SURVEILLANCE OF ANTIBIOTIC CONSUMPTION AND ANTIBIOTIC RESISTANCE IN SWEDISH INTENSIVE CARE UNITS

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ABSTRACT

INTRODUCTION: Nosocomial infections remain a major cause of mortality and morbidity. The problem is most apparent in intensive care units (ICUs). Most ICU patients are compromised and vulnerable as a result of disease or severe trauma. One in ten people admitted to hospital is given an antibiotic for infection. The risk of acquiring a nosocomial infection in a European ICU is approximately 20%. It is vitally important that ways are found to prevent transmission between patients and personnel, and that local hygiene routines and antibiotic policies are developed. This thesis is a holistic work focused particularly on antimicrobial antibiotic resistance, antibiotic consumption and to some extent on hygiene in Swedish ICUs.

AIMS: The general aim of this thesis was to investigate bacterial resistance and antibiotic consumption in Swedish ICUs and to try to correlate ICU demographic data with antibiotic consumption and antibiotic resistance. Additional aims were to investigate on which clinical indications antibacterial drugs are prescribed in the ICU, and to investigate the emergence of resistance and transmission of Pseudomonas aeruginosa in the ICU using cluster analysis based on antibiograms and genotype data obtained by AFLP.

MATERIAL AND METHODS: In paper 1-3, antibiotic consumption data together with bacterial antibiotic resistance data and specific ICU-demographic data were collected from an increasing number of ICUs over the years 1997-2001. Data from ICUs covering up to six million out of Sweden's nine million inhabitants were included. In paper 4, the indications for antibiotic prescribing were studied during two weeks in 2000. Paper 5 investigated Pseudomonas aeruginosa isolates in order to detect cross-transmission with genotype obtained by AFLP, and antibiogram-based cluster analysis was also performed in order to see if this could be a quicker and easier substitute for AFLP.

RESULTS: This thesis has produced three important findings. Firstly, antibiotic consumption in participating ICUs was relatively high during the study period, and every patient received on average more than one antimicrobial drug per day (I-IV). Secondly, levels of antimicrobial drug resistance seen in *S. aureus, E. coli and Klebsiella* spp remained low when data were pooled from all ICUs throughout the study period, despite relatively high antibiotic consumption (I-V). Thirdly, the prevalence of antibiotic resistance in CoNS and *E. faecium*, cefotaxime resistance in *Enterobacter,* and ciprofloxacin and imipenem resistance in *P. aeruginosa* was high enough to cause concern.

CONCLUSION: For the period studied, multidrug resistance in Swedish ICUs was not a major problem. Signs of cross-transmission with non-multiresistant bacteria were observed, indicating a hygiene problem and identifying simple improvements that could be made in patient care guidelines and barrier precautions. A need for better follow up of prescribed antibiotics was evident. With further surveillance studies and monitoring of antibiotics and bacterial resistance patterns in the local setting as well as on a national and international level, some of the strategic goals in the prevention and control of the emergence of antimicrobial-resistant microbes may be achievable.

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LIST OF PUBLICATIONS

This thesis is based on the following original papers:

I Erlandsson C-M, Hanberger H, Eliasson I, Hoffmann M, Isaksson B, Lindgren S, Nilsson L, Sörén L, Walther S. Surveillance of Antibiotic Resistance in ICUs in Southeastern Sweden. Acta Anaesthesiol Scand 1999; 43: 815-820

II Walther SM, Erlandsson M, Burman LG, Cars O, Gill H, Hoffman M, Isaksson B, Kahlmeter G, Lindgren Sune, Nilsson LE, Olsson-Liljequist B, Hanberger H & The Icu-strama Study Group (2002). Antibiotic prescription practices, consumption and bacterial resistance in a cross section of Swedish intensive care units. Acta Anaesthesiologica Scandinavica 2002; 46 (9): 1075-1081

III Hanberger H, Erlandsson M, Burman LG, Cars O, Gill H, Lindgren S, Nilsson LE, Olsson-Liljequist B, Walther S and the ICU-STRAMA Study Group. High Antibiotic Susceptibility Among Bacterial Pathogens In Swedish ICUs. Scand J Infect Dis 2004; 36: 24-30

IV Erlandsson M, Burman LG, Cars O, Gill H, Nilsson LE, Walther S, Hanberger H and the ICU-STRAMA Study Group. Prescription of antibiotic agents in Swedish intensive care units is empiric and adequate.Scand J Infect Dis 2007; 39(1): 63-9

V Erlandsson M, Gill H, Nilsson LE, Walther S, Giske C, Jonas D, Hanberger H and the ICU-STRAMA Study Group. Antibiotic susceptibility patterns and clones of *Pseudomonas aeruginosa* from patients in Swedish ICUs. Submitted to Scand J Infect Dis

ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
APACHE	Acute Physiology And Chronic Health Evaluation
ARPAC	Antibiotic Resistance Prevention And Control
CDC	Centers for Disease Control and Prevention (USA)
CoNS	Coagulase Negative Staphylococci
DDD	Defined Daily Dosages
EARSS	European Antimicrobial Resistance Surveillance System
EDTA	Ethylene Diamine Tetraacetic Acid
EUCAST	European Committee for Antimicrobial Susceptibility Testing
ESBL	Extended Spectrum Betalactamases
ICU	Intensive Care Unit
INSPEAR	International Network for the Study and Prevention of Emerging Antimicrobial Resistance
ICNARC	Intensive Care National Audit & Research Centre
HLGR	High Level Gentamicin Resistant, refers to *Enterococcus* spp
KISS	Krankenhaus Infections Surveillance System
MBL	Metallo-β-Lactamases
MIC	Minimal Inhibitory Concentration
MLST	Multi Locus Sequence Typing
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Susceptible *Staphylococcus aureus*
MSSE	Methicillin Susceptible *Staphylococcus epidermidis*
NNIS	National Nosocomial Infections Surveillance (USA)
NPRS	Nosocomial Resistance Prevalence Study
PFGE	Pulsed Field Gel Electrophoresis
ReAct	Action on Antibiotic Resistance
SARI	Surveillance of Antibiotic Use and Bacterial Resistance in German Intensive Care Units
SIR	Swedish Intensive Care Registry
SMI	Smittskyddsinstitutet (Swedish Institute for Infectious Disease Control)
SRGA	Swedish Reference Group for Antibiotics
STRAMA	Swedish Strategic Programme for the Rational Use of Antimicrobial Agents and Surveillance of Resistance
TMP-SMX	Trimethoprim-sulfamethoxazole
VAP	Ventilator Associated Pneumonia
VRE	Vancomycin Resistant Enterococci

INTRODUCTION

Throughout history, infections have been a major cause of human death. Ignaz Semmelweis, amongst others, discovered the importance of basal hygiene, good antiseptic technique and procedures in the prevention of bacterial spread and nosocomial infections. Eighty years ago, Alexander Fleming provided hope in the fight against infections when he discovered penicillin. Since then, more antibiotics have been discovered and invented, but the struggle against germs remains a great challenge due to their dramatic ability to adapt rapidly to new environments. Today, there are no new classes of antibiotics in sight. Therefore it is becoming increasingly apparent that there is a vital need to gain control over bacterial resistance.

Nosocomial infections remain a major cause of mortality and morbidity. The problem is most apparent in intensive care units (ICUs), which care for the most critically ill patients. Most ICU patients are compromised and vulnerable as a result of disease or severe trauma. According to a prevalence study[1], one in ten people admitted to hospital is given an antibiotic for infection. The risk of acquiring a nosocomial infection in a European ICU is approximately 20% according to a large surveillance study[2]. Ultimately, the pronouncements of globally funded conferences on the problem of increasing nosocomial infections and bacterial resistance matter little if we do not find ways to prevent transmission between patients and personnel, and develop local hygiene routines and antibiotic policies. This thesis is a holistic work on antimicrobial antibiotic resistance, antibiotic consumption and hygiene in Swedish ICUs.

Nosocomial ICU infections

In this thesis the meaning of nosocomial infection is an infection acquired during hospital admission. Nosocomial infections are a problem for hospitals worldwide and for ICUs in particular. ICU patients usually suffer from underlying diseases and are immunocompromised, which makes them especially vulnerable. Most ICU-acquired infections are catheter-related in some way and are dealt with under each subheading and not as a separate entity.

Ventilator associated pneumonia (VAP)

The most common infection in the ICU is ventilator associated pneumonia (VAP). Almost 70% of the pathogens responsible for VAP are Gram negative. Of these, *Pseudomonas aeruginosa* and *Enterobacter spp* are the most common. *Staphylococcus aureus* was responsible for 18% of infections according to Fridkin and co-workers[3].

Urinary tract infections

The second most common cause of nosocomial infection in the ICU is urinary tract infection (UTI). Almost all patients in the ICU have urinary catheters, and therefore the pathogens associated with infection are *Escherichia coli* (18.2%), *Candida albicans* (15.4%), *Enterococcus spp* (14.1%) and *P. aeruginosa* (10.9%)[3].

Bloodstream infections

The third most common nosocomial infection in the ICU setting is bloodstream infection, (BSI) and this is most often associated with intravascular devices. Gram positive bacteria are encountered in 64% and Gram negative bacteria in 19.5%. Fungi account for 11% of pathogens responsible for nosocomial central venous catheter infections. The most common organisms are coagulase-negative staphylococci (CoNS), *S. aureus*, enterococci, *Enterobacter* spp and *Candida* spp according to National Nosocomial Infection Surveillance (NNIS) data[3]. According to the SOAP study (The Sepsis Occurrence in Acutely Ill Patients), which investigated the incidence of sepsis among 3 147 patients in 198 European ICUs during 14 days, the commonest origins were the lung (68%) and the abdomen (22%)[4].

Surgical site infections

According to Fridkin, the microbial organisms associated with surgical site infections (SSI) in the ICU setting may be related to the unique flora in the individual ICU. The problem of SSI is more common in wards outside the ICU[3].

ICU pathogens

Staphylococcus species

Staphylococcus species are Gram positive and include coagulase positive *S. aureus,* coagulase negative (CoNS) *Staphylococcus saprophyticus* and *Staphylococcus epidermidis.*

Staphylococcus aureus

S. aureus causes soft-tissue and skin infections such as impetigo, folliculitis, furuncles, carbuncles and hidradenitis suppurativa. But they also cause pneumonias, sepsis, toxic shock syndrome and are common in late onset VAP. According to the 1995 EPIC study and the recently published SOAP study, *S. aureus* is the most common ICU-bacterium [2, 4]. Methicillin resistant *S. aureus* (MRSA) is a concern for all healthcare personnel. The options for treatment are vancomycin, rifampicin, daptomycin and tigecycline.

Coagulase Negative Staphylococci

S. epidermidis is the major pathogen among CoNS. It is part of the normal skin flora. CoNS is the most common cause of bacteraemia in the ICU[2, 4]. *S. epidermidis* can live for months on medical equipment and devices in the

ICU, and is therefore especially likely to cause catheter-related infections.

Enterococci

Enterococci are facultative anaerobic Gram positive bacteria, which are a natural part of our human intestinal microflora. There are almost 20 species of Enterococci, but it is mainly *Enterococcus faecalis* and *Enterococcus faecium* that are responsible for infections in humans. Enterococci cause urinary tract infections, endocarditis, surgical wound infections, intra-abdominal and pelvic infections, and abscesses[5]. Most vancomycin-resistant enterococci (VRE) are *E. faecium*. There has been a shift over the years among cultured enterococci from *E. faecalis*, which used to be the more common, to *E. faecium*.

Enterobacteriaceae

The enterobacteriaceae are Gram negative bacteria, which are a part of the normal human intestinal flora. Common bacteria are *E. coli, Klebsiella spp* and *Enterobacter spp.* They cause urinary tract infections, intra-abdominal infections and pneumonias. They are often resistant to first-line treatment such as amoxicillin. They are increasingly resistant to ESBLs, providing fewer treatment options other than combination therapies or carbapenems[6].

Pseudomonas aeruginosa
P. aeruginosa is a Gram negative rod.

Most clinical isolates produce pyocyanin and pyoverdin, which are blue and green pigments[7]. The bacteria have a characteristically sweet smell. *P. aeruginosa* is an opportunistic pathogen, both invasive and toxogenic, and rarely causes disease in healthy individuals. The bacteria can survive for long periods in moist environments and on hospital equipment. It is an important pathogen in nosocomial infections, especially in immunocompromised patients, causing respiratory tract infections, urinary tract infections, bacteraemias, and wound infections in burns patients. It also causes external otitis, folliculitis, and keratitis in contact lens wearers[6]. *P. aeruginosa* is adaptive and promiscuous and easily develops antibiotic resistance. Single antibiotic treatment is therefore not the best option.

Stenotrophomonas maltophilia
S. maltophilia is an aerobic Gram negative bacterium of low virulence. It tends to grow in moist environments. It colonizes different solutions used in the hospital setting and may, via these solutions, penetrate and diffuse into wounds, mucosal-barriers and urine. *S. maltophilia* can cause lower respiratory tract infections and bacteraemia, but one has to bear in mind that *S. maltophilia*, due to its low virulence, rarely causes infection and therefore other sources of infection must be excluded[8].

Acinetobacter spp

The term *Acinetobacter* spp usually refers to *Acinetobacter baumannii*. This is an aerobic Gram negative bacterium usually recovered from patients who are immunocompromised or have been subjected to prolonged hospital admission. *A. baumannii* tends to colonize aquatic environments e.g. hospital solutions, sputum, urine and respiratory secretions. It has low virulence and if it infects humans, it affects organs with high water content, e.g. urine, peritoneum, cerebrospinal fluid, the respiratory tract and burns. The bacterium is resistant to many antimicrobial agents, and therefore it presents a challenge for the treating physician[9].

Candida spp

Candida spp are yeast-like fungi with several virulence factors. There are more than 100 species but only a few are clinically relevant in humans. *Candida* spp has the ability to adhere to other cells and surfaces and produces acid proteases. It has the ability to transform into hyphae-like forms. It tends to colonize and infect neonates, elderly patients and the immunocompromised, as well as patients with indwelling catheters. Candida causes a wide variety of infections ranging from skin and soft tissue, respiratory tract, gastrointestinal, genitourinary tract and systemic infections. *Candida albicans* is the most commonly isolated of *Candida* spp and together with *Candida glabrata* makes up between 70-80% of invasive isolates cultured in the USA. *C. glabrata* has become more important due to its greater resistance, especially to azoles and amphotericin B, and it is therefore increasingly found, as are *Candida krusei, Candida tropicalis, Candida lusitaniae, Candida parapsilosis, Candida guilliermondi* and *Candida dubliniensis*[10].

Antimicrobial drugs

Beta-lactam antibiotics

This group consists of penicillins, cephalosporins, carbapenems and monobactams. They have the chemical structure of the β-lactam ring in common.

Penicillins

These were the first in this group of antibiotics to be discovered. They were generally effective against Gram positive bacteria, but groups of penicillins that were effective against Gram negative bacteria were later discovered, and these proved to be potent broad-spectrum antibiotics, especially when combined with β-lactamase inhibitors.

Cephalosporins

This is a group of antibiotics with bactericidal effect. This is achieved by inhibition of peptidoglycan that is needed for cell wall synthesis. The first generation of cephalosporins is primarily effective against Gram

positive bacteria, but the later second and third generations are more effective against Gram negative bacteria. Fourth generation cephalosporins are broad spectrum antibiotics with activity against both Gram negative and Gram positive bacteria.

Carbapenems

These have a chemical structure that makes them highly capable of withstanding β-lactamases. They have the broadest antibacterial spectrum of the β-lactam antibiotics. They are active against both Gram positive and Gram negative bacteria, but not to intracellular bacteria.

Monobactams

These are synthetic monocyclic β-lactam antibiotics, derived from a bacterium. They are inactivated by some β-lactamases and by all extended spectrum beta-lactamases (ESBLs). They are mainly used in *P. aeruginosa* infections, but they are also active against *Enterobacter* spp, *Serratia* spp, *E. coli*, *Klebsiella* spp, *Haemophilus* spp, *Proteus* spp and *Citrobacter* spp.

Fluoroquinolones

The quinolones in clinical use today have a fluoro group attached to their central ring system. Their bactericidal effect is due to inhibition of bacterial DNA-gyrase and topoisomerase IV. Quinolones are often used to treat intracellular microbes because they easily penetrate the cell wall. The kidneys, and to a lesser extent the liver, are the main elimination pathways for quinolones. Ciprofloxacin and levofloxacin are the most commonly used fluoroquinolones in Swedish ICUs. Ciprofloxacin exerts its effect on Gram negative bacteria and therefore is an option in e.g. upper urinary tract infections and exacerbations of chronic bronchitis. In Sweden, levofloxacin is used in atypical pneumonias due to its effect on both aerobic Gram positive and Gram negative bacteria, e.g. *Mycoplasma pneumoniae, Legionella pneumophilia* and *Chlamydia* spp.

Macrolides

Macrolides have a lactone ring to which deoxy sugars are attached. Their main effect is bacteriostatic but in high concentrations they can also be bactericidal. They exert their effect by binding reversibly to the ribosome in the bacteria, inhibiting protein synthesis. They are mainly eliminated through the liver. Macrolides are mainly effective against Gram positive bacteria but not *Enterococcus* spp. In Sweden they are mostly used for treatment of atypical pneumonias and when allergy to penicillin is suspected or established.

Oxazolidinones

Oxazolidinones are organic and contain a ring of 2-Oxazolidone with oxygen and nitrogen. Linezolid was the first antibiotic in this new class, and it exerts its effect by binding to a ribosome sub-unit, inhibiting pro-

tein synthesis in Gram positive bacteria. The elimination of the drug is predominantly renal. It has a bactericidal effect against most *Streptococcus* spp and *Enterococcus* spp, and it has a bacteriostatic effect against *Staphylococcus* spp. Linezolid is mainly prescribed for MRSA infections or other multiresistant bacteria as an alternative to the glycopeptide agent vancomycin.

Glycopeptides

Glycopeptides are non-ribosomal peptides consisting of glycosylated cyclic or polycyclic structures. Vancomycin and teicoplanin are two members of this group that are in clinical use. They inhibit cell wall synthesis by inhibiting the production of peptidoglycan. In ICUs vancomycin is used predominantly. They have a narrow spectrum of action and are toxic to the kidneys and acoustic nerve. Plasma levels must therefore be monitored. Vancomycin is mainly used for severe multiresistant Gram positive infections, e.g. MRSA and MSSE. It is not absorbed when given orally but has a local effect on bacteria, including *Clostridium difficile*.

Aminoglycosides

Aminoglycosides are derived from Streptomyces or Micromonosporas. In the former case they are given suffix –mycin and in the latter –micin. They bind to a sub-unit of the ribosome and block initiation of protein synthesis.

They also makes mRNA misread, which also inhibits protein synthesis. In high doses they have dose-dependent nephro- and ototoxic effects, and therefore serum concentrations have to be monitored carefully. Aminoglycosides are used predominantly to treat infections with aerobic Gram negative bacteria such as *Enterobacter* spp, *P. aeruginosa* and *Acinetobacter* spp.

Amphotericin B

Amphotericin B is derived from *Streptomyces nodosus* and its name is derived from its amphoteric properties. The mechanism of action is through association of amphotericin to fungal membrane ergosterols, which causes leakage of potassium and intracellular components leading to cell death. Higher doses are fungicidal and lower doses are fungistatic. Amphotericin is used in the treatment of systemic fungal infections in immunocompromised patients. The agent is also active in candidiasis, aspergillosis, cryptococcal meningitis and visceral leishmaniasis. It is also used empirically in the treatment of fever in neutropenic patients that do not respond to broad-spectrum antibiotics.

Imidazole and triazole derivates

Imidazole and the newer triazoles have a ring structure consisting of carbon, hydrogen and nitrogen. They exert their fungistatic effects through the inhibition of cytochrome 450 14-α-demythelase,

which is necessary for the conversion of lanosterol to ergosterol, which is used in the fungal cell walls. They are eliminated through the kidneys. Fluconazole is the most commonly used triazole. It is most effective against *C. albicans* and cryptococcal infections. A newer triazole, voriconazole, is effective against *Aspergillus* spp and all *Candida* spp.

Susceptibility breakpoints

In order to express antibiotic resistance, the terms susceptible (S), intermediate/indeterminate (I) and resistant (R) are used, which makes it easier for the treating physician to understand the resistance data obtained from cultures made at the microbiological laboratory. Several different systems are in use worldwide, and many countries have adopted their own. In Sweden, the Swedish Reference Group for Antibiotics (SRGA)[11] is responsible for setting the MIC breakpoints as well as zone diameter breakpoints. Today in Europe, the European Committee for Antimicrobial Susceptibility Testing (EUCAST) is trying to harmonise the MIC breakpoints between the EU countries[12]. Since the beginning of 2007, all breakpoints in Sweden, except for macrolides and penicillins, correspond to EUCAST values. An inquiry is currently looking at the evaluation of the MIC values of the remaining antibiotics.

ATC, DDD and DDD_{1000}

Antibiotic consumption was recorded using the Anatomical Therapeutic and Chemical Classification system (ATC) and Defined Daily Doses (DDD) that were developed during the 60s and 70s and adopted by WHO in 1982[13]. The system was invented and implemented for research on drug usage. The WHO Collaborating Centre for Drug Statistics Methodology classifies drugs according to the ATC-system and establishes DDD for each of these drugs. In order to compare data between different countries and hospital settings, a preferred denominator has to be used. In the hospital setting the denominator most commonly used is 100 or 1000 patient days, giving the measure DDD/1000 patient days (DDD_{1000})[13]. The terms admission days or occupied bed days are often used instead of patient days.

Antimicrobial drug resistance mechanisms, development and spread

Spread of bacterial resistance

The use of antibiotics and antifungals drives the development of resistance in microbes, and several studies have demonstrated an association between increased antibiotic consumption and an increase in bacterial resistance to the drug in question[14]. The converse

relation has yet to be conclusively shown[15, 16]. In order to avoid horizontal spread of resistance, it is of great importance that the prudent use of antibiotics is achieved and maintained. Proper barrier precautions and isolation of patients must be used when indicated. Proper hygiene measures must be undertaken, especially hand disinfection. Age and co-existing diseases are important factors in the development of nosocomial infections, as are length of stay and invasive catheters[17, 18].

Resistance to antimicrobial drugs

Resistance to antimicrobial drugs can be both acquired and intrinsic. The former is due to genetic mutations within the microbe resulting in better protection against the antimicrobial agent. The mechanisms behind this are multiple and complex but four main characteristics can be seen[19]. Firstly, the antimicrobial agent may be inactivated, e.g. by the production of β-lactamases. Secondly, changes in accessibility may occur, by which antibiotics fail to enter the microbe. This happens when downregulation of porins takes place. Thirdly, antibacterial drugs may be excreted, e.g. when efflux pumps are upregulated. Fourthly, mutations can occur in the target for antibiotics rendering the attacking antibiotic ineffective as it lacks a target. The production of alternative targets can shield the microorganism or the target may be protected in other ways[19].

β-lactam resistance

Resistance to β-lactam antibiotics is produced by all of the above mechanisms.

Production of β-lactamases

As mentioned above, the production of enzymes that inactivate antibiotics is one mechanism of protection. This is the most common mechanism of resistance in Gram negative bacteria. β-lactamases inactivate penicillins, cephalosporins and to some extent carbapenems, by the hydrolysis of an amide bond in their β-lactam ring. The governing gene is often an integral part of plasmids and transposons, making them highly transferable between bacteria[20]. β-lactamases can be sub-grouped according to Ambler classes A-D (AmpA-D)[21]. AmpB β-lactamases are metallo-β-lactamases, and they have a broader hydrolytic action against all antibiotics in the β-lactam group[22]. AmpA β-lactamases are most often inhibited by clavulanic acid, but inhibitor resistant enzymes like TEM and SHV are described. In contrast, AmpD β-lactamases are almost fully resistant to inhibition by clavulanic acid[21]. Restricted- spectrum OXA-12 and ImiS are exceptions to this [23], as are the extended spectrum OXA-18 enzymes[24]. AmpC β-lactamases are also of interest as extended spectrum β-lactamases (ESBL) as well as carbapenemases because of their ability to disable most of the β-lactam antibiotics[20].

Effect of porins and efflux pumps on intracellular concentrations of β-lactam antibiotics

Porins in the outer membrane of the bacterium is a channel that some of the β-lactam antibiotics use to enter the microbe. Downregulation of porins or changes in their chemical structure prevents the antibiotic from exerting its effects[25].

Target alteration

The penicillin binding proteins (PBPs) are the targets of β-lactam antibiotics. There are several described types. If they are altered or downregulated, the effects of β-lactam agents will be abolished or reduced[25].

Quinolone resistance

Multiple mechanisms are responsible for the development of quinolone resistance, but the result is a mutation in the genetic structure encoding for the DNA-gyrase termed topoisomerase, specifically, topoisomerase II and IV. In the latter, the mutation occurs in subunits called *gyrA* and *gyrB* or in *parC* or *parE*[19]. The resistance mediated by these changes can be enhanced by efflux pumps and porin permeability. Another mechanism involves the production of a Qnr protein which protects the topoisomerase from quinolones. This is called plasmid-mediated quinolone resistance (PMQR)[26]. All of the mechanisms can co-exist and have an additive effect on resistance levels.

Co-trimoxazole resistance

Co-trimoxazole (TMP-SMX) is a combination drug consisting of trimethoprim and sulfamethoxazole. Both these drugs inhibit folate synthesis but at different stages. Resistance occurs to both drugs. The most important TMP resistance mechanism in Gram negative bacteria involves the alteration of dihydrofolate reductases (DHFR). This is encoded by *dfr*-genes, which are integron-borne genes. Resistance to SMX is mostly mediated by three sulphonamide genes, *sul1-3*, and they are transferred horizontally[27, 28].

Macrolide and lincosamide resistance

Resistance to macrolides and lincosamides are mediated by three different genes. The mefA encodes resistance to erythromycin. The ermB encodes resistance to both erythromycin and clindamycin, and ermA encodes an inducible resistance to clindamycin and resistance to macrolides[29].

Aminoglycoside resistance

Several mechanisms are responsible for the development of aminoglycoside resistance. Firstly, changes in cell permeability and decreased uptake which are chromosomally mediated[30]. Secondly, mutations that produce alterations of ribosomal binding sites can produce resistance[31]. Thirdly and most importantly, modification of

enzymes can produce high level resistance. More than 50 enzymes are known. The genes encoding for these enzymes are usually found in plasmids and transposons[32].

Surveillance of microbial antibiotic resistance, antibiotic consumption and nosocomial infections

There are several surveillance systems for bacterial resistance, antibiotic consumption and nosocomial infection rates. There is a strong focus on these issues as they represent major problems. The 58th World Health Assembly emphasized this in 2005, when it stated that containment of antimicrobial resistance is a priority[33].

Important information systems currently exist, including the Swedish Strategic Programme for the Rational Use of Antimicrobial Agents and Surveillance of Resistance (STRAMA), which was established in 1994[34]. Germany has the Surveillance of Antibiotic Use and Bacterial Resistance in German Intensive Care Units (SARI)[35] and its associated German Hospital Infection Surveillance System (KISS). Denmark has its Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP)[36]. In Europe, several systems co-exist: the European Antimicrobial Resistance Surveillance System (EARRS)[37], the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[12], Action on Antibiotic Resistance (ReAct)[38] and Antibiotic Resistance; Prevention and Control (ARPAC)[39]. In the global arena, we have the surveillance system International Network for the Study and Prevention of Emerging Antimicrobial (INSPEAR)[40].

Hospital hygiene and factors affecting nosocomial infection rates

Several factors influence nosocomial infection rates, and the relations between them are many and complex.

The most important measures are the use of adequate barrier precautions, hand washing and isolation of carriers of multiresistant organisms. These aims can, however, be negated by heavy workload and a high staff turnover[41].

Antibiotic policies affect bacterial drug resistance. It is very important that the correct antibiotic therapy is given[42]. The use of antibiotic cycling has been studied and discussed and may have a role[43, 44]. Selective decontamination of the digestive tract has also been described, but it is not certain whether it has any effect on mortality[45-47]. The restrictive use of antibiotics is also described[48-50].

The Centers for Disease Control and Prevention (CDC) in US has set out a campaign in 12 steps to prevent the spread and transmission of bacterial resistance. The guidance has four cornerstones: Prevent infection, diagnose and treat infection effectively, use antimicrobials wisely and prevent transmission. A summary follows below[51]:

Prevent infection
Vaccinate patients against influenza and pneumococcal infection. Encourage the vaccination of staff as well. Prevent conditions that can lead to infection, e.g. aspiration, pressure sores and dehydration. Remove unnecessary invasive devices and follow relevant guidelines when inserting them[51].

Diagnose and treat infection effectively
Use established criteria for infection, and target empiric and, when possible, definitive treatment. Take appropriate cultures. Use local resources e.g. specialists in infectious diseases when in doubt or complicated scenarios are encountered or foreseen, and know your local data[51].

Use antimicrobials wisely
Use appropriate antibiotics and say no when there is no indication for treatment. Avoid long-term prophylaxis. Treat infections and not colonisation or contamination. Re-evaluate treatment constantly, and stop treatment when infection has resolved or when infection cannot be proven[51].

Prevent transmission
Isolate the pathogen. Break the chain of contagion and use barrier precautions. Perform hand hygiene, preferably with alcoholic hand rub. Identify multiresistant organisms and take appropriate action[51].

AIMS

The general aim of the thesis was to investigate bacterial resistance and antibiotic consumption in Swedish ICUs.

Specific aims were the following:

– To try to correlate ICU demographic data with antibiotic consumption and antibiotic resistance.

– To try to help ICU physicians to interpret antibiotic resistance data so that they can prescribe the most appropriate antibacterial agents for the bacteria commonly found in the ICU.

– To investigate on which clinical indications different antibacterial agents are prescribed in the ICU.

– To investigate if and to what extent bedside physiological and laboratory data influence antibiotic prescribing patterns in the ICU.

– To investigate the emergence of resistance and transmission of Pseudomonas aeruginosa in the ICU using cluster analysis based on antibiograms and genotype data obtained by AFLP.

MATERIALS AND METHODS

Patients and settings

In all papers, participating hospitals and ICUs are grouped into three categories. They are labelled as i) tertiary care centres or university and regional hospitals, ii) district general hospitals, secondary hospitals or county hospitals and iii) local hospitals, primary hospitals or general hospitals.

Paper I

The first paper looks at ICU admissions in southeast Sweden. A total of eight ICUs were included, from five different hospitals. Three were district general hospitals (Norrköping, Jönköping and Kalmar), one was a local hospital (Eksjö), and one was a tertiary care university hospital (Linköping), which contributed patients from the general, burns, cardiothoracic and neurosurgery ICUs. A total of 17 592 patients were included. ICU demographic data were acquired including mean length of stay and total number of admissions, and mean APACHE II scores were obtained from general ICUs.

Paper II, III,

The second and third papers include ICUs taking part in ICU-Strama. For paper II, 38 ICUs participated during 1999, covering approximately six million of Sweden's nine million inhabitants. For paper III, 29 ICUs participated during 1999-2000. ICU demographic data were studied in both

papers, although they were analysed in greater detail in paper II.

Paper IV

This study was conducted during the first two weeks of November 2000 and included 393 patients from 23 Swedish ICUs, of which 7 were tertiary care centres, 11 district general hospitals and 5 local hospitals.

Paper V

Patients admitted to eight Swedish ICUs (five tertiary care academic hospitals located in Stockholm (Karolinska Huddinge and Karolinska Solna), Gothenburg, Malmö and Linköping, and in three district general hospitals located in Stockholm (Södersjukhuset), Jönköping and Skövde).

Paper V was based on material from the multi-centre Nosocomial Prevalence Resistance Surveillance study (NPRS III) carried out in 2002, which investigated aerobic Gram negative bacteria cultured on clinical indication. These hospitals were chosen to represent different geographical areas of Sweden. A total of 505 patients were included in NPRS III. Of these, 88 provided isolates of *P. aeruginosa* and were included in the study.

Susceptibility testing and bacterial isolates

Paper I, II, III, IV and V

In all papers, bacterial samples were taken on clinical indications. Only initial isolates were considered in papers I-IV, whereas repeat isolates were also included in paper V. An isolate in this thesis is defined as bacteria cultured from a patient admitted to an ICU. Susceptibility testing was done at the time of sampling by the disc diffusion (papers 1-4) and E-test (paper V) methods, as recommended by the Swedish Reference Group for Antibiotics (SRGA)(accessed 23/7/2007)[52]. SRGA-recommended breakpoints for susceptible (S), intermediate/indeterminate (I) and resistant (R) were used.

Paper I considered the seven most common bacteria cultured during the study period (*Enterobacter* spp, *Klebsiella* spp, *Enterococcus* spp, *E. coli*, Coagulase-negative staphylococci, *S. aureus* and *P. aeruginosa.).* A total of 800 Gram negative and 2 043 Gram positive isolates were collected.

Paper III specifically investigated *Acinetobacter* spp, CoNS, *Enterobacter* spp, *Enterococcus* spp, *E. coli*, *Klebsiella* spp, *P. aeruginosa*, *Serratia* spp, *S. aureus*, and *S. maltophilia.* In order to define which antibiotics were possible treatment options for each bacterium, a novel index was introduced called Treatment Alternative for more than 90% of tested bacteria (TA_{90}).

In paper V, 101 *P. aeruginosa* isolates from 669 Gram negative isolates from the NPRS III were analysed. All samples were taken on clinical indication and they were cultured and tested at the local microbiological laboratory. In order to be able to detect the emergence of resistance, repeat isolates from each patient were also allowed. Five anti-pseudomonal drugs were investigated (imipenem, gentamicin, ceftazidime, ciprofloxacin, piperacillin-tazobactam). Isolates resistant or intermediately resistant to three or more β-lactam antibiotics were subjected to analysis for the production of metallo-β-lactamases with MBL Etest (AB Biodisk, Solna, Sweden). We defined multidrug resistance (MDR) as resistance to three or more of the tested drugs [53, 54]. SRGA breakpoints for MIC-values were used and accessed 070704[11].

Antibiotic consumption

Paper I, II, III, IV

Data on antibiotic consumption using the Anatomical Therapeutic Chemical (ATC) classification system were provided by hospital pharmacies, expressed as antibiotics delivered in Defined Daily Dose (DDD) to the corresponding ICUs[13]. DDD is calculated as the average maintenance dose per day in adults for the main indication of the drug. In paper IV, administered antibiotic doses were recorded on a daily basis, in addition to the prescribing indication and the stop date or date for evaluation that were set on initiation of treatment.

Laboratory and physiological parameters

Paper IV

Laboratory and physiological parameters were recorded. These were body temperature, heart rate, blood pressure, breathing rate, urinary output, C-reactive protein, blood leucocyte count, platelet count, serum lactate, serum bilirubin, ALAT, arterial base excess, and arterial oxygen tension.

Questionnaire on ICU characteristics and Infection control

Paper I, II, III and IV

In papers I and III, each participating ICU was asked to provide data on length of stay, number of admissions and severity of illness scores (APACHE II and III). In papers II and IV, more specific questionnaires were used to gather information on workload and working procedures in each participating ICU, with questions on the utilisation of hand disinfectant, antibiotic treatment guidelines, regularity of rounds with specialists in infectious diseases, distances between ICU beds, and number of isolation rooms. Information was also gathered about how often feedback about antibiotic consumption was given by the local pharmacy and about local resistance patterns from the hospital microbiology laboratory.

Genotyping of Pseudomonas aeruginosa

Paper V

All bacterial isolates were investigated by amplified fragment length polymorphism PCR (AFLP). The method followed published protocols [55], except that EcoRI-0 primers used for DNA amplification were fluorescently labelled with Cy-5. PCR products were detected by analysis of a 1-μl portion on an ALF Express DNA Sequencer (Amersham Pharmacia) as described previously [56]. Similarity was calculated by Dice coefficients using the BioNumerics uncertain band tool, with 0.5% tolerance and 0.5% optimisation. Cluster analysis was done by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). All groupings with ≥ 90% similarity were inspected visually for the number of fragment differences. Isolates with ≥ 3 fragment differences were assigned to the same genotype.

Detection of Metallo-β-lactamases in Pseudomonas aeruginosa

Paper V

Isolates positive for metallo-β-lactamases (MBL) with MBL Etest were subject to further analysis with imipenem +/- EDTA on Mueller Hinton agar. Isolates with a MIC-ratio imipenem/imipenem+EDTA ≥8 were subjected to multiplex real-time

PCR, targeting genes encoding the five groups of acquired MBLs, i.e. VIM, IMP, GIM, SPM and SIM[57].

Antibiogram-based cluster analysis of Pseudomonas aeruginosa

Paper V

The 101 *P. aeruginosa* isolates from NPRS III were subjected to hierarchical cluster analysis on the basis of $\log_2$ (MIC)-values of susceptibility to five tested antibiotics (imipenem, ceftazidime, piperacillin-tazobactam, ciprofloxacin and gentamicin). The analysis used the MiniTAB software package [58, 59]. The distance measure for each dimension was the absolute value of the difference between the $\log_2$ values, reduced by 1 (except for zero difference) taking the variability in MIC determination into account. The multidimensional measure used was Euclidian distance based on these unidimensional measures. Complete linkage clustering (farthest neighbour) was used, where the distance from a data point to a cluster is measured for the farthest data point in the cluster, and the distance between two clusters is measured using the most distant pair. This was done until the distance was zero and no more clusters could be combined.

Statistical methods

Papers I, II, III, IV and V

In paper I, statistical analysis was done with a non-parametric test (Pearson Chi 2 – test), and p-value was calculated with Monte Carlo approximation. In papers II and III, non-parametric tests (Spearman's rank correlation, Mann-Whitney, Kruskal-Wallis, Fisher's test) were applied to explore relationships and differences[60-64].

In paper V, adjusted Rand coefficient was used for overall concordance and the Wallace coefficient for directional information about the partition relations[65-67].

RESULTS

ICU characteristics and infection control

Paper I

A total of 17 592 patients were included from January 1995 to December 1997. The annual number of patients treated decreased during the observation period, and the number of admission days decreased accordingly from 18 989 in 1995 to 16 850 in 1997. The annual mean length of stay, in days, ranged between 2 and 3.1 for general ICUs, between 1.7 and 1.9 for the cardiothoracic ICU, between 5.1 and 5.2 for the neurosurgery ICU, and between 13.9 and 15.5 for the burns unit. No correlation between APACHE II scores and antibiotic consumption was seen.

Paper II

Thirty-eight ICUs, providing primary services to a population of almost six million, participated in the study. Ten units were located at tertiary care centres (regional/university hospitals), 20 in secondary care centres (county hospitals) and eight were in local hospitals. The number of admissions and the total length of stay differed significantly between the ICU categories (Table 1, page 36). Local hospital ICUs had more admissions and shorter stays compared to county and regional hospital ICUs. 47% collected illness severity scores but only one ICU computed mortality risk from these scores. The mean APACHE II scores were slightly higher in regional hospital than in the local hospitals. 85% of ICUs had alcohol hand disinfection available at the bedside, for more detailed information see Table 1. 44% had regular rounds with a specialist in infectious diseases. This was more common in larger units (p=0.07), see Table 1.

Paper III

Twenty-six ICUs participated and 25 of these had alcohol hand disinfection by each bed. More than 90% had isolation rooms available, 81% had a consultant in infectious diseases available for rounds at least twice weekly and 62% registered severity of illness scores.
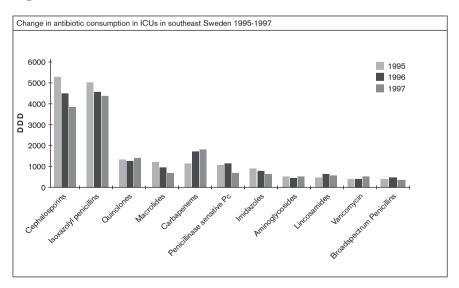
Paper IV

Twenty-three ICUs agreed to participate. Seven were regional/university ICUs, 11 county, and seven local hospital ICUs. Out of a total of 393 patients, 44% were admitted to regional ICUs, 43% to county ICUs and 12% to local hospital ICUs. 22% of the patients were already admitted (inpatients) at the start of the study.

Table 1

Intensive care unit characteristics and selected practice parameters.				
Characteristics*	Local hospital ICU	County hospital ICU	Regional hospital ICU	P-value†
Annual no. of admissions median (range)	2070 (1577–4955) n=5	1746 (591–4950) n=18	1042 (700–1490) n=9	0,03
No. of beds median (range)	8 (6–11) n=5	8.5 (6–19) n=20	9.5 (6–16) n=8	0,53
Mean APACHE II scores median (range)	10.4 (10.0–12.8) n=3	12.0 (8.7–16.0) n=12	12.9 (12.7–13.0) n=2	0,36
Mean length of stay (days) median (range)‡	1.0 (0.3–1.2) n=4	1.4 (0.6–3.2) n=18	2.3 (1.4–4.5) n=9	0,01
Antibiotic consumption (DDD1000) median (range)	1072 (807–1377) n=4	1170 (604–2415) n=17	1541 (584–2247) n=9	0,18
Written guideline on distance between beds	0/5 (0%)	1/20 (5%)	2/8 (25%)	0,19
Written guideline on the use of antibiotics	1/4 (25%)	4/20 (20%)	2/9 (22%)	1
Regular rounds with infectious disease specialist	4/5 (80%)	20/20 (100%)	9/9 (100%)	0,15
Rounds with ID-specialist at least 5 days/week	0/5 (0%)	9/20 (45%)	6/9 (67%)	0,07
Hand disinfection, bedside §	4/5 (80%)	18/20 (90%)	7/9 (78%)	0,51
Report on antibiotic usage at least once a year	3/4 (75%)	16/18 (90%)	7/8 (88%)	0,76
Report on antibiotic usage at least every 3 months	2/4 (50%)	10/18 (56%)	5/8 (63%)	1
Report on bacterial species and drug resistance at least once a year	2/5 (40%)	3/17 (18%)	1/6 (17%)	0,68

* Postoperative patients were included in some units,
  leading to a large number of admissions and short mean lengths of stay.

† P-values refer to comparisons between intensive care unit (ICU) categories.

‡ Correlated with total antibiotic usage (P=0.03, see text).

§ Negatively correlated with total antibiotic usage (P=0.05, see text). DDD1000, defined daily doses per 1000 occupied bed days. The number of units (n) varies as a result of missing values. Unless otherwise stated the ICU characteristics did not correlate with antibiotic consumption.

Figure 1

Change in antibiotic consumption in ICUs in southeast Sweden 1995-1997

Antibiotic consumption and prescriptions

Paper I

Antibiotic consumption expressed as DDD decreased by 13.3%, but this figure fell to 2.5% when corrected for admission days (DDD/1 000 admission days). Consumption of carbapenems increased as the consumption of cephalosporins, macrolides and penicillins decreased (Figure 1). No correlation was found between severity of illness scores (APACHE II) and antibiotic consumption.

Paper II

The median consumption of antibacterial agents was 1 257 DDD/1 000 admission days. No correlation between antibiotic consumption and severity of illness scores was observed. Antibiotic consumption was on average 1.6 times higher in ICUs where no bedside alcohol hand disinfection was available. Total consumption of antibiotics varied up to fourfold between the units but with no differences between the ICU categories (Table 1). ICUs where a consultant in infectious diseases was responsible for antibiotic prescribing had lower consumption rates for glycopeptide antibiotics but for no other antibacterial agents. Local hospitals (primary hospitals) had significantly lower carbapenem consumption compared to university hospitals (tertiary hospitals) (Figure 2, page 38). Cephalosporins were the most prescribed group of antibiotics (median 26%). ICUs with many admissions and short mean length of stay had lower median antibiotic consumption (p=0.01 and p=0.03 respectively). 21% of participating ICUs had written guidelines for antibiotic prescribing.

Paper III

Median antibiotic consumption was 1 391 DDD/1 000 occupied bed days in tertiary care centre ICUs, 1 201 in county hospitals and 983 in local hospitals. These differences were not statistically significant (p=0.125). A wide range was seen, 605-2 143 DDD/1 000 occupied bed days. Cephalosporins were the most prescribed antibiotics with 26% of the median consumption, followed by oxacillins, carbapenems and quinolones with 13%, 10% and 8% respectively. Prescription patterns did not vary between the three ICU categories. Cefuroxime was by far the most used cephalosporin (79%), followed by cefotaxime (13%) and ceftazidime (4.5%).

Paper IV

The median rate of patients on antibiotics was 74% but displaying a wide range of 25-93%. This was especially evident in county and local hospitals where the range was 35-93% (median 67%) and 24-80% (median 38%) respectively. The highest median prescription rate was found in tertiary care centres with 84% (range

Figure 2

Median consumption of antimicrobials in defined daily doses per 1000 occupied bed days (DDD1000) in different categories of intensive care unit. The consumption of carbapenems was significantly lower in local ICUs compared with county and regional ICUs (P<0.05)

NAME OF ANTIBIOTIC

Type of hospital............ DDD/1000 occupied bed days

GLYCOPEPTIDES
Local 12,4
County 24,7
Regional 37,4

AMINOGLYCOSIDES
Local 13,8
County 29,6
Regional 35,0

BETALACTAMASE
SENSITIVE PENICILLINS
Local 86,7
County 56,3
Regional 32,4

IMIDAZOLES
Local 77,9
County 67,9
Regional 49,4

FLUOROQUINOLONES
Local 85,0
County 88,8
Regional 106,1

CARBAPENEMS
Local 58,1
County 116,5
Regional 165,9

ISOXAZOLYL PENICILLINS
Local 168,0
County 162,7
Regional 277,4

CEPHALOSPORINS
Local 314,2
County 330,2
Regional 366,1

Defined daily doses (DDD)/1000 occupied bed days

58-87%). Almost half the patients received monotherapy, 20% had 2 antibiotics and 3% had 3 and occasionally 4 antibiotics prescribed during the study period. Prior to admission, cefuroxime was the most commonly prescribed antimicrobial agent (mean 24%), but after admission carbapenem was the most widely used. Vancomycin was rarely prescribed (2%). Linezolid and teicoplanin were not prescribed at all. Empirical therapy was the most common form of prescription (64%). No correlation was seen between laboratory parameters, such as CRP levels, leucocyte count and thrombocyte count, and antibiotic prescription. Culture-based decisions were less common on days 1-2 than on days 3-14. A date for deciding whether to stop or continue antibiotic treatment was set in 8% of those receiving empirical treatment and 3% of those who received culture-based therapy. 95% of antibiotics prescribed for sepsis were found to be appropriate when compared to antibiograms for blood isolates.

Bacterial species and antibiotic resistance

Paper I
A total of 2 043 Gram positive and 800 Gram negative isolates were taken on clinical indications. Only first isolates were considered. A significant increase in resistance among *Enterococcus* spp was seen between 1996 and 1997 (p<0.001). This was due to a shift from *E. faecalis* towards *E. faecium.* There was a statistically significant increase in ciprofloxacin resistance among *E. coli* and *Enterococcus* spp (p<0.05). An outbreak of methicillin-resistant *S. aureus* was seen in two hospitals during the study period, but no vancomycin resistance was seen in *S. aureus* or coagulase-negative staphylococci (CoNS). Resistance to oxacillin (≈70%), ciprofloxacin (≈50%), fusidic acid (≈50%) and netilmicin (≈30%) in CoNS was seen throughout the study.

Paper II
All ICUs received preliminary information regarding bacterial growth in blood cultures. 74% also received this information for other specimens. More than half the units were given quarterly feedback on local levels of bacterial resistance. Almost 75% received this information at least annually. Clinically significant levels of decreased sensitivity to cephalosporins (second and third generation) were seen in *Enterobacter* spp and to ampicillin in *Enterococcus* spp. 26% of *P. aeruginosa* isolates showed decreased susceptibility (I+R) to imipenem, 11% to ceftazidime and 11% to ciprofloxacin.

Paper III

A total of 12 501 initial isolates were included. All were taken on clinical indications from patients admitted to participating ICUs during 1999-2000. The most common organism isolated was coagulase-negative staphylococci (CoNS) which constituted 17.5% of total isolates and 32.1% of blood isolates. This was followed by *Candida* spp, *E. coli* and *S. aureus*. The mean number of treatment alter-natives $TA_{90,}$ as described in material and methods, for *E. faecium*, CoNS, *P. aeruginosa and S. maltophilia* was 1-2 per organism. Vancomycin was the only option for the first two and ceftazidime and netilmicin for *P. aer-uginosa*. The treatment options for *S. maltophilia* were ceftazidime and tri-methoprim-sulfamethoxazole. There were more treatment alternatives for the other bacteria, see Table 2.

Table 2

Treatment alternatives (TA_{90}[a]) and susceptibility to antibiotics among tested pathogens.														
		Proportion of susceptible isolates[b] (%)												
Organism	TA_{90}	CTX/												
	(n)	CTZ	CXM	CIP	IMI	NET	PTZ	TSU	AMP	CLM	OXA	FUS	RIF	VAN
Acinetobacter spp	**3**	21	6	89	**96**	**91**	40	**96**	-	-	-	-	-	-
Enterobacter spp	**4**	67	41	**93**	**99**	**100**	77	**93**	-	-	-	-	-	-
E. coli	**7**	**99**	**91**	**94**	**100**	**100**	**95**	**92**	79[c]	-	-	-	-	-
Klebsiella spp	**6**	**97**	83	**95**	**100**	**100**	**93**	**95**	-	-	-	-	-	-
P. aeruginosa	**2**	**92**[d]	-	85	70	**99**	85	-	-	-	-	-	-	-
Serratia spp	**3**	88	14	**94**	**98**	**97**	79	89	-	-	-	-	-	-
S. maltophilia	**2**	**91**[d]	-	68	0	-	-	**94**	-	-	-	-	-	-
E. faecalis	**3**	-	-	1	**99**	0	-	-	**100**	-	-	-	-	**99**
E. faecium	**1**	-	-	0	11	0	-	-	22	-	-	-	-	**99**
CoNS	**1**	-	-	4	-	54	-	41	-	42	29	50	84	**100**
S. aureus	**6**	-	-	-	-	**100**	-	-	-	**98**	**98**	**96**	**98**	**100**

[a] TA90 indicates an antibiotic to which > 90% of isolates of a given species or group of bacterial species are susceptible.

[b] Numbers higher than 90 and thus defining TA90 are marked in bold. Ampicillin (AMP), cefotaxime (CTX), ceftazidime (CTZ), cefuroxime (CXM), ciprofloxacin (CIP), clindamycin (CLI), fusidic acid (FUS), imipenem (IMI), netilmicin (NET), oxacillin (OXA), piperacillin-tazobactam (PTZ), rifampicin (RIF), trimethoprim-sulfamethoxazole (TSU) and vancomycin (VAN)

[c] Including S (1%) and I (78%). According to the SRGA, wildtype E. coli are intermediately susceptible to AMP.

[d] CTZ

The maximum numbers of isolates tested per antibiotic was 128 for Acinetobacter spp, 410 for Enterobacter spp, 778 for E.coli, 498 for Klebsiella spp, 602 for P. aeruginosa, 90 for Serratia spp, 198 for S. maltophilia, 805 for E. faecalis, 434 for E. faecium, 2238 for CoNS, 1063 for S. aureus.

Paper IV

Among blood isolates (n=58) *S. aureus*, *C. albicans* and CoNS were the most common organisms followed by *Enterobacter* spp. In urine cultures (n=38) *E. coli* (32%) and *Enterobacter* spp were the most prevalent findings, followed by *P. aeruginosa* (7%), *E. faecalis* (7%) and *Candida* spp (4%). Respiratory tract isolates (n=44) had *Klebsiella* spp (18%), *P. aeruginosa* (14%), CoNS (11%), *S. aureus* (11%) and *H. influenzae* (9%) as the most common microbes.

Empirical treatment was correct in 55/58 (95%) of bacteraemias according to corresponding antibiograms. The instances of incorrect therapy included fluconazole for infection due to resistant *C. albicans*, meropenem for resistant CoNS, and cefuroxime for a naturally resistant *E. faecium*.

Paper V

The main source of isolates was the respiratory tract, from which 36 (35.6%) isolates were obtained. Of these, 15 (14.9%) came from the upper airway (nasopharynx and tracheostoma) and 21 (20.8%) from the lower respiratory tract. Skin and soft tissue produced 25 (24.6%) isolates. Thirteen (12.9%) isolates were collected from abdominal wounds and drains. The urinary tract and blood contributed 12 (11.9%) and 10 (9.9%) isolates respectively, and five (5.0%) isolates were obtained from other body sites.

Six (5.9%) of the isolates were multidrug resistant (MDR), rising to eight (7.9%) when both intermediate and resistant isolates were considered.

MIC distributions of all tested antibiotics are given in Table 3. No gentamicin-resistant strains were seen. Seventeen (16.8%) of investigated *P. aeruginosa* isolates were resistant, and four (4.0%) were intermediately susceptible to imipenem. Corresponding resistant and intermediate num-

Table 3

Pseudomonas Aeruginosa									
Antimicrobial drug	Minimal Inhibitory Concentration (MIC) mg/L[a]								
	≤0,125	0,25	0,5	1	2	4	8	16	32	≥64
Imipenem	2	5	16	41	11	5	**5**	4	7	5
Ceftazidime		4	2	32	37	16	2	3	2	3
Piperacillin-Tazobactam				10	26	33	17	5	5	5
Ciprofloxacin	52	14	14	**6**	**2**	4	2	3	4	
Gentamycin		6	7	29	48	11				

Bold – Intermediate resistant (I)

Grey – Resistant (R)

[a] MIC-breakpoints according to SRGA and EUCAST 14/07/07 (www.srga.org, www.escmid.org)

bers for ciprofloxacin were 10 (9.9%) and 6 (5.9%). For ceftazidime, eight (7.9%) resistant isolates were seen but no intermediates. The same was observed for piperacillin-tazobactam where 11 (10.9%) of the isolates were resistant.

Six isolates showed resistance to all investigated beta-lactam antibiotics and were subjected to phenotypic analysis for metallo-β-lactamases (MBL) with Etest. One isolate showed a MBL-phenotype, but no VIM, IMP, SIM, GIM or SPM genes were detected with multiplex real-time PCR.

Eight patients with repeat isolates of *P. aeruginosa* were found. In one patient *P. aeruginosa* was isolated from two samples taken from different body sites on the same day, and these isolates were therefore not considered as a true repeat isolate.

Genotyping of Pseudomonas aeruginosa

Amplified fragment length polymorphism analysis (AFLP) of 101 *P. aeruginosa* isolates identified 68 genotypes. Fifty-one isolates were unique genotypes, and 17 genotypes displayed identical or similar patterns to one or several other isolates. Of these 17 genotypes, five were present in more than one ICU. Genotype A, C, H, M and N were present in 3, 2, 4, 3, and 2 hospitals respectively. We did not find any clonal spread of MDR clones in this study, but cross transmission between nine of 88 patients (10.2%) was seen.

Antibiogram-based cluster analysis of Pseudomonas aeruginosa

The cluster analysis of the phenotypes based on MIC-values of *P. aeruginosa* for the key antibiotics investigated showed 40 different phenotypes.

DISCUSSION

Main findings

There are three important findings in this thesis. Firstly, antibiotic consumption in participating ICUs was relatively high during the study period, and every patient received on average more than one antimicrobial drug per day (I-IV). Secondly, levels of antimicrobial resistance in *S. aureus, E. coli,* and *Klebsiella* spp remained low when data were pooled from all ICUs throughout the study period, despite relatively high antibiotic consumption (I-V). Thirdly, the prevalence of antibiotic resistance in CoNS and *E. faecium*, cefotaxime resistance in *Enterobacter* and ciprofloxacin and imipenem resistance in *P. aeruginosa* was high enough to cause clinical concern.

Settings

Papers I, II and III

In the first pilot study, eight ICUs participated from the southeast region of Sweden, and thereafter successively more ICUs were included in ICU-STRAMA. This gives a fairly good view of Swedish ICUs. The first study included almost all the ICUs in the region, and paper II covered six million out of a total population of nine million. The third paper included ICUs providing primary service to more than half of the Swedish population.

There is a preponderance of district general (county) hospitals in the material, but this mirrors the distribution of Swedish ICUs described by the Swedish Intensive Registry (SIR)[68].

The median length of stay in paper II was short (1.5 days, range 0.5-4.5), which can be explained by a high proportion of postoperative recovery cases. In local hospital ICUs, coronary care patients and post-operative patients both influence the mean length of stay. Both patient categories contribute to short length of stay and also lower antibiotic consumption, which is related to the total admission days. This was seen in papers I and III as well as in the five-year study[69].

The general (mixed) ICUs were categorized into groups according to three types of hospital. These were local (general/primary) hospitals, district general (secondary) hospitals and regional (tertiary) care centres. The case-mix is very difficult to verify for each ICU. Differences between ICUs in each category must be taken into account. The ICUs belonging to each category are situated in similar regions, operate in similar health service frameworks, and provide services to inhabitants from similar socioeconomic mixes. In this thesis the assumption is made that the case-mix is comparable in the three ICU categories.

In a recently published study from 14 Swedish ICUs, the mean APACHE II scores per unit and year (1999-2003) were between 9.0 and 18.4, and the mean (SD) was 14.1 (2.7). This closely resembles the APACHE II scores of the Swedish Intensive Care Registry (mean: 13.4, 25-75 percentiles: 7-19 over the past 4 years). However, in the second paper (II) the median APACHE II score were measured and presented separately for ICUs in tertiary care centres (12.9), county hospitals (12.0) and local hospitals (10.4). Although these differences were not significant it can be explained by the presence of a different case mix in the local hospital ICUs (larger proportion of postoperative patients) which is confirmed by the shorter mean length of stay (range) in these ICUs of 1.0 days (0.3-1.2) compared to 1.4 days (0.6-3.2) in the county hospital and 2.3 days in the tertiary care centres (1.4-4.5).

Papers IV and V

In the prospective study performed in 2000 to investigate the appropriateness of antibiotic prescribing in Swedish ICUs, a total of 393 patients from 23 ICUs in different hospital categories were included. These were located in tertiary care centres (177 patients/7 centres), secondary hospitals (169/11) and primary hospitals (47/5). Of the 393 patients included, 44% were in ICUs in tertiary care centres, 43% in secondary hospitals and 12% in primary hospitals, which is fairly representative of Swedish adult critical care.

The fifth paper is based on the prospective collection of aerobic Gram negative bacteria isolated from patients admitted to Swedish ICUs during 2002. ICUs located in five tertiary care hospitals and in three secondary care hospitals were enrolled. These hospitals were chosen to represent different geographical areas of Sweden, but ICUs in tertiary care centres were relatively over-represented compared to the mean for Swedish ICUs.

Antibiotic consumption

ATC/DDD and DDD$_{1000}$

Antibiotic consumption was registered according to the ATC/DDD defined by the WHO[13]. DDD is a highly standardized measure that allows comparison of antibiotic consumption between different settings and countries, as long as a common definition is also used for length of stay. The length of stay (admission days) was in the first paper based on whole days and did not differentiate between admission for 25 hours or 2 days. Registration of length of stay in minutes has gradually been implemented and the minutes have been converted to 24 hour periods in order to make more reliable comparisons.

Antibiotic use in papers I-III is based on the quantities of drugs delivered by each hospital pharmacy, and this method produces several potential sources of error, e.g. drugs may be delivered but not administered, which may cause an overestimation of the antibiotics given to patients. However, in paper IV, antibiotic use was studied in 23 Swedish ICUs during 2 weeks in 2000, and 74% of 393 patients were on antibiotics. When taking into account that 1 in 3 patients were treated with more than one antibiotic, antibiotic consumption was almost as high as the median consumption shown in papers I –III. In a recent study performed in 14 ICUs from 1999 to 2003, it was shown that mean total antibiotic use increased from 1 245 DDD_{1000} in 1999 to 1 510 DDD_{1000} in 2003 (p < 0.11), an alarming but not statistically significant trend[69]. Antibiotic consumption of greater than 1 000 DDD_{1000} is common in European and US ICUs[70, 71], but lower rates were found in a Swiss ICU study (462 DDD_{1000} in the surgical ICU and 683 DDD_{1000} in the medical ICU)[72]. Antibiotic use described in Swiss surgical ICUs may be due to good adherence to strict indications for treatment and infection control[72].

Furthermore, some limitations of comparing DDD in the ICU setting have to be taken into consideration. Firstly, the defined doses are based on doses for the most common indication given to a 70 kilogram male. Therefore, the doses are not always correct for the individual critically ill patient. For example, intravenous administration of cefuroxime is most often prescribed at 4.5 g/day, but the DDD calculation is based on 3 g/day. Levofloxacin is prescribed in Sweden at 1 g/day but at only 500 mg/day in the DDD system[13]. For meropenem the dose for meningitis is 2g x 3 (6 g/day), but for sepsis 1g x 3 (3 g/day) is commonly given, as opposed to the DDD defined by WHO of 2 g[13]. On the other hand, the dose is often reduced in ICU patients with impaired renal function. However, there are studies that indicate that antibiotic consumption is overestimated when DDD_{1000} or DDD_{100} is used compared to actual prescribed daily doses (PDD) per 100 patient days[73, 74]. A study in Jönköping, Sweden, of antibiotics actually given to ICU patients as compared to the antibiotics delivered from the pharmacy found a 10% overestimation in all antibiotics, and for oxacillins the difference was even greater (not published). The explanation is that high dependency units and theatres are always situated in close proximity, and theatre staff tend to take antibiotics from the ICU even though this is not encouraged as they have different budgets. This pattern was even more prominent at weekends (personal communication Fredrik Hammarskjöld). When analysing trends and shifts in antibiotic

use, all the factors influencing consumption statistics have to be taken in consideration. This is best done by local validation.

Antibiotic consumption during the study period with a focus on carbapenems and cephalosporins

Antibiotic consumption decreased somewhat during the first study. This may, in part, have been due to an increased awareness of the issues under investigation, but the decrease could equally well have been coincidental. However, there was an increase in carbapenem consumption, but no corresponding increase in carbapenem resistance among Gram negative bacteria was seen during the 3-year study period. A correlation between high carbapenem consumption and carbapenem resistance has been shown for *P. aeruginosa* by others[75, 76], but the studies in this thesis (I-III) were not designed to investigate the impact of high carbapenem consumption on carbapenem resistance. The highest risk for emergence of resistance during carbapenem treatment is probably seen in *P. aeruginosa*, and this was demonstrated in paper IV in a few cases. The use of carbapenems has increased during the last decade in Swedish ICUs[77, 78]. In paper II carbapenems accounted for 9% of total antibiotic consumption as measured by DDD_{1000}, with greater use in county and regional ICUs. There have been

some early data supporting the theory that increased carbapenem consumption need not necessarily produce an increase in Gram negative resistance [79, 80]. But in later studies, carbapenem resistance in *P. aeruginosa* becomes more prevalent, as it also does in other bacteria [81-83].

In the early 1990s, cephalosporins were the most frequently administered antibiotics in European ICUs, as shown in the EPIC study[2]. This was still true in the cohort of ICUs in papers I-IV. Such high consumption may be a matter of concern, as evidence accumulates that cephalosporin usage is an important determinant of selection and propagation of multiresistant bacteria [84, 85]. The proportion of different cephalosporins varied, but few units used anything other than second and third generation compounds. In paper III, cefuroxime – a second generation cephalosporin – accounted for 80% of total cephalosporin consumption. Possible ecological side effects of this may include an increased number of infections by enterococci and increased prevalence of enterobacteriaceae with the ESBL phenotype. This however has not been demonstrated within the scope of our investigation. In general, resistance among *E. coli* and *Klebsiella* spp isolated in Swedish ICUs is low, and this fact may in part explained by the extensive prescribing of cefuroxime noted in our studies. Although con-

troversial, recent data suggest that fourth generation cephalosporins are less conducive to the development of bacterial multiresistance[86-88]. Clinically significant resistance to antibiotics was found in *Enterobacter* spp with decreased sensitivity to second and third generation cephalosporins. This might explain the increasing use of carbapenems, and it suggests that use of second and third generation cephalosporins should be reduced in Swedish ICUs. In comparison with data from other European countries, data from papers II and III show that antibiotic prescribing is lower in Sweden, the other Nordic countries and in the Netherlands, and penicillin is still commonly used outside hospitals[89]. This fact probably produces a low entry of resistant epidemic clones like MRSA, VRE and ESBL-producing *Klebsiella* spp and *P. aeruginosa* from the community (accessed 06/08/2007)[37].

Factors affecting antibiotic consumption

As mentioned above, data on delivered antibiotics from the pharmacy may overestimate true antibiotic consumption. This has always to be taken into consideration.

It is possible that significant differences in case-mix within the same hospital category contributed to the large difference (up to four-fold) in consumption of antibiotics as seen in the second paper. The importance of specific case-mix is highlighted by the findings that cardiothoracic ICUs were among the highest consumers of antibiotics per 1 000 occupied bed days. When analysed in more detail, it became apparent that this stemmed from the large consumption of isoxazolyl-penicillins, which are given routinely postoperatively, combined with short lengths of stay.

Whilst we were able to identify some ICU characteristics linked to high antibiotic consumption, as in the case mentioned above, we also identified that the median use of carbapenems was lower in local hospitals compared to county (district general) and regional hospitals. The ICUs having a specialist in infectious diseases responsible for antibiotic treatment had significantly lower use of glycopeptides compared to other units. Surprisingly, there was no obvious association between total antibiotic consumption and ICU category or case-mix of admissions based on APACHE II scores. Such a relationship could have been concealed because we had difficulties in obtaining a satisfactory picture of the case-mix from individual units. However, the results are consistent with the theory that factors other than patient-related factors determine the use of antibiotics[18]. None of the ICUs practised selective decontamination of the digestive tract as described by de Jonge and co-workers, so this fac-

tor could not explain the large differences between units[46]. We also looked for associations with a set of diverse clinical practices and hygiene control measures. But, in contrast to preliminary reports from the European Strategy for Antibiotic Prophylaxis[90], we were unable to establish any association between these selected practice parameters and antibiotic consumption except for a higher consumption in the ICUs without bedside facilities for hand disinfection. The ESAP also found considerable heterogeneity in the use of antibiotics in 21 European ICUs of six European countries[90]. In addition, that study observed that the prescription of antibiotics was less when approval was needed from a senior physician/microbiologist and when a list of restricted compounds was provided. Restricted compounds were defined as third and fourth generation cephalosporins, ticarcillin-clavulanate, piperacillin-tazobactam, carbapenems, amikacin, fluoroquinolones and glycopeptides. Increased consumption of these antibiotics was, in the ESAP study, associated with surveillance of colonisation in the ICU and with sponsoring of meetings and other PR activities by the pharmaceutical industry, suggesting that factors other than patient-related factors determine the use and choice of antibiotic therapy[90].

The heterogeneity in total consumption of antibiotics normalised per occupied bed days suggest that the prescription and use of antibiotics can be improved. Optimisation of prophylaxis and the choice and duration of empiric therapy remain critical goals to reduce antibiotic pressure within ICUs. Although this is typically achieved by having regularly updated written guidelines and feedback to all physicians, only 20% of the ICUs in the second study had such formal policies, and in paper IV only 9% had a set date for re-evaluation. Therefore, prescription of antibiotics in the ICU was largely up to the individual, increasing the risk of antibiotic overuse and the selection of inadequate agents and dosage regimens[91]. In a follow up by ICU-STRAMA in 2003, more than 50% of the ICUs had such formal guidelines, and 24% had routines for re-evaluation of treatment (unpublished data)[77]. While there is a lack of studies of optimal antibiotic strategies for preventing the emergence of bacterial resistance, there is consensus that knowledge of trends in usage and costs, coupled with insights into local patterns of bacterial resistance, are steps toward the prevention and control of emerging bacterial resistance[18].

Glycopeptide antibiotics in Swedish ICUs

The consumption of glycopeptides was low in comparison with a fairly recent French study[92]. Units relying on a specialist in infectious diseases

for the prescription of antibiotics had a generally lower use of glycopeptides, which might be due to an awareness among specialists in infectious diseases of the need for restricted glycopeptide use. Furthermore, the low glycopeptide consumption can be explained by the very low (1%) prevalence of methicillin-resistant *S. aureus* (MRSA) in Swedish ICUs, which is also the reason for the comparatively high (15%) isoxazolyl-penicillin consumption described in the second paper.

Antibiotic prescribing in Swedish ICUs

Studies of antibiotic consumption in ICU patients have generally been based on retrospective data[43, 93-96]. In the large prevalence study on nosocomial infections in European ICUs from 1992, 62% of the patients were receiving antibiotics, and of these patients 51% were receiving more than one agent[2]. The median antibiotic consumption (1 147 DDD_{1000}, range 605-2 143) in paper III was apparently as high as in the European Prevalence of Infection in Intensive Care (EPIC) study from 1992 where two-thirds of 6 250 patients were on antibiotic treatment on the day of the study[2]. Compared to other European countries, the overall prescribing of antibiotics is lower in the Nordic countries and in the Netherlands, and penicillin is still commonly used outside hospitals[89]. However, the EPIC study

looked not only at ICU consumption, but also included hospitals as a whole and outpatients, and may therefore not be directly comparable with our research.

In papers I-III, no corrections have been made for patients receiving more than one antimicrobial agent. In the second paper it was shown that ICU patients were, on average, continuously treated with one or more antibiotics. In paper I, consumption is higher than in the EPIC study, but when compared to more recent studies, consumption seems to be much the same[73, 97-99]. However it seems difficult to obtain reliable antibiotic hospital consumption data, due to different case-mixes and lack of clarity about which wards are included, leading to an obvious overestimation of consumed antibiotics. In Sweden, efforts have been made to create a new register for SIR in which all data are linked to the individual patient, based on the unique personal identity number given to every Swedish citizen. For non-Swedish citizens a unique number is created, based on the date of birth. In Europe, there are more data on antibiotic consumption in outpatients than in inpatients[100]. Prospective collection of such data at patient level as performed in paper IV has several advantages. A more accurate picture of consumed/prescribed antibiotics can be obtained using PDD instead of DDD. The decision

process can be studied, and it allows for the expression of antibiotic use in terms of exposure, either as a number of antibiotic exposure-days per 1 000 patient-days or as a percentage of ICU patients who received 1 or more antimicrobial drugs [101]. Paper IV produced 3 principal findings. Firstly, antibiotic treatment was very common with a mean 74% of ICU patients (mean 84% in tertiary care units) receiving at least one antibiotic. Secondly, only 30% of initial treatment decisions were based on positive microbiological data. Thirdly, although most decisions on antibiotic prescription were empirical, and cefuroxime was the most commonly selected antibiotic, decisions turned out to be adequate in 95% of bacteraemia cases according to the antibiogram. There was a wide range of antibiotic prescribing rates among ICUs, and this was also apparent in papers II and III. The antibiotic consumption rate found in paper IV was higher (74% of all ICU patients) than that seen in EPIC (62%) and a Dutch study from 1997 (59%)[102]. This is consistent with papers I-III. The second generation cephalosporin, cefuroxime, and third generation cefotaxime and ceftazidime were, together with carbapenems, the most commonly prescribed antimicrobial agents on and after admission to the ICUs in our study. Too few patients were included in this study to evaluate the risk of treatment failure using cefuroxime, but according to antibio-

grams for blood isolates this risk appeared to be low.

Guidelines, computerised decision support, and rapid feedback from the microbiology laboratory can promote appropriate antibiotic usage[103]. Providing the physician with data on pathogen frequency and susceptibility at ward level, in addition to information on the site of infection and patient-specific clinical information has been shown to improve antibiotic selection, control antibiotic cost, and slow the emergence of resistance[104, 105], and should also minimise adverse outcomes due to inadequate empirical therapy.

Testing for bacterial antibiotic resistance and breakpoints

In papers I-IV all isolates were collected on clinical indication at the discretion of clinicians attending the ICU. It was beyond the scope of this thesis to determine whether the isolates caused infection or only reflected colonisation of the critically ill. In the first paper, breakpoints for susceptible (S), intermediate/indeterminate (I) and resistant (R), according to SRGA breakpoints[11], were considered as separate entities. However, in papers II and III the intermediate/indeterminate and resistant isolates were grouped together and referred

to as non-susceptible or isolates with decreased susceptibility. In 1997, a Swedish study was carried out using Etest to assess MIC-distributions. This method is based on a high inoculum and the native population can be more easily distinguished from strains with decreased susceptibility than with the disc diffusion method used in the first four papers[93]. In the Etest study, 2% of *E. coli* isolates had reduced susceptibility to ciprofloxacin according to NCCLS (CLSI) breakpoints compared to 8% using SRGA breakpoints[93] and 6% in paper III using disc diffusion. In order to improve early detection of decreased susceptibility, the SRGA species-related zone breakpoints were used, aimed at defining deviations from the native (wild type) susceptible population of a species as I or R, irrespective of the pharmacokinetics of the drug[11,52]. Thus, by defining decreased antibiotic susceptibility as the sum of I and R isolates, all isolates not belonging to the native population were classified as non-susceptible, yielding a higher non-susceptible rate compared to NCCLS (CLSI) breakpoints. This avoids the risk of underestimating the emergence of isolates with moderately reduced sensitivity, but it complicates a comparison with other studies. And there is also a risk of mixing up the terms when they mean almost the same. Since breakpoints may be changed, the value of studies reporting antimicrobial susceptibility data

in terms of percentages of susceptible or resistant are limited in the longer perspective.

When testing for methicillin resistance in Swedish laboratories oxacillin is used instead of methicillin because of its greater durability. The term *methicillin resistant* is nevertheless used also in Sweden.

When comparing breakpoints from SRGA, BSAC and CLSI (former NCCLS) for enterococci, good concordance was seen according to a Swedish study[106]. On the other hand, when another study published in 2001 compared these systems on enterococci plus another three other systems, major differences were seen between the SRGA, BSAC and CLSI, but also in comparison with the other systems[107]. Today, there is work in progress to harmonise the breakpoints in Europe into one, the EUCAST-system[12]. However, it is a work that takes time due to the fact that many interests have to be taken into consideration. It is of great importance that this work is completed. And in a utopian scenario, this harmonisation will also happen globally.

One limitation with the first four studies is that antibiotic susceptibility was measured with a disc diffusion method using zone breakpoints for S, I and R according to SRGA, as mentioned above, which means that small

changes in susceptibility may not be detectable. However, using disc diffusion histograms reduces the risk of missing changes in susceptibility. Another factor to consider is the experience from the SARI project, which suggests that many bacteria can have varying MIC-values over time, and this may not necessarily imply an expression of increased resistance (personal communication Daniel Jonas). The same has also been observed in the ICU STRAMA project as well as in the MYSTIC study, with increases in resistance levels for some bacteria in some years, which may be due to small outbreaks, and decreases during other years resulting in no significant change over time[65, 108] (Phil Turner personal communication).

Bacterial isolates, antibacterial drug resistance and the emergence of resistance

Adverse outcomes, such as increased mortality, resulting from inadequate antimicrobial treatment of in-hospital infections caused by antibiotic-resistant bacteria have been demonstrated in other studies[109, 110]. However, this thesis was not intended to study patient outcome.

Gram positive bacteria

Coagulase-negative Staphylococci

CoNS were the most frequently encountered bacteria in our studies (with a median 17.5% of all isolates and 32% of blood isolates in paper III), but no attempt was made to determine their clinical relevance as mentioned previously. In American intensive care units, CoNS represented the most common cause of bloodstream infection according to two US studies[96, 111]. Oxacillin-resistant strains of CoNS are endemic worldwide and, as in previous northern European studies[112, 113], and in papers I and III, 70-80% of CoNS were resistant to oxacillin and often to other antibiotic classes as well. Thus, 50% of the CoNS isolates were resistant to clindamycin, netilmicin and fusidic acid, and one out of five to rifampicin, but none showed decreased susceptibility to glycopeptides.

Staphylococcus aureus

In *S. aureus*, methicillin (oxacillin) resistance is a major problem worldwide, e.g. 60% of isolates in the EPIC study[2]. The prevalence of MRSA varies considerably between countries from high frequencies in ICUs in southern Europe (up to 80%) and England (16%) to low rates in The Netherlands (< 5%) and in the Nordic countries (1%)[2, 112, 114, 115]. The finding of 2% MRSA in paper III is similar to papers I and II.

Enterococcus spp

An increase in ampicillin resistance was seen in *Enterococcus* spp, due to a shift from *E. faecalis* to *E. faecium*. In contrast to non-Scandinavian ICUs, little or no vancomycin resistance was seen in *E. faecium* in a previous Scandinavian ICU study based on Etest MIC[112]. Furthermore, by using a disc diffusion test containing 5 µg instead of 30µg, it is possible to detect vancomycin-resistant *E. faecium* [11]. The low prevalence of vancomycin-resistant *E. faecium* in Sweden is probably due to low glycopeptide consumption in humans and the avoidance of using antibiotics, in particular avoparacin, as growth promoters. Continuously high cephalosporin consumption will select inherently resistant enterococci and could contribute to the emergence of HLGR enterococci in Swedish ICUs[116]. However, a recent study by Hallgren et al indicated that the clonal spread of HLGR enterococci reflected an infection control problem rather than the misuse or overuse of cephalosporins[117].

Gram negative bacteria

The low incidence of resistance to carbapenems in Gram negative bacteria shown in paper I was consistent with results from ICU studies in Belgium, France, Portugal, Spain and the USA[79, 80]. This was also true in papers II and III, and when compared to an-

other Swedish ICU prevalence study, the non-susceptible rates were also moderate using the SRGA species-related zone breakpoints as opposed to NCCLS (CLSI)[93].

Pseudomonas aeruginosa and
Stenotrophomonas maltophilia

In the second study from 1999, an emergence of resistance to carbapenems was noted among isolates of *P. aeruginosa* in association with increased use[112]. We found that 26% of *P. aeruginosa* isolates demonstrated intermediate susceptibility or were resistant to imipenem. An additional problem with the increased use of carbapenem is the selection of *S. maltophilia* in ICU patients. While alarming, *P. aeruginosa* and *S. maltophilia* were only found in a small proportion (4% and 2%, respectively) of all positive cultures in that paper. Since the median length of stay in the ICUs in our studies is relatively short, many of the isolates are probably from the endogenous flora of the patient, but nosocomial isolates such as *P. aeruginosa*, *Acinetobacter* spp and *S. maltophilia* may to a larger extent be hospital-acquired.

P. aeruginosa is reported to develop resistance during therapy in about 10% of treated patients (more often with imipenem than with ceftazidime)[118]. *P. aeruginosa* represented only a median of 3% (range 0-11%) of all isolates in the third paper and a median

of 0% (range 0-6%) of blood isolates. Some ICUs showed very low resistance levels to imipenem, ceftazidime and ciprofloxacin, whereas those from other ICUs had resistance levels as high as 57%. This has not been observed in previous Swedish or Nordic ICU studies including paper I [79, 93]. This indicates the possible current local spread of resistant strains. However, this could not be proven in our first four studies.

The antibiotic resistance patterns in *P. aeruginosa* found in paper V did not differ substantially from those found in previous antibiotic resistance surveillance studies carried out in Swedish ICUs[69, 93, 119]. Lower frequencies of MDR were observed in this study compared to data from the MYSTIC study[119] and US data[54, 120]. However, comparison between studies is difficult due to differences in breakpoints used for susceptibility and resistance, and some studies include not only resistant isolates, as in this study, but also intermediately susceptible isolates. The frequencies of antibiotic resistance have also varied over time during the ICU-Strama and MYSTIC studies[69, 119] (Phil Turner, personal communication).

Enterobacter spp

In *Enterobacter* spp isolates, susceptibility to cefuroxime was low in the first three papers. A trend towards decreased resistance to cefotaxime in *Enterobacter* spp was observed in the third paper in parallel with a decrease in the total consumption of cephalosporins. This was probably due to increased efforts to avoid ß-lactam antibiotics, except carbapenems, for treatment of infections caused by *Enterobacter* spp. Susceptibility to third generation cephalosporins among *Enterobacter* spp was 67% in that study and 32-67% in recent European ICU studies[112, 121]. Previous use of third generation cephalosporins has been found to cause the selection of blood isolates of *Enterobacter* spp resistant to several ß-lactam antibiotics and associated with high mortality[122]. Emergence of ceftazidime resistance in previously susceptible *Enterobacter* spp strains (by mutation) appears to occur more frequently than horizontal transmission in periods of non-outbreak[123]. The relatively high consumption of cephalosporins in Swedish ICUs could explain the frequent cephalosporin resistance in *Enterobacter* spp, but as no studies of the rate of transmission of such strains between patients have been carried out in Swedish ICUs, the role of this mechanism in the development of resistance remains unclear. A recent Nordic 16-centre study showed an absence of *Enterobacter* spp with decreased susceptibility to ceftriaxone among patients treated in primary care centres, compared to 13% in general hospital wards and 27% in ICUs[124]. Resistance to ceftazidime in enterobacteriaceae with inducible ß-

lactamase was significantly reduced in ICUs and haematology wards where ceftazidime was replaced with cefepime (+/– amikacin)[125-127]. A similar intervention may also be successful in Nordic hospitals since there will be little or no inflow of resistant strains borne by patients coming from the community. The prevalence of cephalosporin resistance in *Enterobacter* spp may be underestimated in our studies, since another multicentre study using Etest carried out in 1997 in Swedish ICUs showed 30% of *Enterobacter* spp to be resistant to third generation cephalosporins[93].

Acinetobacter species

The number of nosocomial infections caused by *Acinetobacter* spp, notably *A. baumannii*, has increased in recent years, probably because of their intrinsic resistance to many commonly used antimicrobial agents[128]. However, in critically ill patients, *A. baumannii* bacteraemia is not associated with a significantly increased mortality rate but may be a surrogate marker for disease severity [129, 130]. In paper III, these bacteria constituted only a median of 0.6% (range 0-3%) of all isolates and a median of 0% (range 0-4%) of blood isolates. Most isolates of *Acinetobacter* spp were susceptible to the carbapenems, but there have been reports of ICU outbreaks with strains multiply resistant to drugs including carbapenems[131, 132]. In the third study, imipenem susceptibility in *Acinetobacter* spp was >96% compared to only 42% in the most recent European ICU study[112].

Fungi

The ecological impact of increased carbapenem and quinolone consumption on faecal, skin and mucous membrane flora is difficult to estimate. However, the relatively high prevalence of *Candida* spp may be an ecological impact of the use of broad-spectrum drugs such as carbapenems and ciprofloxacin. A possible ecological side-effect of the high usage of cefuroxime could be an increased number of infections caused by enterococci, but this has not been evaluated.

Genotyping methods

PFGE and AFLP are considered appropriate methods for the investigation of clonal spread because of their high resolution powers. Results derived from PFGE and AFLP are usually considered comparable, although some authors argue that AFLP is more precise [15, 29]. We therefore believe that AFLP is an appropriate method for our study. However, in the case of isolates with the same genotype appearing in completely different ICUs, it would have been useful to have carried out MLST in order to provide greater discriminatory power and to establish whether epidemic clones

were present, as described by others [30-33]. MLST also enables easier comparison of sequences held by international MLST databases [33-38].

Validation of antibiogram-based cluster analysis

The theory that cluster phenotype analysis based on MIC data could be used to identify clusters based on genotype data is an appealing one, given the easy availability of routine susceptibility testing. Unfortunately there was no concordance between the phenotype and the AFLP genotype. Our analysis was based on the five antimicrobial key drugs (imipenem, ceftazidime, piperacillin-tazobactam, ciprofloxacin and gentamicin). We also reduced the risk of error caused by variability in MIC measurement by defining one dilution step as no difference. The results of this study lead us to suggest that if there is a suspicion of clonal spread of *P. aeruginosa*, further investigation should be done with a relevant genotyping method, as phenotypes based on MIC values are not concordant with genotypes of *P. aeruginosa*. If an outbreak with *P. aeruginosa* occurs, information about the diversity of genotypes will help the physician and, especially if there is a dominating clone, help to determine the most appropriate intervention. *saeruginosa* and only 1 for *Klebsiella*

spp[112, 121]. In general, resistance to cefuroxime increased in Gram negative bacteria, and this should therefore not be considered as a treatment option in late ICU-acquired infections. There are, in most cases, several treatment alternatives in most cases if an infection with Gram negative bacteria is suspected. *S. aureus* with reduced susceptibility to vancomycin was not found in our study and its number of TA_{90} was as high as six. The low rate of ampicillin susceptibility in *E. faecium* shown in this study (TA_{90} 1) is in agreement with the results shown in previous studies, including papers I and II, carried out in Swedish and European ICUs[112, 113]. TA_{90} could be a useful instrument for simplifying antibiotic prescribing for the individual ICU physician as it is illustrative of the current situation and perhaps could increase the interest in antibiotic policy issues as well as hospital hygiene.

Adherence to hospital hygiene procedures, hygiene factors and infection control measures

In the first study, an outbreak of MRSA and multiresistant *P. aeruginosa* was seen and handled successfully by intervention from the local hospital infection control team. In Sweden as well as in the Netherlands,

strict MRSA control measures are introduced (the "search and destroy" strategy)[133], and this is apparently keeping the problem at a minimum level. Based on experience from this, the suggestion is that, in an optimal program for antibiotic resistance surveillance, the data should be linked to the individual patient and unit. Spread of multiresistant bacteria in ICUs can be minimised with infection control routines such as efficient hand disinfection between patient contacts, barrier precautions, and isolation of patients infected with resistant organisms, although studies to identify more effective strategies are needed.

Failure to use basic infection control techniques with isolation precautions have been repeatedly shown to be associated with the spread of nosocomial infections within intensive care environments. While most ICUs in Sweden have one or two single rooms, a few units lack such facilities in keeping with a trend noted during the last decade in Sweden[134]. Intensive care unit beds are typically spaced widely apart, as indicated by the long median distances between beds in paper II. However, in a couple of ICUs, the distances were probably too short to allow for effective barrier nursing. Because hand hygiene remains the most important measure to prevent the transmission of microbes[135], a link has been sought in the second paper

between the number of bedside devices for hand disinfection, frequency of clinical infection and consumption of antibiotics. There was no relation between positive cultures and the availability or consumption of hand disinfectant. Among numerous possibilities this may indicate that the frequency of positive cultures per occupied bed days was a poor surrogate for a clinically significant infection. However, we noted an association between large antibiotic consumption and a lack of devices for hand disinfection at the bedside. This is one of the relationships found in the current work that needs to be assessed carefully in prospectively designed studies before a causal relationship can be established.

The susceptibility to important drugs of clinical isolates, expressed as the number of TA_{90}, from Swedish ICUs was high, despite comparatively high consumption of antibiotics. This suggests that the ecological impact of the drugs chosen was only moderate and suggests a positive impact of hospital hygiene on resistance rates. However, experience from other countries shows that this situation can change rapidly. Recently Bonten and Mascini summarised the forces involved in the emergence of resistance in ICUs and described why the type of microorganism, the mechanism of resistance and different epidemiological

variables determine the likelihood of successful intervention[136]. Reduction in antibiotic use will reduce costs and can reduce the development of resistance caused by mutations during antibiotic therapy, but to stop the transmission of e.g. MRSA and VRE other interventions are needed. In paper V clonal spread was not seen, but cross transmission between nine of 88 patients (10.2%) was observed, indicating a nosocomial infection control problem.

Further studies are needed to find more effective strategies to improve antibiotic use and hospital hygiene in order to minimise the emergence and spread of resistant organisms in ICUs.

Validation of the ICU-STRAMA database

In the United Kingdom, the Directory of Clinical Databases (DoCDat) is an institution established to provide information about available clinical databases and to make an independent assessment of their scope and quality[137]. The Intensive Care Society in the UK set up a centre for national audit and has carried out validation using a locally appropriate protocol [138]. This has been applied to the ICU-STRAMA database. Ten questions are asked and graded into four levels, where level one represents the least rigorous method and four the most rigorous. Six of the questions assess validity and reliability and four assess coverage. Question one looks at the extent to which the eligible population is representative for the country. Level four requires that the total population of the country is represented. Level one means that the population is unlikely to be representative for the country. ICU-STRAMA scores vary over the years. During 99-03 data is representative for the country, but the whole population is not included and therefore they are classified as level three. The database was subsequently adapted to fit care-ICU, and although representation fell during this process, it has now returned to former levels. The second question relates to completeness of recruitment of eligible population. Level four demands more than 97%, and ICNARC gives itself level four. All isolates are included and the antibiotic consumption is given for all participating units. Therefore ICU-STRAMA is also rated at level four. The third question deals with variables included in the database, and here ICU-STRAMA only reaches level one because no outcomes are measured. However, this is not in the scope of this database, but the SIR database possesses all the tools required to achieve level four. It is very hard to validate the ICU-STRAMA database without also validating the SIR database. Work is under way to inte-

grate those databases, which will in the long run improve results in similar validations. The fourth question deals with completeness of data where variables are at least 95% complete. For level four more than 97% has to be complete. This is fulfilled by the ICU-STRAMA database. Question five relates to the percentage of data collected as raw data. All or almost all are collected in this manner and therefore level four is achieved. Question six asks whether explicit definitions exist for the variables. If they exist for more than 97%, level four is reached. Level two implies less than 50%, and level one implies that none exist. ICU-STRAMA has definitions for all variables, reaching level four. Question seven asks if explicit rules exist for the recording of variables. This is true for all data in the ICU-STRAMA database, achieving level four. Question eight relates to reliability of coding and interventions. This is not tested, which puts ICU-STRAMA at level one. Question nine concerns independence of observations of primary outcome. This question is not appropriate for the ICU-STRAMA database, but the SIR database would be rated at level two because they neither have independent observers, nor are they blind to interventions as it is the treating physician that is responsible for providing the data set. The last and tenth question asks to what extent data are validated. No valida-

tions have hitherto been performed, putting the ICU-STRAMA database at level one. This gives a mean score of 2.5. The median score of the DoCDat databases is 3.0. ICNARC scores 3.4 [138]. The future holds the promise of a seamless integration of SIR and the ICU-STRAMA databases, based on a patient and individual code as well as the Swedish personal identity number – enabling short term and long term follow up. This work is in progress and validation of that database will score significantly better than today, but there are always risks with retrospective data collection where so many factors can interfere with the input of data onto the database.

How to continue the battle against multiresistant microbes in Swedish ICUs

At present, antimicrobial drug resistance in Swedish ICUs is at a fairly low level compared to other European countries. However, as highlighted by this thesis, there is room for improvement in several aspects. Better surveillance systems are being developed as mentioned previously, with the merger of the ICU-STRAMA and SIR databases. But it is of vital importance that feedback of surveillance data is given to and used by the local unit in their daily work, so that each prescribing physician is familiar

with their department's microbial flora. A discussion needs to take place in Sweden about whether colonisation cultures on patients with longer stays in the ICU department should be routine, and whether the threshold clinical indications for the taking of cultures should be lowered. However, it is important that antibiotic treatment is started only when criteria for infection are fulfilled and that colonization without infection is not treated. Written guidelines governing choice of prescription and follow up can be improved in most ICUs. A discussion about whether antibiotic cycling programmes could be an alternative should take place in Sweden. It is too early to tell whether selective decontamination of the digestive tract (SDD) should be an option. The important factors to consider are the basis on which patients are selected for SDD in the ICU and whether a genuine decrease in mortality can be achieved. The current "search and destroy" strategy implemented in Swedish ICUs when outbreaks occur seems to have been effective according to the studies in this thesis. As regards hygiene factors, continuing efforts are needed, including staff education and involvement, because the importance of hand disinfection and basic hygiene measures cannot be overemphasised.

CONCLUSION

For the period studied, multidrug resistance in Swedish ICUs was not a major problem. Signs of cross-transmission with non-multiresistant bacteria were observed, indicating a hygiene problem and identifying simple improvements that could be made in patient care guidelines and barrier precautions. A need for better follow up of prescribed antibiotics was evident. With further surveillance studies and monitoring of antibiotics and bacterial resistance patterns in the local setting as well as on a national and international level, some of the strategic goals in the prevention and control of the emergence of antimicrobial-resistant microbes may be achievable.

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