strains despite that the latter were resistant to fosfomycin. Fosfomycin + meropenem + colistin showed synergistic effect against all strains and bactericidal effect against 3 of 4 strains.

Rifampin + colistin demonstrated synergy and >2.5 $\log_{10}$ decrease in cfu/mL after 24 h compared to the starting inoculum against both NDM-strains. The triple combination rifampin + meropenem + colistin resulted in synergistic and bactericidal effects against all strains despite high MICs for rifampin in all strains and that two of the strains were highly resistant to meropenem alone.

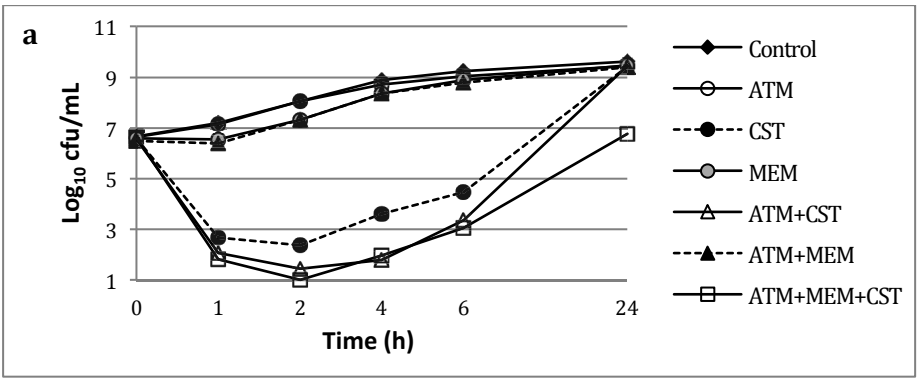
The antibacterial activity of tigecycline, ciprofloxacin, daptomycin, vancomycin and telavancin, alone and in combination with colistin, against the VIM-KP strains was evaluated in time-kill experiments performed in triplicate. However, no synergistic or bactericidal activity was detected with these antibiotic regimens and they were not further studied against the NDM-KP strains.

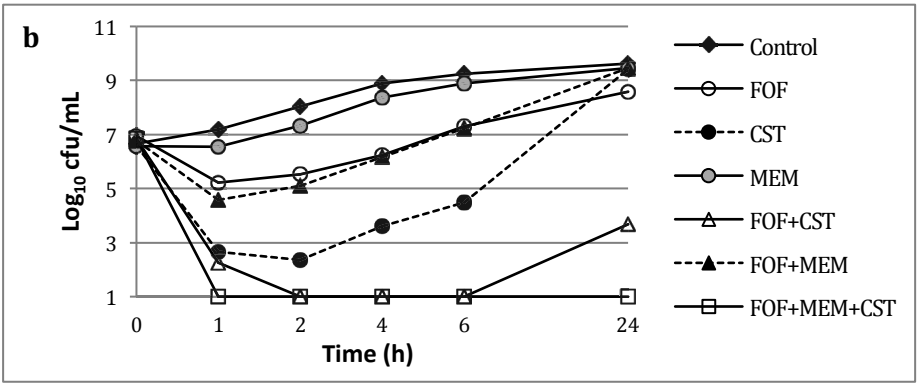
Antibiotic	**VIM-KP T14789**	**VIM-KP ÖN-2211**	**NDM-KP IR8**	**NDM-KP IR62E**
ATM+CST		+		
ATM+MEM				
ATM+MEM+CST		+	+	+
MEM+CST				+
FOF+CST	+		+	+
FOF+MEM				
FOF+MEM+CST	+	+	+	+
RIF+CST			+	+
RIF+MEM+CST	+	+	+	+

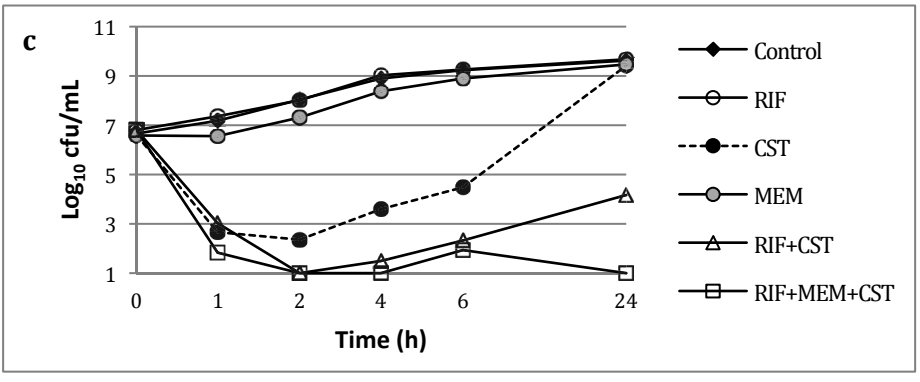
+, synergy.

ATM, aztreonam; CST, colistin; MEM, meropenem; FOF, fosfomycin; RIF, rifampin.

Table 9. Results from time-kill experiments with combinations including aztreonam, fosfomycin, meropenem, rifampin and colistin against VIM- and NDM-producing *K. pneumoniae*. Synergy (≥2 $\log_{10}$ reduction in cfu/mL compared to the most effective individual drug) is depicted with "+". Bactericidal effect (≥3 $\log_{10}$ decrease in cfu/mL after 24 h compared to the starting inoculum) is highlighted with grey.







ATM, aztreonam; CST, colistin; MEM, meropenem; FOF, fosfomycin; RIF, rifampin

Figure 4. Results from time-kill experiments for NDM-producing *Klebsiella pneumoniae* strain NDM-KP IR62E exposed to (a) aztreonam combinations, (b) fosfomycin combinations and (c) rifampin combinations. Each point represents the mean value of two experiments.

Overall, rifampicin + meropenem + colistin was the most effective regimen in this study. A similar triple combination of rifampin + doripenem + colistin has in a previous study shown bactericidal effect against two KPC-producing *K. pneumoniae* strains in time-kill experiments.[130] Rifampin + colistin, which did not demonstrate bactericidal effect against any of the strains has been effective against the majority of KPC-producing *K. pneumoniae* isolates in previous time-kill and chequerboard studies.[171,201]

Fosfomycin + meropenem + colistin showed synergistic effect against all four strains and was bactericidal against three of the four strains. Fosfomycin + colistin had synergistic and bactericidal effect against both NDM-KP strains and one VIM-KP strain. This combination has previously demonstrated synergy against 2 of 10 NDM-producing *Klebsiella* spp. in a study with chequerboard method.[172]

Aztreonam + colistin and aztreonam + meropenem + colistin was bactericidal against both VIM-KP strains. The triple combination also had synergistic effect against the NDM-KP strains despite their high resistance to aztreonam and non-susceptibility to meropenem. One possible explanation for this finding is that aztreonam acts as a competitive MBL-inhibitor and meropenem as a competitive inhibitor for other co-produced beta-lactamases, such as ESBLs or AmpC beta-lactamases, that hydrolyze aztreonam but not meropenem.[24,173,176,177] Aztreonam + meropenem did not demonstrate synergy. However, addition of colistin to this combination resulted in improved antibacterial activity, probably because of increased permeability of the other antibiotics.[169]

Some of the antibiotic combinations that have been effective against carbapenamase-producing *K. pneumoniae* in previous studies and been recommended for clinical use showed no synergistic or bactericidal effects in our experiments. For example, colistin and meropenem was not bactericidal against any of these strains. Tigecycline + colistin, which has shown synergistic and bacteriostatic effects against KPC-producing *K. pneumoniae*,[171,202] was not effective against the VIM-KP isolates in our study. Also, meropenem + fosfomycin, which demonstrated bactericidal effects against the majority of KPC-producing *K. pneumoniae* isolates in a previous time-kill study,[166166] was not effective in our experiments.

In summary, we have found several double and triple combinations of aztreonam, fosfomycin, meropenem, rifampin and colistin with synergistic and bactericidal effects against MBL-producing *K. pneumoniae*. More studies are needed to confirm the efficacy and evaluate the clinical use of these combinations. Still, our results are encouraging and indicate that there is a largely unexplored antibacterial potential of antibiotic combinations against MBL-producing *K. pneumoniae*.

General discussion and future perspectives

The results from these studies have contributed to a better understanding of the dynamics of multidrug-resistant *E. coli* and *K. pneumoniae* in relation to epidemiology, selection and susceptibility to antibiotic therapy.

This thesis includes the first prospective study to show how easily multidrug-resistant Enterobacteriaceae spread between countries. Notably, the vast majority of the volunteers in this study were ordinary short-time travelers on vacation. It is estimated that 350,000 Swedes travel to Thailand every year, and based on our findings, approximately 100,000 of these individuals will acquire ESBL-producing *E. coli*. Although none of the volunteers in our study suffered from clinical infections with these bacteria during the follow-up period, colonization with ESBL-producing Enterobacteriaceae is known to increase the risk for future clinical infections with these strains.

Therefore, patients with severe infections that are likely to be caused by Gram-negative pathogens should be asked for a history of recent travel to areas with higher prevalence of resistant bacteria. Additional cultures might be appropriate for these patients and the decision of empirical antibiotic therapy might have to be adjusted to cover antimicrobial-resistant pathogens. Knowing that a large number of healthy people in the community are colonized with resistant bacteria, hygienic precautions sufficient to prevent the spread of multidrug-resistant bacteria must be used for every patient admitted to a hospital or treated at other health care facilities. To do this, more health care workers and a sufficient number of isolation rooms at the hospitals are required. Still, the spread of antibiotic resistance cannot be prevented and therefore international cooperation is essential to meet the emergence of multidrug resistance.

Before conducting an antibiotic intervention, appropriate replacement antibiotics should be considered and be selected to minimize the risk for collateral damage. This approach was successfully achieved in our intervention in that the consumption of fluoroquinolones and carbapenems did not increase. An unintended effect was that the cephalosporins were replaced mainly by piperacillin/tazobactam rather than penicillin G. To date, resistance to piperacillin/tazobactam has not increased at our hospital. However, replacing a broad-spectrum antibiotic by another one does not solve the problem in the long run but will eventually cause other resistance. Therefore, continuous efforts are needed to reduce the total antibiotic use and promote a shift to narrow-spectrum antibiotics.

Selection of porin-deficient subpopulations with non-susceptibility to ertapenem frequently emerged in ESBL-producing *E. coli* exposed to ertapenem at concentrations simulating human pharmacokinetics. Importantly, these subpopulations still had relatively low MIC values for all carbapenems and thus might be treatable with ertapenem at higher concentrations. Combination with a beta-lactamase inhibitor effective against ESBLs would be an alternative approach to improve the antibacterial activity of ertapenem and reduce selection of porin-deficient ESBL-producing subpopulations. Appropriate dosage regimens or combinations might prolong the lifespan of new antibiotics. Therefore, when new antibiotics are developed, the preclinical development should not only include studies on their efficacy but also the risk for emerging resistance with alternative dosage regimens or combinations.

The results from our time-kill experiments indicate that there are huge potential benefits for antibiotic combination therapy against carbapenemase-producing *K. pneumoniae*. Antibiotics, to which the bacteria were resistant, accomplished synergistic and bactericidal activity when combined. The strains used in our study were susceptible to colistin and all antibiotic combinations demonstrating synergistic and bactericidal effects included this drug. Although still rare, colistin resistance has increased in *K. pneumoniae* during recent years and few effective therapeutic options remain for infections with these bacteria. However, unexplored combinations of two or three antibiotics, which have insufficient antibacterial activity when used alone, might still accomplish antibacterial activity against these strains.

In an era of increasing antibiotic resistance and no new antibiotics, combination therapy will be increasingly used for infections with Gram-negative bacteria. Treating physicians must have knowledge on the local resistance epidemiology, prevalent resistance mechanisms and the properties of existing antibiotics. The choice of combination therapy for resistant Gram-negative bacteria will often be based on results from in vitro studies because prospective clinical studies are not easily performed with these patients and retrospective studies are too slow. Therefore, well-designed in vitro studies are urgently needed to supplement other studies. These studies should evaluate antibiotic combinations that might be considered in clinical practice, taking into account toxicity and other factors, and use clinically relevant static antibiotic concentrations or concentrations simulating human pharmacokinetics.

Sammanfattning på svenska

Escherichia coli och *Klebsiella pneumoniae* tillhör gruppen Gram-negativa bakterier. De utgör en viktig del av tarmens normalflora men kan också ge upphov till urinvägsinfektioner, sepsis och andra infektioner. *E. coli* är den mest aggressiva av dessa bakterier och är den vanligaste orsaken till urinvägsinfektioner även hos i övrigt friska patienter. *K. pneumoniae* orsakar framförallt infektioner hos patienter med andra bakomliggande sjukdomar eller nedsatt immunförsvar.

Cefalosporiner och karbapenemer är förstahandsval vid behandling av allvarliga infektioner med dessa bakterier. Resistens mot dessa antibiotika orsakas nästan uteslutande av att bakterierna bildar enzymer, ESBL eller karbapenemaser, som förändrar den aktiva delen av antibiotikamolekylen genom hydrolys. Detta gör att antibiotikamolekylen inte längre kan binda och utöva sin effekt på målmolekylen i bakterien. ESBL orsakar resistens mot cefalosporiner medan karbapenemaser orsakar resistens mot både cefalosporiner och karbapenemer. Andra mekanismer, till exempel förändringar i bakteriernas yttre membran som förhindrar att antibiotika når fram till målmolekylen, kan också bidra till resistens mot dessa antibiotika.

Resistenta bakterier ökar i antal under antibiotikabehandling (selekteras) därför att de i närvaro av antibiotika har en konkurrensfördel gentemot de känsliga bakterierna i normalfloran. Därför medför antibiotikabehandling en ökad risk för framtida infektioner med resistenta bakterier hos den enskilda patienten och ökad risk för spridning av resistenta bakterier till andra individer.

Förekomsten av ESBL har ökat kraftigt över hela världen under de senaste tio åren men varierar stort mellan olika länder och världsdelar. Från Indien och vissa länder i södra Europa rapporteras att mer än hälften av *E. coli* och *K. pneumoniae* producerar ESBL medan Sverige fortfarande har en relativt låg nivå (<3%). Karbapenemaser förekommer i mycket lägre utsträckning än ESBL men har orsakat sjukhusutbrott i olika delar av världen sedan början av 2000-talet. På senare tid har man sett en oroväckande ökning av karbapenemaser av typen metallo-betalaktamaser (MBL) som ger höggradig resistens mot karbapenemer. MBL-producerande bakterier är svårbehandlade därför att de i mycket hög utsträckning är multiresistenta (resistenta mot tre eller flera relevanta antibiotikagrupper).

Den övergripande målsättningen med avhandlingen var att öka kunskapen om ESBL- och karbapenemas-producerande *E. coli* and *K. pneumoniae* med avseende på spridning, selektion och känslighet för behandling med antibiotika.

Arbete I visade att ESBL-producerande *E. coli* sprids lätt mellan länder med hjälp av resenärer. Tjugofyra av 100 friska frivilliga som inkluderades i studien blev bärare av ESBL-producerande *E. coli* i tarmfloran under resor till länder utanför norra Europa. Risken var högst för resenärer som hade varit i Indien (7 av 8) och för resenärer som hade varit magsjuka under resan. En hög andel blev bärare även efter resa till övriga länder i Asien (10 av 31) och till Mellanöstern (4 av 14). Vid uppföljningen efter sex månader var 5 av 21 ESBL-positiva fortfarande bärare av ESBL-producerande *E. coli*.

Arbete II beskrev en antibiotikaintervention som genomfördes på Akademiska sjukhuset 2006 under ett stort utbrott med ESBL-bildande *K. pneumoniae*. Syftet var att minska användningen av cefalosporiner som selekterar fram ESBL-producerande bakterier i större utsträckning än andra antibiotika. Förändringen av antibiotikaförskrivningen på sjukhuset analyserades med så kallad tidsserieanalys (ITS, Interrupted Time Series analysis). Användningen av cefalosporiner halverades inom loppet av några månader medan användningen av ersättningspreparatet piperacillin/tazobactam ökade med 253%. Utbrottet avtog strax efter detta, men det är svårt att dra några säkra slutsatser om betydelsen av antibiotikainterventionen eftersom samtidiga vårdhygieniska insatser och andra kända eller okända faktorer också kan ha påverkat förloppet.

Arbete III är en in vitro studie där vi studerade effekten av ertapenem, en av karbapenemerna, mot ESBL-producerande *E. coli*. Ertapenem används ofta vid urinvägsinfektioner orsakade av dessa bakterier. Resistensen mot ertapenem har ökat på senare år, särskilt bland ESBL-producerande stammar. Våra resultat talar för att ESBL-produktion, som normalt sett inte orsakar resistens mot ertapenem, kan bidra till nedsatt känslighet mot ertapenem om bakterien dessutom har resistensmekanismer som minskar antibiotikas möjligheter att penetrera bakteriens yttre membran. Efter 5 av 9 experiment med ESBL-producerande *E. coli* som exponerades for ertapenem kunde vi efter 48 timmar påvisa bakterier med nedsatt känslighet för ertapenem. I samtliga fall fanns mutationer i bakteriernas DNA som orsakar minskad möjlighet för ertapenem att penetrera det yttre bakteriemembranet (minskat uttryck av så kallade poriner).

Målsättningen med Arbete IV var att hitta effektiva kombinationer av antibiotika för behandling av MBL-producerande *K. pneumoniae*. Sammanlagt genomfördes mer än 200 experiment med 14 olika antibiotikakombinationer mot fyra MBL-producerande stammar. Sju av antibiotikakombinationerna var effektiva trots att bakterierna i många fall var resistenta mot ett eller flera av de preparat som ingick i kombinationen. Flera antibiotikakombinationer som har haft effekt i andra studier var inte effektiva i våra försök, vilket kan

bero på att man tidigare nästan uteslutande har inkluderat bakterier som producerar en annan typ av karbapenemaser (KPC). Kliniska studier är svåra att genomföra på patienter infekterade med multiresistenta bakterier och val av antibiotikabehandling måste ofta baseras på resultat från in vitro studier i dessa fall. Därför behövs fler in vitro studier som kan bekräfta våra resultat och hitta andra effektiva kombinationer för behandling av MBL-producerande stammar.

Sammanfattningsvis visar våra resultat att:

I. ESBL-producerande bakterier sprids lätt över världen med hjälp av resenärer.
II. Det går att förändra antibiotikaförskrivningen snabbt och radikalt på ett stort universtetssjukhus, åtminstone i en utbrottssituation.
III. Mutanter med nedsatt känslighet för ertapenem uppkommer ofta in vitro under pågående behandling av ESBL-bildande *E. coli.*
IV. Kombinationer av två eller tre antibiotika kan vara effektiva mot multiresistenta *K. pneumoniae* trots att bakterierna är resistenta mot ett eller flera av de enskilda preparaten.

Acknowledgements

This thesis is the result of many people's efforts. Thank you all! In particular, I am deeply grateful to:

The **travelers** who volunteered to share some of their time and feces. You´re famous now!

My **colleagues and friends at the "clinic"**, for taking care of the patients while I have been gone. I guess I´ll see you around, it's payback time...

Otto Cars, wherever you are, for believing in me and opening the doors to the world of antibiotics and resistance and for teaching me how to translate complicated research findings into something that might actually be understood and used.

Elisabeth Löwdin, for your energy, brilliant ideas, numerous cups of espresso and our talks about research, patients and everything else that has to do with life and the complexity of being an infectious disease physician.

Åsa Melhus, for sharing some of your great knowledge on microbiology and resistance. Thank you for being honest and always crystal clear in our discussions, although this has sometimes frightened me.

Britt-Marie Eriksson, for your courage to participate in the implementation of the antibiotic intervention and for your efforts to keep the local Strama group intact during my parental leave.

Jan Sjölin, for encouraging me to start my research career early on, for your remarkable patience with me during the (almost) never-ending work on Gram-negative meningitis and for introducing me to the intrinsic rules and conflicts of the university.

Birgitta Sembrant, for understanding and guiding me through the intrinsic rules of the university (and always with a smile, too).

Malin Vretblad Plesse, for your help with questionnaires, cinema tickets and other things during the travel study.

Pernilla Lagerbäck, for being present at the laboratory, actually reading and answering my e-mails and contributing with valuable discussions and comments, especially on the synergy project.

Matti Karvanen, for the unforgettable last-minute calculations on the appropriate pump flow in the ertapenem project and for thoroughly answering all my questions on colistin during the synergy project.

Charlotte Annerstedt, Juliana Larsson, Sabina Fontana, Rachel Hickman, Petter Forsberg and **Christer Malmberg** at the Antibiotic Research Unit for your cooperation and excellent laboratory work. Without your help, this thesis would never have been brought to completion.

Elisabet Nielsen, for always taking the time to help me with pharmacokinetic issues on short notice.

Linus Sandegren and **Marlen Adler**, for fruitful collaboration during the long-lasting work on the ertapenem study.

Christian Giske, for bringing aztreonam and some of the world's most resistant strains to the antibiotic combination study and for providing rapid and valuable input throughout this project.

Bodil Svennblad, for much needed help with the statistics, particularly with the interrupted time series analysis.

Birgitta Lytsy, Angela Lagerqvist Vidh, Anna Schwan and others at the Department of Clinical Microbiology for your help with the detection of ESBLs in the travel study and for providing data on the ESBL outbreak.

My old, new, singing and wine-loving **friends**, who I hope to see a lot more of now.

Hedvig, my not so understanding wife (when it comes to doing research during nights and weekends) and **Vilgot** and **Valter**, my sons, cats, cars, wolves, knights, robots or whatever you like. You´ve helped me to remember what is important in life during these years. I love you.

This work was funded in part by unrestricted grants from Strama (Paper I), Merck and Co. (Paper I) and the Family Olinder-Nielsen Foundation (III).

References

1. de Kraker, M.E.*, et al.* Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to Escherichia coli resistant to third-generation cephalosporins. *J Antimicrob Chemother* **66**, 398-407 (2011).
2. Miyakis, S., Pefanis, A. & Tsakris, A. The challenges of antimicrobial drug resistance in Greece. *Clin Infect Dis* **53**, 177-184.
3. Hsueh, P.R.*, et al.* Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. *J Infect* **63**, 114-123.
4. Rossi, F. The challenges of antimicrobial resistance in Brazil. *Clin Infect Dis* **52**, 1138-1143.
5. Schwaber, M.J. & Carmeli, Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. *J Antimicrob Chemother* **60**, 913-920 (2007).
6. Schwaber, M.J.*, et al.* Clinical and economic impact of bacteremia with extended- spectrum-beta-lactamase-producing Enterobacteriaceae. *Antimicrob Agents Chemother* **50**, 1257-1262 (2006).
7. Paterson, D.L.*, et al.* Antibiotic therapy for Klebsiella pneumoniae bacteremia: implications of production of extended-spectrum beta-lactamases. *Clin Infect Dis* **39**, 31-37 (2004).
8. So, A.D., Ruiz-Esparza, Q., Gupta, N. & Cars, O. 3Rs for innovating novel antibiotics: sharing resources, risks, and rewards. *BMJ* **344**, e1782 (2012).
9. Hirsch, E.B. & Tam, V.H. Detection and treatment options for Klebsiella pneumoniae carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. *J Antimicrob Chemother* **65**, 1119-1125.
10. Hawkey, P.M. Molecular epidemiology of clinically significant antibiotic resistance genes. *Br J Pharmacol* **153 Suppl 1**, S406-413 (2008).
11. Zinner, S.H. Overview of antibiotic use and resistance: setting the stage for tigecycline. *Clin Infect Dis* **41 Suppl 5**, S289-292 (2005).
12. Colodner, R.*, et al.* Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. *Eur J Clin Microbiol Infect Dis* **23**, 163-167 (2004).

13. Tacconelli, E., De Angelis, G., Cataldo, M.A., Pozzi, E. & Cauda, R. Does antibiotic exposure increase the risk of methicillin-resistant Staphylococcus aureus (MRSA) isolation? A systematic review and meta-analysis. *J Antimicrob Chemother* **61**, 26-38 (2008).
14. Hillier, S.*, et al.* Prior antibiotics and risk of antibiotic-resistant community-acquired urinary tract infection: a case-control study. *J Antimicrob Chemother* **60**, 92-99 (2007).
15. Bronzwaer, S.L.*, et al.* A European study on the relationship between antimicrobial use and antimicrobial resistance. *Emerg Infect Dis* **8**, 278-282 (2002).
16. Livermore, D.M. Beta-lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* **8**, 557-584 (1995).
17. Paterson, D.L. & Bonomo, R.A. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* **18**, 657-686 (2005).
18. Livermore, D.M. Defining an extended-spectrum beta-lactamase. *Clin Microbiol Infect* **14 Suppl 1**, 3-10 (2008).
19. Falagas, M.E. & Karageorgopoulos, D.E. Extended-spectrum beta-lactamase-producing organisms. *J Hosp Infect* **73**, 345-354 (2009).
20. Knothe, H., Shah, P., Krcmery, V., Antal, M. & Mitsuhashi, S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. *Infection* **11**, 315-317 (1983).
21. Ambler, R.P. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci* **289**, 321-331 (1980).
22. Bush, K., Jacoby, G.A. & Medeiros, A.A. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* **39**, 1211-1233 (1995).
23. Walsh, T.R. Clinically significant carbapenemases: an update. *Curr Opin Infect Dis* **21**, 367-371 (2008).
24. Walsh, T.R., Toleman, M.A., Poirel, L. & Nordmann, P. Metallo-beta-lactamases: the quiet before the storm? *Clin Microbiol Rev* **18**, 306-325 (2005).
25. Tumbarello, M.*, et al.* Bloodstream infections caused by extended-spectrum-beta-lactamase-producing Klebsiella pneumoniae: risk factors, molecular epidemiology, and clinical outcome. *Antimicrob Agents Chemother* **50**, 498-504 (2006).
26. Rodriguez-Bano, J.*, et al.* Risk-factors for emerging bloodstream infections caused by extended-spectrum beta-lactamase-producing Escherichia coli. *Clin Microbiol Infect* **14**, 180-183 (2008).
27. Rodriguez-Bano, J.*, et al.* Clinical and molecular epidemiology of extended-spectrum beta-lactamase-producing Escherichia coli as a cause of nosocomial infection or colonization: implications for control. *Clin Infect Dis* **42**, 37-45 (2006).
28. Paterson, D.L.*, et al.* International prospective study of Klebsiella pneumoniae bacteremia: implications of extended-spectrum beta-

lactamase production in nosocomial Infections. *Ann Intern Med* **140**, 26-32 (2004).
29. Lautenbach, E., Patel, J.B., Bilker, W.B., Edelstein, P.H. & Fishman, N.O. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* **32**, 1162-1171 (2001).
30. Skippen, I.*, et al.* Epidemiology of infections caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella spp.: a nested case-control study from a tertiary hospital in London. *J Hosp Infect* **64**, 115-123 (2006).
31. Martinez, J.A.*, et al.* Prior use of carbapenems may be a significant risk factor for extended-spectrum beta-lactamase-producing Escherichia coli or Klebsiella spp. in patients with bacteraemia. *J Antimicrob Chemother* **58**, 1082-1085 (2006).
32. Ho, P.L.*, et al.* Community emergence of CTX-M type extended-spectrum beta-lactamases among urinary Escherichia coli from women. *J Antimicrob Chemother* **60**, 140-144 (2007).
33. Pitout, J.D., Nordmann, P., Laupland, K.B. & Poirel, L. Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. *J Antimicrob Chemother* **56**, 52-59 (2005).
34. Rodriguez-Bano, J.*, et al.* Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing Escherichia coli in nonhospitalized patients. *J Clin Microbiol* **42**, 1089-1094 (2004).
35. Rodriguez-Bano, J., Lopez-Cerero, L., Navarro, M.D., Diaz de Alba, P. & Pascual, A. Faecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli: prevalence, risk factors and molecular epidemiology. *J Antimicrob Chemother* **62**, 1142-1149 (2008).
36. Pitout, J.D.*, et al.* Molecular epidemiology of CTX-M-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob Agents Chemother* **51**, 1281-1286 (2007).
37. Mathai, D., Rhomberg, P.R., Biedenbach, D.J. & Jones, R.N. Evaluation of the in vitro activity of six broad-spectrum beta-lactam antimicrobial agents tested against recent clinical isolates from India: a survey of ten medical center laboratories. *Diagn Microbiol Infect Dis* **44**, 367-377 (2002).
38. Hawser, S.P.*, et al.* Emergence of high levels of extended-spectrum-beta-lactamase-producing gram-negative bacilli in the Asia-Pacific region: data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program, 2007. *Antimicrob Agents Chemother* **53**, 3280-3284 (2009).
39. Winokur, P.L., Canton, R., Casellas, J.M. & Legakis, N. Variations in the prevalence of strains expressing an extended-spectrum beta-

lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. *Clin Infect Dis* **32 Suppl 2**, S94-103 (2001).
40. Hirakata, Y.*, et al.* Regional variation in the prevalence of extended-spectrum beta-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002). *Diagn Microbiol Infect Dis* **52**, 323-329 (2005).
41. Baquero, F.*, et al.* In vitro susceptibilities of aerobic and facultatively anaerobic gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: 2005 results from Study for Monitoring Antimicrobial Resistance Trends (SMART). *Surg Infect (Larchmt)* **10**, 99-104 (2009).
42. Norskov-Lauritsen, N., Marchandin, H. & Dowzicky, M.J. Antimicrobial susceptibility of tigecycline and comparators against bacterial isolates collected as part of the TEST study in Europe (2004-2007). *Int J Antimicrob Agents* **34**, 121-130 (2009).
43. Goossens, H. & Grabein, B. Prevalence and antimicrobial susceptibility data for extended-spectrum beta-lactamase- and AmpC-producing Enterobacteriaceae from the MYSTIC Program in Europe and the United States (1997-2004). *Diagn Microbiol Infect Dis* **53**, 257-264 (2005).
44. Anonymous. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). http://www.ecdc.europa.eu (2010).
45. Anonymous. SWEDRES 2011, a report on antimicrobial utilisation and resistance in humans in Sweden. http://www.smittskyddsinstitutet.se. (2012).
46. Lytsy, B.*, et al.* The first major extended-spectrum beta-lactamase outbreak in Scandinavia was caused by clonal spread of a multiresistant Klebsiella pneumoniae producing CTX-M-15. *APMIS* **116**, 302-308 (2008).
47. Ransjo, U.*, et al.* Hospital outbreak control requires joint efforts from hospital management, microbiology and infection control. *J Hosp Infect* **76**, 26-31 (2010).
48. Tangden, T., Eriksson, B.M., Melhus, A., Svennblad, B. & Cars, O. Radical reduction of cephalosporin use at a tertiary hospital after educational antibiotic intervention during an outbreak of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae. *J Antimicrob Chemother* **66**, 1161-1167 (2011).
49. Alsterlund, R., Carlsson, B., Gezelius, L., Haeggman, S. & Olsson-Liljequist, B. Multiresistant CTX-M-15 ESBL-producing Escherichia coli in southern Sweden: Description of an outbreak. *Scand J Infect Dis* **41**, 410-415 (2009).
50. Woksepp, H.*, et al.* High-resolution melting-curve analysis of ligation-mediated real-time PCR for rapid evaluation of an epidemiological outbreak of extended-spectrum-beta-lactamase-producing Escherichia coli. *J Clin Microbiol* **49**, 4032-4039 (2011).

51. Fang, H., *et al.* Molecular epidemiological analysis of Escherichia coli isolates producing extended-spectrum beta-lactamases for identification of nosocomial outbreaks in Stockholm, Sweden. *J Clin Microbiol* **42**, 5917-5920 (2004).
52. Hawkey, P.M. Prevalence and clonality of extended-spectrum beta-lactamases in Asia. *Clin Microbiol Infect* **14 Suppl 1**, 159-165 (2008).
53. Kader, A.A., Kumar, A. & Kamath, K.A. Fecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in patients and asymptomatic healthy individuals. *Infect Control Hosp Epidemiol* **28**, 1114-1116 (2007).
54. Tangden, T., Cars, O., Melhus, A. & Lowdin, E. Foreign travel is a major risk factor for colonization with Escherichia coli producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob Agents Chemother* **54**, 3564-3568 (2010).
55. Stromdahl, H., *et al.* 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 (2011).
56. Rhomberg, P.R. & Jones, R.N. Summary trends for the Meropenem Yearly Susceptibility Test Information Collection Program: a 10-year experience in the United States (1999-2008). *Diagn Microbiol Infect Dis* **65**, 414-426 (2009).
57. Gales, A.C., Jones, R.N. & Sader, H.S. Contemporary activity of colistin and polymyxin B against a worldwide collection of Gram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006-09). *J Antimicrob Chemother* **66**, 2070-2074.
58. Davies, T.A., *et al.* Longitudinal survey of carbapenem resistance and resistance mechanisms in Enterobacteriaceae and non-fermenters from the USA in 2007-09. *J Antimicrob Chemother*.
59. Bratu, S., *et al.* Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New York City: a new threat to our antibiotic armamentarium. *Arch Intern Med* **165**, 1430-1435 (2005).
60. Yigit, H., *et al.* Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrob Agents Chemother* **45**, 1151-1161 (2001).
61. Walther-Rasmussen, J. & Hoiby, N. Class A carbapenemases. *J Antimicrob Chemother* **60**, 470-482 (2007).
62. Woodford, N., *et al.* Outbreak of Klebsiella pneumoniae producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical Center. *Antimicrob Agents Chemother* **48**, 4793-4799 (2004).
63. Deshpande, L.M., Rhomberg, P.R., Sader, H.S. & Jones, R.N. Emergence of serine carbapenemases (KPC and SME) among clinical strains of Enterobacteriaceae isolated in the United States

Medical Centers: report from the MYSTIC Program (1999-2005). *Diagn Microbiol Infect Dis* **56**, 367-372 (2006).
64. Naas, T., Nordmann, P., Vedel, G. & Poyart, C. Plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC in a Klebsiella pneumoniae isolate from France. *Antimicrob Agents Chemother* **49**, 4423-4424 (2005).
65. Dortet, L.*, et al.* Intercontinental travels of patients and dissemination of plasmid-mediated carbapenemase KPC-3 associated with OXA-9 and TEM-1. *J Antimicrob Chemother* **61**, 455-457 (2008).
66. Souli, M.*, et al.* An outbreak of infection due to beta-Lactamase Klebsiella pneumoniae Carbapenemase 2-producing K. pneumoniae in a Greek University Hospital: molecular characterization, epidemiology, and outcomes. *Clin Infect Dis* **50**, 364-373 (2010).
67. Fontana, C.*, et al.* Emergence of KPC-producing Klebsiella pneumoniae in Italy. *BMC research notes* **3**, 40 (2010).
68. Giani, T.*, et al.* Emergence in Italy of Klebsiella pneumoniae sequence type 258 producing KPC-3 Carbapenemase. *J Clin Microbiol* **47**, 3793-3794 (2009).
69. Samuelsen, O.*, et al.* Emergence of clonally related Klebsiella pneumoniae isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden. *J Antimicrob Chemother* **63**, 654-658 (2009).
70. Toth, A.*, et al.* Emergence of a colistin-resistant KPC-2-producing Klebsiella pneumoniae ST258 clone in Hungary. *Eur J Clin Microbiol Infect Dis* **29**, 765-769.
71. Poirel, L., Naas, T. & Nordmann, P. Diversity, epidemiology, and genetics of class D beta-lactamases. *Antimicrob Agents Chemother* **54**, 24-38 (2010).
72. Poirel, L., Heritier, C., Tolun, V. & Nordmann, P. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. *Antimicrob Agents Chemother* **48**, 15-22 (2004).
73. Giakkoupi, P.*, et al.* VIM-1 Metallo-beta-lactamase-producing Klebsiella pneumoniae strains in Greek hospitals. *J Clin Microbiol* **41**, 3893-3896 (2003).
74. Kassis-Chikhani, N.*, et al.* First outbreak of multidrug-resistant Klebsiella pneumoniae carrying blaVIM-1 and blaSHV-5 in a French university hospital. *J Antimicrob Chemother* **57**, 142-145 (2006).
75. Cagnacci, S.*, et al.* Bloodstream infections caused by multidrug-resistant Klebsiella pneumoniae producing the carbapenem-hydrolysing VIM-1 metallo-beta-lactamase: first Italian outbreak. *J Antimicrob Chemother* **61**, 296-300 (2008).
76. Shibata, N.*, et al.* PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. *J Clin Microbiol* **41**, 5407-5413 (2003).

77. Yong, D., *et al.* Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother* **53**, 5046-5054 (2009).
78. Kumarasamy, K.K., *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* **10**, 597-602 (2010).
79. Nordmann, P., Poirel, L., Toleman, M.A. & Walsh, T.R. Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? *J Antimicrob Chemother* **66**, 689-692.
80. Perry, J.D., *et al.* Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. *J Antimicrob Chemother* **66**, 2288-2294.
81. Freeman, J.T., *et al.* Community-onset genitourinary tract infection due to CTX-M-15-Producing Escherichia coli among travelers to the Indian subcontinent in New Zealand. *Clin Infect Dis* **47**, 689-692 (2008).
82. Sannes, M.R., *et al.* Predictors of antimicrobial-resistant Escherichia coli in the feces of vegetarians and newly hospitalized adults in Minnesota and Wisconsin. *J Infect Dis* **197**, 430-434 (2008).
83. Laupland, K.B., Church, D.L., Vidakovich, J., Mucenski, M. & Pitout, J.D. Community-onset extended-spectrum beta-lactamase (ESBL) producing Escherichia coli: importance of international travel. *J Infect* **57**, 441-448 (2008).
84. Tham, J., *et al.* Extended-spectrum beta-lactamase-producing Escherichia coli in patients with travellers' diarrhoea. *Scand J Infect Dis* **42**, 275-280.
85. Kennedy, K. & Collignon, P. Colonisation with Escherichia coli resistant to "critically important" antibiotics: a high risk for international travellers. *Eur J Clin Microbiol Infect Dis* **29**, 1501-1506 (2010).
86. Samuelsen, O., *et al.* Molecular characterization of VIM-producing Klebsiella pneumoniae from Scandinavia reveals genetic relatedness with international clonal complexes encoding transferable multidrug resistance. *Clin Microbiol Infect* **17**, 1811-1816 (2011).
87. Rogers, B.A., Aminzadeh, Z., Hayashi, Y. & Paterson, D.L. Country-to-country transfer of patients and the risk of multi-resistant bacterial infection. *Clin Infect Dis* **53**, 49-56 (2011).
88. Cao, V.T., *et al.* Emergence of imipenem resistance in Klebsiella pneumoniae owing to combination of plasmid-mediated CMY-4 and permeability alteration. *J Antimicrob Chemother* **46**, 895-900 (2000).
89. Jacoby, G.A., Mills, D.M. & Chow, N. Role of beta-lactamases and porins in resistance to ertapenem and other beta-lactams in

Klebsiella pneumoniae. *Antimicrob Agents Chemother* **48**, 3203-3206 (2004).
90. Hernandez-Alles, S.*, et al.* Relationship between outer membrane alterations and susceptibility to antimicrobial agents in isogenic strains of Klebsiella pneumoniae. *J Antimicrob Chemother* **46**, 273-277 (2000).
91. Ahmad, M.*, et al.* Clinical characteristics and molecular epidemiology associated with imipenem-resistant Klebsiella pneumoniae. *Clin Infect Dis* **29**, 352-355 (1999).
92. Bornet, C., Davin-Regli, A., Bosi, C., Pages, J.M. & Bollet, C. Imipenem resistance of enterobacter aerogenes mediated by outer membrane permeability. *J Clin Microbiol* **38**, 1048-1052 (2000).
93. Mena, A.*, et al.* Characterization of a large outbreak by CTX-M-1-producing Klebsiella pneumoniae and mechanisms leading to in vivo carbapenem resistance development. *J Clin Microbiol* **44**, 2831-2837 (2006).
94. Livermore, D.M., Oakton, K.J., Carter, M.W. & Warner, M. Activity of ertapenem (MK-0826) versus Enterobacteriaceae with potent beta-lactamases. *Antimicrob Agents Chemother* **45**, 2831-2837 (2001).
95. Livermore, D.M., Sefton, A.M. & Scott, G.M. Properties and potential of ertapenem. *J Antimicrob Chemother* **52**, 331-344 (2003).
96. Livermore, D.M.*, et al.* Activities of NXL104 combinations with ceftazidime and aztreonam against carbapenemase-Producing Enterobacteriaceae. *Antimicrob Agents Chemother* **55**, 390-394 (2011).
97. Woodford, N.*, et al.* Ertapenem resistance among Klebsiella and Enterobacter submitted in the UK to a reference laboratory. *Int J Antimicrob Agents* **29**, 456-459 (2007).
98. Hawser, S.P.*, et al.* Susceptibility of Klebsiella pneumoniae isolates from intra-abdominal infections and molecular characterization of ertapenem-resistant isolates. *Antimicrob Agents Chemother* **55**, 3917-3921.
99. Hawser, S.P.*, et al.* Susceptibility of European Escherichia coli clinical isolates from intra-abdominal infections, extended-spectrum beta-lactamase occurrence, resistance distribution, and molecular characterization of ertapenem-resistant isolates (SMART 2008-2009). *Clin Microbiol Infect*.
100. Adler, M., Anjum, M., Andersson, D.I. & Sandegren, L. Influence of acquired beta-lactamases on the evolution of spontaneous carbapenem resistance in Escherichia coli. *J Antimicrob Chemother* (2012).
101. Yoshida, T., Qin, L., Egger, L.A. & Inouye, M. Transcription regulation of ompF and ompC by a single transcription factor, OmpR. *J Biol Chem* **281**, 17114-17123 (2006).

102. Apisarnthanarak, A.*, et al.* Effectiveness of education and an antibiotic-control program in a tertiary care hospital in Thailand. *Clin Infect Dis* **42**, 768-775 (2006).
103. Bantar, C.*, et al.* Replacement of broad-spectrum cephalosporins by piperacillin-tazobactam: impact on sustained high rates of bacterial resistance. *Antimicrob Agents Chemother* **48**, 392-395 (2004).
104. Brahmi, N.*, et al.* Impact of ceftazidime restriction on gram-negative bacterial resistance in an intensive care unit. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* **12**, 190-194 (2006).
105. Furtado, G.H.*, et al.* Impact of a hospital-wide antimicrobial formulary intervention on the incidence of multidrug-resistant gram-negative bacteria. *American journal of infection control* **36**, 661-664 (2008).
106. Kim, J.Y.*, et al.* Control of extended-spectrum {beta}-lactamase-producing Klebsiella pneumoniae using a computer-assisted management program to restrict third-generation cephalosporin use. *J Antimicrob Chemother* **62**, 416-421 (2008).
107. Lee, J.*, et al.* Control of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a children's hospital by changing antimicrobial agent usage policy. *J Antimicrob Chemother* **60**, 629-637 (2007).
108. Lipworth, A.D.*, et al.* Limiting the emergence of extended-spectrum Beta-lactamase-producing enterobacteriaceae: influence of patient population characteristics on the response to antimicrobial formulary interventions. *Infect Control Hosp Epidemiol* **27**, 279-286 (2006).
109. Meyer, E.*, et al.* Impact of restriction of third generation cephalosporins on the burden of third generation cephalosporin resistant K. pneumoniae and E. coli in an ICU. *Intensive Care Med* **35**, 862-870 (2009).
110. Nijssen, S.*, et al.* Effects of reducing beta-lactam antibiotic pressure on intestinal colonization of antibiotic-resistant gram-negative bacteria. *Intensive Care Med* **36**, 512-519 (2010).
111. Patterson, J.E., Hardin, T.C., Kelly, C.A., Garcia, R.C. & Jorgensen, J.H. Association of antibiotic utilization measures and control of multiple-drug resistance in Klebsiella pneumoniae. *Infect Control Hosp Epidemiol* **21**, 455-458 (2000).
112. Pena, C.*, et al.* Epidemiology and successful control of a large outbreak due to Klebsiella pneumoniae producing extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* **42**, 53-58 (1998).
113. Petrikkos, G.*, et al.* Differences in the changes in resistance patterns to third- and fourth-generation cephalosporins and piperacillin/tazobactam among Klebsiella pneumoniae and Escherichia coli clinical isolates following a restriction policy in a Greek tertiary care hospital. *Int J Antimicrob Agents* **29**, 34-38 (2007).

114. Rahal, J.J., *et al.* Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial Klebsiella. *Jama* **280**, 1233-1237 (1998).
115. Wen, Z., *et al.* Intervention study of the association of antibiotic utilization measures with control of extended-spectrum beta-lactamase (ESBL)-producing bacteria. *Microbes Infect* **12**, 710-715 (2010).
116. Murki, S., Jonnala, S., Mohammed, F. & Reddy, A. Restriction of Cephalosporins and Control of Extended Spectrum Beta Lactamase Producing Gram Negative Bacteria in a Neonatal Intensive Care Unit. *Indian pediatrics* (2010).
117. Davey, P., *et al.* Interventions to improve antibiotic prescribing practices for hospital inpatients. *Cochrane Database Syst Rev*, CD003543 (2005).
118. Davey, P., *et al.* Systematic review of antimicrobial drug prescribing in hospitals. *Emerg Infect Dis* **12**, 211-216 (2006).
119. Ramsay, C., Brown, E., Hartman, G. & Davey, P. Room for improvement: a systematic review of the quality of evaluations of interventions to improve hospital antibiotic prescribing. *J Antimicrob Chemother* **52**, 764-771 (2003).
120. Wagner, A.K., Soumerai, S.B., Zhang, F. & Ross-Degnan, D. Segmented regression analysis of interrupted time series studies in medication use research. *J Clin Pharm Ther* **27**, 299-309 (2002).
121. Lan, C.K., *et al.* Association of antibiotic utilization measures and reduced incidence of infections with extended-spectrum beta-lactamase-producing organisms. *J Microbiol Immunol Infect* **36**, 182-186 (2003).
122. Rossolini, G.M., D'Andrea, M.M. & Mugnaioli, C. The spread of CTX-M-type extended-spectrum beta-lactamases. *Clin Microbiol Infect* **14 Suppl 1**, 33-41 (2008).
123. Magiorakos, A.P., *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*.
124. Rodriguez-Bano, J. & Navarro, M.D. Extended-spectrum beta-lactamases in ambulatory care: a clinical perspective. *Clin Microbiol Infect* **14 Suppl 1**, 104-110 (2008).
125. Chaubey, V.P., *et al.* Clinical outcome of empiric antimicrobial therapy of bacteremia due to extended-spectrum beta-lactamase producing Escherichia coli and Klebsiella pneumoniae. *BMC research notes* **3**, 116 (2010).
126. Paterson, D. Infections with organisms producing extended-spectrum β -lactamase. in *Antimicrobial resistance - Beyond the breakpoint.* , Vol. 2010 21-34 (Issues Infect Dis, Basel, Karger., 2010).
127. Bazaz, R., Chapman, A.L. & Winstanley, T.G. Ertapenem administered as outpatient parenteral antibiotic therapy for urinary

tract infections caused by extended-spectrum-beta-lactamase-producing Gram-negative organisms. *J Antimicrob Chemother* **65**, 1510-1513 (2010).
128. Dalgic, N., Sancar, M., Bayraktar, B., Dincer, E. & Pelit, S. Ertapenem for the treatment of urinary tract infections caused by extended-spectrum beta-lactamase-producing bacteria in children. *Scand J Infect Dis* **43**, 339-343 (2011).
129. Riera, E.*, et al.* Pseudomonas aeruginosa carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem and doripenem. *J Antimicrob Chemother* **66**, 2022-2027.
130. Urban, C., Mariano, N. & Rahal, J.J. In vitro double and triple bactericidal activities of doripenem, polymyxin B, and rifampin against multidrug-resistant Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. *Antimicrob Agents Chemother* **54**, 2732-2734.
131. Paterson, D.L.*, et al.* Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. *J Clin Microbiol* **39**, 2206-2212 (2001).
132. Suankratay, C., Jutivorakool, K. & Jirajariyavej, S. A prospective study of ceftriaxone treatment in acute pyelonephritis caused by extended-spectrum beta-lactamase-producing bacteria. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet* **91**, 1172-1181 (2008).
133. Ramphal, R. & Ambrose, P.G. Extended-spectrum beta-lactamases and clinical outcomes: current data. *Clin Infect Dis* **42 Suppl 4**, S164-172 (2006).
134. Goethaert, K.*, et al.* High-dose cefepime as an alternative treatment for infections caused by TEM-24 ESBL-producing Enterobacter aerogenes in severely-ill patients. *Clin Microbiol Infect* **12**, 56-62 (2006).
135. Wong-Beringer, A.*, et al.* Molecular correlation for the treatment outcomes in bloodstream infections caused by Escherichia coli and Klebsiella pneumoniae with reduced susceptibility to ceftazidime. *Clin Infect Dis* **34**, 135-146 (2002).
136. Maglio, D.*, et al.* Determination of the in vivo pharmacodynamic profile of cefepime against extended-spectrum-beta-lactamase-producing Escherichia coli at various inocula. *Antimicrob Agents Chemother* **48**, 1941-1947 (2004).
137. Thomson, K.S. & Moland, E.S. Cefepime, piperacillin-tazobactam, and the inoculum effect in tests with extended-spectrum beta-lactamase-producing Enterobacteriaceae. *Antimicrob Agents Chemother* **45**, 3548-3554 (2001).
138. Gavin, P.J.*, et al.* Clinical correlation of the CLSI susceptibility breakpoint for piperacillin- tazobactam against extended-spectrum-

beta-lactamase-producing Escherichia coli and Klebsiella species. *Antimicrob Agents Chemother* **50**, 2244-2247 (2006).
139. Chen, Y.H.*, et al.* Antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region according to currently established susceptibility interpretive criteria. *J Infect* **62**, 280-291 (2011).
140. Hoban, D.J.*, et al.* Susceptibility of gram-negative pathogens isolated from patients with complicated intra-abdominal infections in the United States, 2007-2008: results of the Study for Monitoring Antimicrobial Resistance Trends (SMART). *Antimicrob Agents Chemother* **54**, 3031-3034 (2010).
141. Rodriguez-Bano, J.*, et al.* Bacteremia due to extended-spectrum beta -lactamase-producing Escherichia coli in the CTX-M era: a new clinical challenge. *Clin Infect Dis* **43**, 1407-1414 (2006).
142. Perez, F. & Bonomo, R.A. Can we really use beta-lactam/beta-lactam inhibitor combinations for the treatment of infections caused by extended-spectrum beta-lactamase-producing bacteria? *Clin Infect Dis* **54**, 175-177 (2012).
143. Rodriguez-Bano, J., Navarro, M.D., Retamar, P., Picon, E. & Pascual, A. Beta-lactam/beta-lactam inhibitor combinations for the treatment of bacteremia due to extended-spectrum beta-lactamase-producing Escherichia coli: a post hoc analysis of prospective cohorts. *Clin Infect Dis* **54**, 167-174 (2012).
144. Paterson, D.L. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). *Clin Microbiol Infect* **6**, 460-463 (2000).
145. Jones, R.N. & Pfaller, M.A. Antimicrobial activity against strains of Escherichia coli and Klebsiella spp. with resistance phenotypes consistent with an extended-spectrum beta-lactamase in Europe. *Clin Microbiol Infect* **9**, 708-712 (2003).
146. Endimiani, A.*, et al.* Bacteremia due to Klebsiella pneumoniae isolates producing the TEM-52 extended-spectrum beta-lactamase: treatment outcome of patients receiving imipenem or ciprofloxacin. *Clin Infect Dis* **38**, 243-251 (2004).
147. Vidal, L.*, et al.* Efficacy and safety of aminoglycoside monotherapy: systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother* **60**, 247-257 (2007).
148. Kelesidis, T., Karageorgopoulos, D.E., Kelesidis, I. & Falagas, M.E. Tigecycline for the treatment of multidrug-resistant Enterobacteriaceae: a systematic review of the evidence from microbiological and clinical studies. *J Antimicrob Chemother* **62**, 895-904 (2008).
149. Zarkotou, O.*, et al.* Predictors of mortality in patients with bloodstream infections caused by KPC-producing Klebsiella pneumoniae and impact of appropriate antimicrobial treatment. *Clin Microbiol Infect* **17**, 1798-1803 (2011).

150. Souli, M., *et al.* Clinical experience of serious infections caused by Enterobacteriaceae producing VIM-1 metallo-beta-lactamase in a Greek University Hospital. *Clin Infect Dis* **46**, 847-854 (2008).
151. Freire, A.T., *et al.* Comparison of tigecycline with imipenem/cilastatin for the treatment of hospital-acquired pneumonia. *Diagn Microbiol Infect Dis* **68**, 140-151.
152. Anonymous. FDA Drug Safety Communication: Increased risk of death with Tygacil (tigecycline) compared to other antibiotics used to treat similar infections. (2010).
153. Lee, J., Patel, G., Huprikar, S., Calfee, D.P. & Jenkins, S.G. Decreased susceptibility to polymyxin B during treatment for carbapenem-resistant Klebsiella pneumoniae infection. *J Clin Microbiol* **47**, 1611-1612 (2009).
154. Kontopidou, F., *et al.* Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients. *Clin Microbiol Infect* **17**, E9-E11 (2011).
155. Chen, S., *et al.* Independent emergence of colistin-resistant Enterobacteriaceae clinical isolates without colistin treatment. *J Clin Microbiol* **49**, 4022-4023 (2011).
156. Paul, M., *et al.* Effectiveness and safety of colistin: prospective comparative cohort study. *J Antimicrob Chemother* **65**, 1019-1027.
157. Qureshi, Z.A., *et al.* Treatment outcome of bacteremia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens. *Antimicrob Agents Chemother* **56**, 2108-2113 (2012).
158. Kallel, H., *et al.* Safety and efficacy of colistin compared with imipenem in the treatment of ventilator-associated pneumonia: a matched case-control study. *Intensive Care Med* **33**, 1162-1167 (2007).
159. Plachouras, D., *et al.* Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. *Antimicrob Agents Chemother* **53**, 3430-3436 (2009).
160. Antachopoulos, C., *et al.* Serum and cerebrospinal fluid levels of colistin in pediatric patients. *Antimicrob Agents Chemother* **54**, 3985-3987 (2010).
161. Garonzik, S.M., *et al.* Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* **55**, 3284-3294.
162. Hartzell, J.D., *et al.* Nephrotoxicity associated with intravenous colistin (colistimethate sodium) treatment at a tertiary care medical center. *Clin Infect Dis* **48**, 1724-1728 (2009).
163. Auer, S., Wojna, A. & Hell, M. Oral treatment options for ambulatory patients with urinary tract infections caused by extended-spectrum-beta-lactamase-producing Escherichia coli. *Antimicrob Agents Chemother* **54**, 4006-4008 (2010).

164. Falagas, M.E., Kastoris, A.C., Kapaskelis, A.M. & Karageorgopoulos, D.E. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. *Lancet Infect Dis* **10**, 43-50 (2010).
165. Michalopoulos, A.*, et al.* Intravenous fosfomycin for the treatment of nosocomial infections caused by carbapenem-resistant Klebsiella pneumoniae in critically ill patients: a prospective evaluation. *Clin Microbiol Infect* **16**, 184-186 (2010).
166. Souli, M.*, et al.* In vitro interactions of antimicrobial combinations with fosfomycin against KPC-2-producing Klebsiella pneumoniae and protection of resistance development. *Antimicrob Agents Chemother* **55**, 2395-2397 (2011).
167. Chen, L.Y.*, et al.* High-dose daptomycin and fosfomycin treatment of a patient with endocarditis caused by daptomycin-nonsusceptible Staphylococcus aureus: case report. *BMC Infect Dis* **11**, 152 (2011).
168. Pfausler, B.*, et al.* Concentrations of fosfomycin in the cerebrospinal fluid of neurointensive care patients with ventriculostomy-associated ventriculitis. *J Antimicrob Chemother* **53**, 848-852 (2004).
169. Rahal, J.J. Antimicrobial resistance among and therapeutic options against gram-negative pathogens. *Clin Infect Dis* **49 Suppl 1**, S4-S10 (2009).
170. Daikos, G.L. & Markogiannakis, A. Carbapenemase-producing Klebsiella pneumoniae: (when) might we still consider treating with carbapenems? *Clin Microbiol Infect* **17**, 1135-1141.
171. Elemam, A., Rahimian, J. & Doymaz, M. In vitro evaluation of antibiotic synergy for polymyxin B-resistant carbapenemase-producing Klebsiella pneumoniae. *J Clin Microbiol* **48**, 3558-3562 (2010).
172. Bercot, B., Poirel, L., Dortet, L. & Nordmann, P. In vitro evaluation of antibiotic synergy for NDM-1-producing Enterobacteriaceae. *J Antimicrob Chemother* **66**, 2295-2297.
173. Drawz, S.M. & Bonomo, R.A. Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev* **23**, 160-201 (2010).
174. Endimiani, A., Choudhary, Y. & Bonomo, R.A. In vitro activity of NXL104 in combination with beta-lactams against Klebsiella pneumoniae isolates producing KPC carbapenemases. *Antimicrob Agents Chemother* **53**, 3599-3601 (2009).
175. Endimiani, A.*, et al.* Evaluation of ceftazidime and NXL104 in two murine models of infection due to KPC-producing Klebsiella pneumoniae. *Antimicrob Agents Chemother* **55**, 82-85 (2011).
176. Murphy, T.A., Simm, A.M., Toleman, M.A., Jones, R.N. & Walsh, T.R. Biochemical characterization of the acquired metallo-beta-lactamase SPM-1 from Pseudomonas aeruginosa. *Antimicrob Agents Chemother* **47**, 582-587 (2003).
177. Franceschini, N.*, et al.* Meropenem stability to beta-lactamase hydrolysis and comparative in vitro activity against several beta-

lactamase-producing Gram-negative strains. *J Antimicrob Chemother* **49**, 395-398 (2002).
178. Giske, C.G.*, et al.* Diverse sequence types of Klebsiella pneumoniae contribute to the dissemination of blaNDM-1 in India, Sweden, and the United Kingdom. *Antimicrob Agents Chemother* **56**, 2735-2738 (2012).
179. Majumdar, A.K.*, et al.* Pharmacokinetics of ertapenem in healthy young volunteers. *Antimicrob Agents Chemother* **46**, 3506-3511 (2002).
180. Meyers, B.R.*, et al.* Pharmacokinetics of aztreonam in healthy elderly and young adult volunteers. *J Clin Pharmacol* **33**, 470-474 (1993).
181. Lettieri, J.T., Rogge, M.C., Kaiser, L., Echols, R.M. & Heller, A.H. Pharmacokinetic profiles of ciprofloxacin after single intravenous and oral doses. *Antimicrob Agents Chemother* **36**, 993-996 (1992).
182. Dvorchik, B.H., Brazier, D., DeBruin, M.F. & Arbeit, R.D. Daptomycin pharmacokinetics and safety following administration of escalating doses once daily to healthy subjects. *Antimicrob Agents Chemother* **47**, 1318-1323 (2003).
183. Matzi, V.*, et al.* Extracellular concentrations of fosfomycin in lung tissue of septic patients. *J Antimicrob Chemother* **65**, 995-998 (2010).
184. Conte, J.E., Jr., Golden, J.A., Kelley, M.G. & Zurlinden, E. Intrapulmonary pharmacokinetics and pharmacodynamics of meropenem. *Int J Antimicrob Agents* **26**, 449-456 (2005).
185. Anonymous. Rifampin, package insert. (2003).
186. Anonymous. Vibativ, package insert. (2009).
187. Meagher, A.K., Ambrose, P.G., Grasela, T.H. & Ellis-Grosse, E.J. Pharmacokinetic/pharmacodynamic profile for tigecycline-a new glycylcycline antimicrobial agent. *Diagn Microbiol Infect Dis* **52**, 165-171 (2005).
188. James, J.K., Palmer, S.M., Levine, D.P. & Rybak, M.J. Comparison of conventional dosing versus continuous-infusion vancomycin therapy for patients with suspected or documented gram-positive infections. *Antimicrob Agents Chemother* **40**, 696-700 (1996).
189. Cars, O., Henning, C. & Holm, S.E. Penetration of ampicillin and dicloxacillin into tissue cage fluid in rabbits: relation to serum and tissue protein binding. *Scand J Infect Dis* **13**, 69-74 (1981).
190. Lowdin, E., Odenholt, I., Bengtsson, S. & Cars, O. Pharmacodynamic effects of sub-MICs of benzylpenicillin against Streptococcus pyogenes in a newly developed in vitro kinetic model. *Antimicrob Agents Chemother* **40**, 2478-2482 (1996).
191. Gustafsson, I., Lowdin, E., Odenholt, I. & Cars, O. Pharmacokinetic and pharmacodynamic parameters for antimicrobial effects of cefotaxime and amoxicillin in an in vitro kinetic model. *Antimicrob Agents Chemother* **45**, 2436-2440 (2001).

192. Durkalski, V.L., Palesch, Y.Y., Lipsitz, S.R. & Rust, P.F. Analysis of clustered matched-pair data. *Stat Med* **22**, 2417-2428 (2003).
193. Hogberg, L.*, et al.* Reduction in outpatient antibiotic sales for pre-school children: interrupted time series analysis of weekly antibiotic sales data in Sweden 1992-2002. *J Antimicrob Chemother* **56**, 208-215 (2005).
194. Newey, W.K.K.D.W. Automatic Lag Selection in Covariance matrix Estimation *Review of Economic Studies, 61: 631-653* **61**, 631-653 (1994).
195. Bush, K. Extended-spectrum beta-lactamases in North America, 1987-2006. *Clin Microbiol Infect* **14 Suppl 1**, 134-143 (2008).
196. Canton, R.*, et al.* Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. *Clin Microbiol Infect* **14 Suppl 1**, 144-153 (2008).
197. Villegas, M.V., Kattan, J.N., Quinteros, M.G. & Casellas, J.M. Prevalence of extended-spectrum beta-lactamases in South America. *Clin Microbiol Infect* **14 Suppl 1**, 154-158 (2008).
198. Munday, C.J., Xiong, J., Li, C., Shen, D. & Hawkey, P.M. Dissemination of CTX-M type beta-lactamases in Enterobacteriaceae isolates in the People's Republic of China. *Int J Antimicrob Agents* **23**, 175-180 (2004).
199. Landman, D., Chockalingam, M. & Quale, J.M. Reduction in the incidence of methicillin-resistant Staphylococcus aureus and ceftazidime-resistant Klebsiella pneumoniae following changes in a hospital antibiotic formulary. *Clin Infect Dis* **28**, 1062-1066 (1999).
200. Keren, I., Kaldalu, N., Spoering, A., Wang, Y. & Lewis, K. Persister cells and tolerance to antimicrobials. *FEMS Microbiol Lett* **230**, 13-18 (2004).
201. Bratu, S.*, et al.* Carbapenemase-producing Klebsiella pneumoniae in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents. *J Antimicrob Chemother* **56**, 128-132 (2005).
202. Pournaras, S.*, et al.* Activity of tigecycline alone and in combination with colistin and meropenem against Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae strains by time-kill assay. *Int J Antimicrob Agents* **37**, 244-247 (2011).

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 840

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2012

Distribution: publications.uu.se
urn:nbn:se:uu:diva-182897