From the Department of Laboratory Medicine, Division of Clinical Pharmacology
Karolinska Institutet, Stockholm, Sweden

# CLINICAL STUDIES ON DRUG TREATMENT OF HOSPITALISED PATIENTS:
## GENERAL INFECTIOUS DISEASES AND ACUTE MYOCARIDAL INFARCTION

Ksenia Zagorodnikova (Goryachkina)


Karolinska Institutet

Stockholm 2013

Published by Karolinska Institutet. Printed by Larserics Digital Print AB


ISBN 978-91-7549-171-4

**To my parents and my teachers**

**“A teacher affects eternity**
**He can never tell where his influence stops”**
**(Henry Adams)**

## ABSTRACT

Treatment of hospitalised patients is generally governed by the pre-developed algorithms and common guidelines. These approaches are helpful in most, but not all cases. Treatment of hospitalised patients is limited to the time of hospital stay and is therefore directed to immediate help. There are diseases for which immediate help is as important as its long-term consequences. General infections and ischemic heart disease are among the most prominent examples. Cardiovascular diseases (CVD) remain the leading cause of death in developed countries. While immediate treatment of acute myocardial infarction (AMI) is currently dependent on rapid surgery and management of thrombosis, adequate long-term treatment with other agents including beta-blockers may prolong time to further cardiovascular events and therefore prolong patients' life. It is important to achieve adequate effects as early as possible and avoid adverse drug reactions (ADR) to fulfill primary goals of the treatment. Factors that affect individual treatment response may be inherited (genetic polymorphisms) or exogenous (drug interactions). General infectious diseases represent another problem where hospital lethality is traditionally high and is dependent on a number of factors, mainly timeliness of diagnosing and susceptibility of pathogenic microorganisms to available antibiotics. This susceptibility is a changing parameter and may be dependent on the pattern of traditional antibiotic use in a given hospital, which is related to selection of resistant pathogens potentially worsening patients' survival. This also has a more global consequence of cultivation of multiple resistant pathogens, which may then be spread over the hospital, region and even country borders.

General aim of the current thesis was to increase knowledge of specific factors that may affect quality of hospital care in the treatment of general infections and acute myocardial infarction and suggest methods to minimize their negative influence in hospitalised patients.

We found that CYP2D6 is a major factor of metoprolol disposition and effects in AMI patients and also a major determinant of individual variability of response to the treatment. Common exogenous medications prescribed for treatment of depression complicating AMI namely selective serotonin reuptake inhibitor (SSRI) paroxetin significantly increase metoprolol concentrations in patients and put them at risk of excessive bradycardia. Based on our findings we suggested that CYP2D6 genetically defined activity may be related to ventricular rhythm disorders (VRD) complicating early period after acute myocardial infarction, though not in patients undergoing percutaneous coronary intervention.

In our studies on surveillance of antibiotic use and resistance we applied a method of Drug Utilization 90% (DU90%), and modified it with cumulative microbial resistance data. From this combination it was clear that most widely utilized antibiotics are not suitable for treatment of registered infections due to high resistance of the microbes. We showed that this method of combined presentation of antibiotic 90% use and microbial resistance reflects existing situation in a comprehensive and easy way both – for prescribers and authorities. When this method was tested in a Russian hospital we observed antibiotic use and resistance during five consecutive years, we could not see any change in resistance despite obvious changes in utilization profile. We considered these changes attributable to our intervention because they were not observed in a control Russian hospital. We also observed antibiotic utilization and key

microorganisms resistance in a Swedish hospital. Overall antibiotic use was much higher in that hospital, antibiotics active against multiple resistant microorganisms were present within DU90% segment, despite that resistance of key microorganisms in this segment was low during the whole observation period. We concluded that the instrument of combined presentation of antibiotic use and cumulative resistance is an effective tool to show in an easy and comprehensive way rationality of antibiotic use and change utilization profile. It was also clear that in hospitals with high resistance of microorganisms to the most agents used other methods of infection control are required.

Our studies demonstrate two principally different approaches to improvement of drug treatment of hospitalised patients. In cardiovascular diseases we showed clinical importance of pharmacogenetics and drug interactions, which may further be continued by studies defining place for pharmacogenetic tests in clinics. For patients suffering from general infections we proposed a more general approach of antibiotic use and resistance surveillance that will help to define existing problems. It is a crucial step to improved treatment of patients in a particular hospital but also may have global contribution to containment of world dissemination of resistant microbes.

## LIST OF PUBLICATIONS

This thesis is based on the following publications, which will be referred to by their Roman numerals as indicated below:

I. **Goryachkina K**, Burbello A, Boldueva S, Babak S, Bergman U, Bertilsson L. Inhibition of metoprolol metabolism and potentiation of its effects by paroxetine in routinely treated patients with acute myocardial infarction (AMI). Eur J Clin Pharmacol. 2008 Mar;64(3):275-82

II. **Goryachkina K**, Burbello A, Boldueva S, Babak S, Bergman U, Bertilsson L. CYP2D6 is a major determinant of metoprolol disposition and effects in hospitalised Russian patients treated for acute myocardial infarction Eur J Clin Pharmacol. 2008 Dec;64(12):1163-73

III. **Goryachkina K**, Babak S, Burbello A, Wettemark B, Bergman U. Quality use of medicines: a new method of combining antibiotic consumption and sensitivity data--application in a Russian hospital Pharmacoepidemiol Drug Saf. 2008 Jun;17(6):636-44

IV. **Zagorodnikova (Goryachkina) K**., Bergman U., Hahlin A., Burbello A., Fedorenko A., Zaharova N., Giske C. Combined presentation of the Drug Utilization 90% segment of antibiotic use and cumulative resistance – a tool for quality assessment and intervention. Manuscript

# CONTENTS

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACE | Angiotensin-Converting Enzyme |
| ADR | Adverse Drug Reactions |
| AMI | Acute Myocardial Infarction |
| ASP | Antibiotic Stewardship Program |
| ATC | Anatomic Therapeutic Chemical classification |
| AUC | Area Under the Curve |
| BAB | Beta-Adrenergic Blocker |
| CABG | Coronry Artery Bypass Grafting |
| CAD | Coronary Artery Disease |
| CLSI | Clinical and Laboratory Standards Institute |
| CVD | Cardiovascular Disease |
| CYP2D6 | Cytochrome P450 2D6 |
| DDD | Defined Daily Dose |
| DU90% | Drug Utilization 90% |
| DURG | Drug Utilization Research Group |
| EARS-Net | European Antimicrobial Resistance Surveillance Network |
| EM | Extensive Metazolizer |
| EPA | Early Postinfarction Angina |
| ESBL | Extended-Spectrum Beta-Lactamases |
| EU | European Union |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| HADS | Hospital Anxiety and Depression Scale |
| HamDRS | Hamilton Depression Rating Scale |
| HR | Heart Rate |
| ICU | Intensive Care Unit |
| IHD | Ischemic Heart Disease |
| IM | Intermediate metabolizer |
| LVEF | Left Ventricular Ejection Fraction |
| MDR | Multiple Drug Resistance |
| MR | Metabolic Ratio |
| MRSA | Methicillin Resistant Staphylococcus Aureus |
| PCI | Percutaneous Coronary Intervention |
| PM | Poor Metabolizer |
| SNP | Single Nucleotide Polymorphism |
| SSRI | Selective Serotonin Reuptake Inhibitor |
| TDM | Therapeutic Drug Monitoring |
| UM | Ultrarapid metabolizer |
| VRD | Ventricular Rhythm Disorder |
| VRE | Vancomycin Resistant Enterococci |
| WHO | World Health Organization |

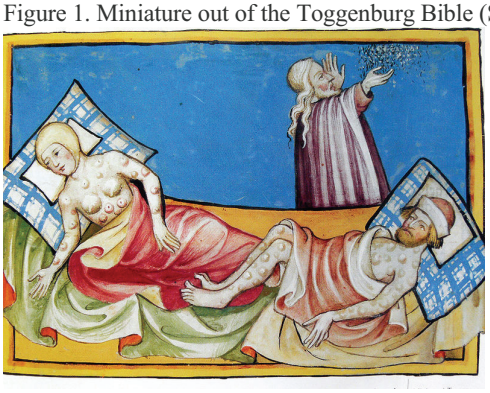
# 1 BACKGROUND

## 1.1 GENERAL INFECTIONS AND ISCHEMIC HEART DISEASE ARE THE LEADING CAUSES OF HOSPITAL DEATH

*Infectious diseases*

Infectious diseases are defined by the WHO as «caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi; the diseases can be spread, directly or indirectly, from one person to another»[1]. Infections led to deaths of millions of people starting from the antiquity, first mentions of plague are found in the ancient texts and first pandemics known - the Justinian plague – refer to 541-544 A.D[2].

Figure 1. Miniature out of the Toggenburg Bible (Switzerland) of 1411.



Many non-pandemic diseases were accompanied by fever, as it follows from the Hippocrates works, many of these patients died. Already in the 18th century the Scottish physician A. Gordon described the proofs of contagious origin of in-hospital Erysepilas and puerperal fever, which was later described in more details by an American Dr Homes and a Hungarian Dr Semmelweis [3]. Treatment strategies changed from calomel and izal antiseptics [4] to antibiotics, but the problem remained. Currently infectious diseases represent an unsolved problem in the lower income countries with malaria and tuberculosis being the most common; however, lower respiratory infections, being primarily treated in hospitals[5], were the 5th leading cause of death in the high-income countries in 2008[6].

*Cardiovascular diseases*

With the improvement of hygiene and treatment strategies of communicable diseases the duration of life increased and so increased the global prevalence of noncommunicable diseases. According to the WHO report 2012 63% of all death in 2008 were due to noncommunicable diseases among which the largest proportion belongs to cardiovascular diseases (48%). It was projected that cardiovascular deaths will increase from 17 million in 2008 to 25 million in 2030. The same report showed that in Eastern European countries a large proportion of such deaths constitute people of the younger (30-70) age. Ischemic heart disease represents 42% of all cardiovascular deaths globally and 48% in Europe[5]. All acute situations require immediate hospital care, which makes cardiovascular diseases a leading cause of deaths in hospitals.

These two problems are closely related. Healthcare associated infections can occur in any hospitalised patient. According to the annual report of European Center for Disease Control and Prevention in 2010 7.4% of patients staying longer than 2 days in ICU acquire pneumonia, and 3.4% - bloodstream infections[7]. A recent review published in the Lancet discusses a contrary relation saying that about a quarter of adults hospitalised with pneumonia develop major cardiac complications with 60% increase in cardiac mortality[8].

## 1.2 MICROBIAL RESISTANCE

### 1.2.1 General mechanisms

Microbial resistance is a kind of drug resistance that develops in microbes towards antibiotics. Resistance may be intrinsic or acquired. Molecular basis for the latter is mutation and selection of the mutated strain. These mutations may occur in genes encoding proteins or promoter genes regulating gene expression of target proteins, so that the drug can not bind, proteins involved in drug transport, changing microbial wall permeability, enzymes deactivating or altering drugs.

Microbial resistance represents random mutations being factor of survival in presence of antibiotics. Resistance genes can rapidly move within bacterial populations. First resistance of *Staphylococcus aureus* to penicillin was described before the antibiotic was deployed[9]. Rapid development of resistance stimulated chemical modifications of existing antibiotics in attempts to increase their effects against new pathogens and make them "resistant to resistance". Resistance, however, appeared to newer agents and consequent to that and also because of high costs of drug development many pharmaceutical companies abandoned research on new antibiotics[10]. At the same time spread of resistance does not stop so that currently one microbe may carry up to 12 genes of resistance to different agents, which is called multidrug resistance. These multidrug resistant agents may frequently be more virulent than others. Some of these microbes with resistance to several antibiotic classes represent a serious clinical problem leading to increased mortality, namely Extended-Spectrum Beta—Lactamases (ESBL), Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococci* (VRE), multidrug resistant *Pseudomonas aeruginosa*, carbapenemase and AmpC producers.

### 1.2.2 ESBL producers

Extended-spectrum beta-lactamases (ESBL) is a group of enzymes capable of inhibiting a wide range of cephalosporin anti-bacterial agents[11]. They have been described in the 70s, the main mechanism of transfer is plasmids. ESBL producers are generally defined as being resistant to all cephalosporins and monobactams but cephamycins (cefoxitin, cefotetan) or carbapenems. These enzymes are inhibited by classical beta-lactamase inhibitors (clavulanic acid, sulbactam, tazobactam). ESBLs are commonly produced by *Klebsiella* and *E.coli*. Their virulence is not enhanced by ESBL presence, but infections caused by ESBL producers are associated with increase rates of treatment failure, mortality and hospital costs[12]. The rate of ESBL production by enterobacteriaceae varies worldwide. According to data from European Antimicrobial Resistance Surveillance Network (EARS-Net) ESBL producing *Klebsiella* is a more common problem than ESBL producing *E.coli*. The frequency of detection varies between countries from 1.7% to 75.6% for *Klebsiella* and from 2.6% to 24.8% for *E.coli* [7]. ESBL genes are commonly associated with other resistance genes, which makes multidrug resistance not uncommon. Previous antibiotic use, especially beta-lactams, have been identified as a risk factor for ESBL blood stream infection with odds ratios raging from 1.9 to 11.8[12]. Investigators conclude that use of 3$^{rd}$ generation cephalosporins is a strong factor of selection of ESBL producers[13]. Also fluoroquinolone use was an independent risk factor for ESLB-producing enterobacteriaceae blood stream infections. Also a prolonged hospital stay is identified in studies as a risk factor for ESBL-producing enterobacteriaceae infection with odds ratio 1.3 to 14.9. Other factors associated with likelihood of ESBL producing enterobacteriaceae infections are previous invasive procedures or catheterizations, old age, liver cirrhosis, chronic obstructive pulmonary disease and others. Mortality rates in patients with blood stream infections caused by ESBL producing enterobacteriaceae is reported to vary from 8 to 43%[12]. These infections are generally considered to be sensitive to beta-lactam/beta-lactamase inhibitor combinations, although due to unexpected clinical failure despite in vitro effectiveness observed carbapenems are becoming in many places a recommended group for initial therapy of severe infections.

### 1.2.3 Carbapenemase and AmpC producers

AmpC producing enterobacteriaceae are considered to be resistant to all cephalosporins and aztreonam, but not to carbapenems. The specific feature is that they are phenotypically expressed under the pressure of antibiotics, mostly cephalosporins[11]. Selection of AmpC producers is associated with a two times higher mortality, longer mean hospital stay and treatment costs[14]. Cefepim and carbapenems are considered more stable and less selective. High use of carbapenems has consequently lead to appearance of carbapenem-resistant enterobacteriaceae. This is mostly due to loss of porins – channels required for antibiotic penetration through the bacterial cell wall. Enterobacteriaceae that produce enzymes destroying carbapenems have recently become a real danger in hospitals. The most common carbapenemase producers are *E.coli* and *Klebsiella spp.*. In contrast to ESBL producers these microbes cause severe infections and spread rapidly around the world[11]. The highest national rates of enterobacteriaceae producing carbapenemases published is 40% reported in Greece, metallobetalactamases (class B) are detected with frequency of 2-8% in India and up to 27% in Pakistan[14]. Many of carbapenemase producing strains are sensitive to tigecycline, colistin and fosfomycin, but resistant strains to these agents exist as well.

### 1.2.4 P.aeruginosa

Pseudomonas aeruginosa is a microorganism that is generally affecting severely or chronically ill patients. Resistance of this microorganism to aminoglycosides, fluoroquinolones, cephalosporins and carbapenems vary in different countries. They are lowest in Scadinavia (<10%) and highest in southeast of Europe (25-50%). The most common mechanisms of resistance are loss of porins, derepression of AmpC and activation of efflux. It is characterized by an ability to develop resistance to many antimicrobial agents. MDR (Multiple Drug Resistance) in *Ps.aeruginosa* is usually defined as resistance to three or more of the following agents – antipseudomonal penicillins, antipseudomonal cephalosporins, fluoroquinolones, carbapenems and aminoglycosides[15].

### 1.2.5 MRSA and VRE

The first strain of *Staphylococcus aureus* resistant to methicillin was discovered in a British hospital in 1961, and existing evidence show that further distribution was due to clonal spread, which means that these strains have a strong ability to overcome non-resistant counterparts[11]. According to the latest European reports frequency of MRSA is 1-5% in Scandinavian countries, and is around 25% in Germany, Poland, France and other countries in central Europe. It is reported to be even higher (up to 50%) in Southern Europe[16]. According to the surveillance data there is no increase in the majority of European countries, but high resistance levels are remaining. According to the data from an international study collecting data from ICUs in Latin America, Asia, Europe and Africa reported frequencies were 74% for catheter associated urinary tract infections and 84% for central-line associated blood stream infections[17]. Methicillin resistant staphylococcal infections are associated with a worse prognosis[18]. Currently MRSA infections are registered also in patients with community-acquired infections.

The mechanism of enterococcal insensitivity to vancomycin is in the amino acid change in the cellular wall of the microbe, which makes the affinity of vancomycin over 1000 times lower[11]. Vancomycin resistance was not observed until the late 80s.

Resistance rate is different for different types of enterococci – in some studies is is reported to be as high as 76% for *Enterococcus faecium* and 6.5% for *Enterococcus Faecalis*[17].

## 1.3 ANTIBIOTIC USE AND RELATION TO RESISTANCE

Resistance of microbes is a matter of their survival, which means that resistance they develop represents inevitable consequence of antibiotic use. First strains resistant to penicillin were identified prior to wide distribution of this agent. Some resistance is occurring naturally, but antibiotic use is a well recognized driving force. When antibiotics are used there are two mechanisms of resistance distribution – first is that being toxic to microbes antibiotics stimulate evolutionary process and development of protection mechanisms, second is selection of resistant strains by eliminating sensitive competitors. These processes are not only related to human antibiotic use – agriculture and cattle raising are also utilizing antibiotics which may be another source of resistance development. It is generally recognized that amount of antibiotic use in food animals may have impact on public health. It is well recognized that in order to improve something you have to be able to measure it. A number of metrics have been proposed to measure antibiotic use in agriculture. These are antibiotic sales, drug mass in kilograms, number of animals treated, treatment rate, animal defined daily dose (DDD), and animal DDD per 1000 animals[19]. Easy access to antibiotics in humans is a problem in some countries of the world, which makes measurements almost impossible and antibiotic exposure and resistance wider.

There have been quite a number of studies showing close relation of antibiotic use and resistance. In the most recent study previous antibiotic use was the only independent risk factor of acquisition of carbapenem-resistant enterobacteriaceae[20]. Clear relation between decreased use of carbapenems and carbapenem resistant pseudomonas aeruginosa was shown in 6-year observation in Japan[21]; in a Danish study increased consumption of fluoroquinolones and cephalosporins was related to increased resistance of *E.coli* from blood cultures in a 4-year observation[22]. Increased use of fluoroquinolones in Spain during 10 years was related to increased resistance of enterobacteriaceae to fluoroquinolones and 3rd generation cephalosporins[23]. Similarly previous use of aminopenicillins combined with enzyme inhibitors was a risk factor for carbapenem resistant *Klebsiella pneumonia* in a controlled study in Greece[24]. In some cases, however, resistance can not be directly related to utilization , like it was shown in a study involving a large number of hospitals, where aminoglycoside use decrease was related to decrease of resistant *P.aeruginosa* but increase of resistant *E.coli*[25] and in a Serbian study where relation of carbapenems was not found to be related to *Pseudomonas aeruginosa* and *Acinetobacter* resistance[26].

## 1.4 CONTAINMENT OF RESISTANCE BY IMPROVING QUALITY OF ANTIBIOTIC USE

Microbial resistance was for the first time defined as a global healthcare problem by the WHO in 1983 in the memorandum on "Control of antibiotic-resistant bacteria"[27]. It was then followed by the European Union Copenhagen recommendations "The microbial threat" released in 1998[28]. In 1999 WHO has issued a "wake-up call" against microbial threats [29] where it was stated that "the world has dangerously underestimated the threat bacteria and viruses are posing to national security and economic growth, and may soon miss its opportunity to protect people from this risk". Finally in 2001 the "Global strategy for containment of antimicrobial resistance" was published[30]. The interventions supported by this strategy are: "reducing the disease burden and the spread of infection, improving access to appropriate antimicrobials, improving use of antimicrobials, strengthening health systems and their surveillance capabilities, enforcing regulations and legislation, encouraging the development of appropriate new drugs and vaccines". As we can see rational antibiotic use is one of the most important goals together with microbiology surveillance and control if distribution of resistant strains.

## 1.5 EVALUATION OF ANTIMICROBIAL CONSUMPTION

*"If you cannot measure it you cannot improve it"*
*Lord Kelvin*

Antibiotic utilization studies were mentioned as the best way to influence rational antibiotic use in the WHO memorandum[27]. A number of indicators were proposed for drug utilization studies. The first studies on comparison of drug utilization between different settings were performed in the 60s . In these studies significant differences in antibiotic use between six European countries were demonstrated[31]. This publication lead to the organization of the first WHO meeting on "Drug consumption" which was held in Oslo in 1969[32] and establishment of European Drug Utilization Research Group (DURG)[33]. The concept was then developed in order to make drug utilization data comparable. The unit developed was initially called Agreed Daily Dose, and then the name was transferred to Defined Daily Dose (DDD). DDD is the average maintenance dose of the drug when used on its major indication in adults[31]. A number of related definitions were given then by the WHO. These are: drug utilization research – "the marketing, prescription and distribution of

drugs in the society with special emphasis on the resulting medical, social and economic consequencies"; pharmacoepidemiology – "the study of the use and effects/side effects of drugs in large numbers of people with the purpose of supporting the rational and cost-effective use of drugs in the population thereby improving health outcomes"[31]. Another important achievement was adoption of the common drug classification – Anatomical Therapeutic Chemical (ATC)[34] classification where the active substances are divided into different groups according to the organ or system on which they act, and their therapeutic, pharmacological and chemical properties.

Currently the methodology of ATC/DDD is widely used in drug utilization research not only in Europe, but also in Eastern countries[35]. It is used with varying denominators – DDD/1000 inhabitants for out-patient populations and DDD/100 bed-days for hospitals. Other measures include Days Of Therapy (DOTs) – a measure indicating any dose of a drug received by a patient during a 24-hour period[25]. Other measures including numbers of packages, tablets, numbers of prescriptions, or physical units – kilograms, grams or liters – are sometimes used for presentation of utilization volume but are not suitable for cross-national comparisons[31].

Based on the majority of studies indicating that antibiotic use is capable of influencing microbial resistance there have been a number of global initiatives launched on the monitoring of antibiotic use. The largest program in Europe is the European Surveillance of Antimicrobial Consumption Network (ESAC-net)[36] representing a "Europe-wide network of international surveillance systems, providing European reference data on antimicrobial consumption". It collects and analyzes information on antimicrobial consumption from European Union and European Economic Area countries and is currently affiliated to the European Center for Disease Prevention and Control, located in Stockholm. The data are collected and analyzed in order to define indicators of antibiotic use and monitor process of prudent antibiotic use. The data indicate that antibiotic use patterns vary widely in different European countries with total consumption being more intensive in Southern Europe and least intensive in Scandinavian countries[36]. It was for the first time shown in the work by Otto Cars published in Lancet in 2001 [37]. There are no national antibiotic utilization surveillance programs in many other countries like Russia, Japan[38], and the United States[39], which makes international comparison of antibiotic utilization complicated. Hospital surveillance of antibiotic use in American hospitals is usually presented as some rate with certain measure unit like cost, grams, DDD or DOT as a numerator, and time as a denominator. This approach, however, is not suitable for nation-wide comparisons[39].

Antibiotic utilization studies have been recommended in the WHO strategy for hospital routine use. These studies are the basement of different strategies to improve quality of antibiotic use. The most widely used is adherence to local guidelines. In Sweden there is a renewable list of first-line medications for different conditions including infections. It is available publicly and is used as a source for adherence evaluation[40]. In order to improve this methodology it was proposed to concentrate on the bulk of utilization volume represented within 90% of Drug Utilization (DU90%)[41]. Based on Pareto's principle the use of 90% segment is considered to be the most important. It has been tested as quality measure in different kinds of drugs including antibiotics[42,43], which was positively met by prescribers[44].

Benchmarking techniques have also been used for drug use monitoring and improvement since the 90s. In Sweden the benchmarking process was initiated on a national level within the national strategic program on rational use of antibiotics and reduction of antibiotic resistance (STRAMA). The data on antibiotic consumption collected from seven hospitals were presented on the web-site as a way of providing feedback[45]. Point-prevalence studies have also been utilized[46,47] that were, however, not related to any defined quality indicator since different countries participated with varying guidelines and antibiotic distribution rules. More universal quality indicators than guidelines were developed within the ESAC-net project[48,49] for outpatient use. On the hospital

level there has been some criticism against data for antibiotic benchmarking, because a number of factors do not let identify really irrational use of antibiotics based on aggregate data[50]. In order to overcome these difficulties methodologies have been proposed in benchmarking of antibiotics like Case Mix Index – an index considering type of patients, their age, medications and other parameters making the patient category more homogenous; risk adjustment methodologies have been used in some regression models in benchmarking of antibiotic use, however these standardized methods are rarely used[50]. There have been scattered attempts to benchmark antibiotic use and resistance together. It may be exemplified by a SARI (Surveillance of Antimicrobial Resistance in Intensive Care Units) program in Germany. In the United States antibiotic use for selected conditions is a standardized, nationally reported, publicly available quality metrics. Surgical Care Improvement Program is directed towards improved selection, timing and discontinuation of peri-operative prophylaxis. The mechanism used for quality improvement is development of guidelines, and the instrument to measure quality is adherence to these guidelines. A 27% reduction of preventable surgical site infections was noted as a result of this initiative [39]. Another quality metrics used was time to first antibiotic dose for community acquired pneumonia. The timeframe was set for giving the first antibiotic dose based on prospective observations. Adherence to this time frame was monitored by the insurance companies, which led, however, to unnecessary antibiotics received by patients with suspected but not confirmed pneumonia. At the same time there were no direct benefits observed. Antibiotic stewardship programs (ASP) have become popular in many parts of the world. Their major indicators are antibiotic use, clinical parameters, antibiotic resistance and costs. Effects of measuring antibiotic consumption and providing feedback evaluated in prospective studies were capable of changing antibiotic utilization profile but not outcomes[51,52]. Differential reimbursement program was also used within benchmarking of antibiotic use in Belgium[53]. Formulary restrictions and pre-order approval are the other two methods to influence quality of antibiotic use. Other interventions include antibiotic cycling, educational initiatives and decision supporting tools.

The most popular methods of local containment of antimicrobial resistance are infection control and antibiotic stewardship teams and programs. Antibiotic Stewardship Program (ASP) is usually defined as a program that supports selection, dosing, rout of administration and duration of antimicrobial therapy[54]. ASPs are supported by the guidelines released by the Infectious Disease Society of America and the Society for Healthcare Epidemiology of America[55]. These guidelines emphasize the two strategies as having the best volume of evidence; these are prospective audit and feedback and formulary restrictions with preauthorization for selected antibiotics. These strategies have shown effects in reducing costs for treatment and patient safety. To make these programs most efficient it is recommended that each hospital has an antibiotic stewardship team consisting of a clinical pharmacist and a physician – both trained in infectious diseases[54]. Antimicrobial resistance itself, however, is not recommended as an outcome because other factors but antibiotic use may influence resistance. Selective restrictions of antibiotic use have shown effects – in the study by Rahal et al. restriction of cephalosporin use led to 44% decreased resistance to these agents, but compensatory increase of carbapenems use led to 69% increase of resistance to these agents[56]. Clinical decision supporting software may also be useful within stewardship programs. There is evidence that such computer-based programs can decrease the number of patients requiring review by 84%[57]. In comparison of active and passive antibiotic surveillance it was shown that active feedback was associated with shorter duration of inappropriate antibiotic use and length of hospital stay[58]. In the Cochrane review of interventions directed to improvement of antibiotic prescribing in hospitalised patients published in 2005 it was concluded that these interventions are generally effective and are capable of reducing

microbial resistance[59]. However in any intervention it is useful to remember statements popular in business: “Businesses recognize that people do not change their behaviors because they are shown an analysis that shifts their thinking. Rather, people change their behaviors because they are shown a truth that influences their feelings”[39].

## 1.6 ACUTE MYOCARDIAL INFARCTION – DEFINITION AND TREATMENT STRATEGIES

According to the current guidelines the term AMI should be used “when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischaemia”. It is a cause of most cardiovascular deaths and a major cause of death and disability worldwide. [60]. AMI is currently classified by its origin into 5 types, where type 1 refers to spontaneous event related to atherosclerotic plaque rupture or damage with resulting thrombus in one or more of the coronary arteries; type 2 is AMI secondary to an imbalance between myocardial oxygen supply and/or demand, where myocardial damage occurs not due to underlying coronary artery disease (CAD) but may happen in critically ill patients, in patients undergoing non-cardiac surgery, or may also be due to vasospasm or endothelial dysfunction; type 3 implies cardiac death with symptoms suggestive of myocardial ischemia when blood markers were not available; type 4 is AMI related to Percutaneous Coronary Intervention (PCI) (4a) or stent thrombosis (4b); and type 5 refers to AMI associated with Coronary Artery Bypass Grafting (CABG).

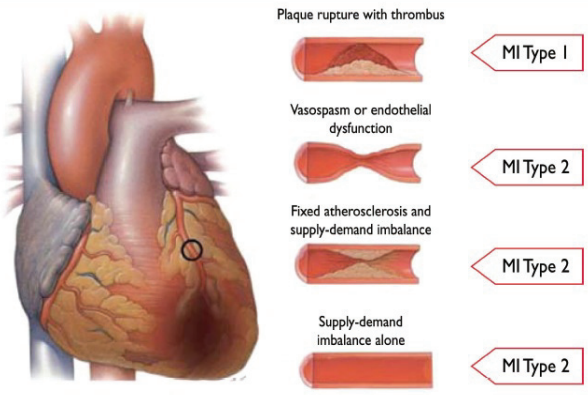



Figure 3. Types of AMI. (reproduced from Thygesen et al, 2012[60])

A recent study of cardiac deaths after AMI based on the population from the clinical trial TRITON-TIMI 38[61] showed that with the use of the most modern treatment strategies 6.5% of AMI patients die within 180 days, but when death rates are analyzed by AMI subtype, it becomes clear that while naturally occurring AMI (type 1) is associated with 8.3% 180 days cardiac mortality, in patients with AMI associated with stent thrombosis and CABG (types 4b and 5) mortality is as high as 15.4 and 14.3% respectively. Besides practical importance this may indirectly indicate that even after surgery medications directed to prevention of cardiac deaths are essential.

There are well defined treatment strategies for AMI patients. In the acute stage treatment is largely based on restoration of blood flow in the culprit vessel and use of fibrinolytics, antiplatelet agents and anticoagulants as soon as possible after the beginning of symptoms[62]. Long-term therapies are, however, important for long-term prognosis because these patients are at high risk of new events and premature death. These treatments should be maintained by the general practitioner, but

to a large extent its success is dependent on early initiation of therapy before discharge from the hospital. According to current recommendations the highest level of evidence (IA) belongs to the following strategies: antiplatelet therapy with low dose aspirin (75-100 mg); dual antiplatetel therapy with aspirin and ticagrelor or aspirin and prasugrel in patients who were treated with PCI; oral treatment with beta-blockers for patients with heart failure or left ventricular dysfunction; high dose statins early after admission in all patients regardless of initial cholesterol; Angiotensin-Converting Enzyme (ACE) inhibitors starting within first 24 hours after AMI in patients with heart failure, left ventricular systolic dysfunction, diabetes or an anterior infarct[62]. Typical complications of AMI in early period after AMI include chronic and acute heart failure, arrhythmias and conduction disturbances (28% atrial fibrillation, 13% non-sustained ventricular tachycardia, 10% high-degree atrioventricular block, 7% sinus bradycardia, 5% sinus arrest, 3% sustained ventricular tachycardia and 3% ventricular fibrillation); mitral valve regurgitation, cardiac rupture, left ventricular aneurysm, left ventricular thrombus or pericarditis.
Another complication in AMI is depression. Its estimated frequency after AMI varies from 1.5 to 50%[63,64]. It was demonstrated to be an independent risk factor of for all-cause cardiac mortality and sudden cardiac death[65].

### 1.6.1 Beta blockers in AMI

Beta-adrenergic blockers remain the cornerstone of treatment in all different stages of ischemic heart disease and they are also a standard treatment for a variety of other conditions including hypertension, various arrhythmias, cardiomyopathy and chronic heart failure[66,67]. Beta-blockers were originally designed by Sir James Black – the Nobel Prize winner. His idea of designing a drug that would counteract adverse effects of adrenergic stimulation gave life to the prototype beta-blocker propranolol. By blocking beta-1 adrenergic receptors this agent could produce negative chronotropic, dromotropic and inotropic effects (respectively inhibition of the sinus node, atrioventricular node and myocardial contraction). Bradycardia and negative inotropic effects are especially important for CAD because these changes decrease the myocardial oxygen demand. Benefits of beta-blockers in patients with ST-elevation AMI have been well demonstrated in terms of long-term mortality in pre-reperfusion era [68–70] and also in high risk patients who have undergone reperfusion[71]. In a smaller number of studies were beneficial effects of beta-blockers demonstrated in unstable angina[72]. The only precaution should be taken when beta-blockers are used in AMI with regard to blood pressure stability, because higher incidence of cardiogenic shock was observed in patients receiving beta-blockers within first 24 hours of AMI[73]. Currently beta-blockers are indicated in case of ST-elevation AMI: for all patients without contraindications (level of recommendations IIa B); for all patients with heart failure and left ventricular dysfunction (level I A); intravenously at the time of presentation in patients without contraindications with high blood pressure, tachycardia, and no signs of heart failure[62]. In patients with acute coronary syndrome without persistent ST-elevation: patients on beta-blocker treatment should be continued on this treatment if no heart failure Killip class ≥III (level I B); oral beta-blockers should be initiated in all patients with left-ventricular dysfunction without contraindications (level I B); intravenous beta-blockers should be considered in patients at admission in a stable haemodynamic condition (Killip class <III) with hypertension and/or tachycardia[74].

## 1.7 METOPROLOL DISPOSITION AND CLINICAL USE

### 1.7.1 Evidence of benefits

Metoprolol was the first beta-blocker selective to beta adrenergic receptors type 1 that demonstrated its benefits in AMI[68,69]. The first randomized controlled study results were

published in Lancet in 1981, where researchers showed 36% of reduced mortality in the metoprolol group compared to placebo[68]. The same group later published the results of the Gothenburg metoprolol trial that reproduced initial results[69]. Then 29% reduction of mortality on metoprolol was reproduced in a bigger international study – the MIAMI (Metoprolol In Acute Myocardial Infarction) trial[70]. Lopressor Intervention Trial group followed the patients with recent AMI on oral metoprolol aiming to evaluate its effects on mortality, but this study did not show any benefits, and showed high rates of withdrawal of metoprolol due to adverse effects[75]. TIMI-IIB study evaluated effects of metoprolol introduced early after thrombolysis. It showed benefits in decreasing myocardial ischemia and reinfarction, but not of mortality and ventricular function[76]. Another big COMMIT study was published in 2005, this study clarified that early intravenous metoprolol is related to increased risk of cardiogenic shock, but decreases reinfarction and risk of ventricular fibrillation[73]. Metorpolol is still unique with regard to the duration of observation – it showed its beneficial effects in 10-year long observation of patients from the Gothenburg study[77]. Despite wide variability of beta-blockers available there are no other beta-blockers with the same evidence as metoprolol for use in AMI patients[78]. The interest to this beta-blocker is maintained, which is evidenced by the announced study METOCARD-CNIC testing the hypothesis that early pre-reperfusion initiation of metoprolol might reduce infarct size as compared to oral post-reperfusion administration[79].

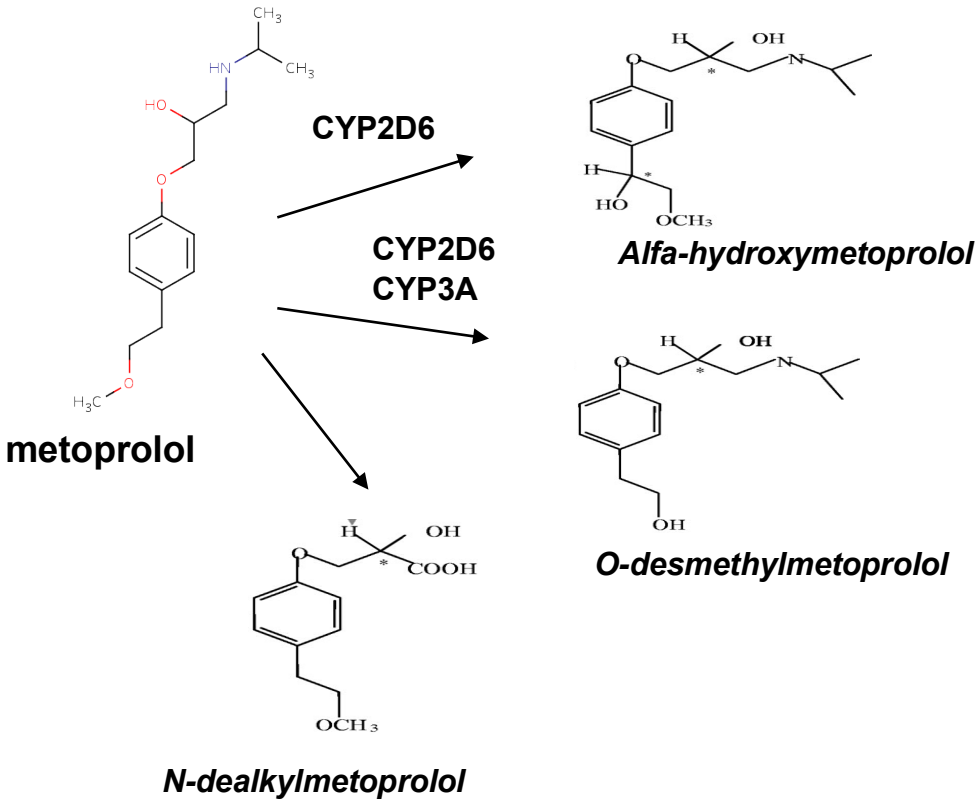
### 1.7.2 Variability of clinical effects

In the Gothenburg study, however, where metoprolol was used in the highest recommended dose of 200 mg per day not all the patients were maintained on this in a long-term perspective[77]. This problem was summarized in the more recent study by Herman who demonstrated that target heart rates are generally not achieved during hospital stay in patients with acute coronary syndrome receiving beta-blockers[80]. While it has been clearly demonstrated that resting heart rate is an independent prognostic factor of cardiovascular risk including sudden death[81]. On the other extreme there are observations of early studies with metoprolol that identified high rates of adverse effects. Taking into consideration wide therapeutic range of metoprolol one could explain these differences in clinical effects by significant variability in metoprolol disposition.

### 1.7.3 Metoprolol pharmacokinetics

Metoprolol is a lipophilic molecule that was produced as two salts – succinate and tartrate, which does not change its pharmacokinetics and two types of formulations – immediate and extended release with the latter providing smoother concentration-time curve and potentially effects[82]. Its pharmacokinetics was thoroughly studied in the 80s[83]. Metoprolol is rapidly and completely absorbed from gastrointestinal tract after oral intake. Maximal concentrations are achieved in 1-3 hours after immediate release formulation intake and in 3-5 hours after extended release formulation. Absorption is taking place over the large distance in intestines, providing complete absorption even for extended release formulations. After absorption metoprolol is subjected to a significant hepatic first-pass extraction. It is readily distributed in the body, the volume of distribution equals 3.2 l/kg. Only 12% of metoprolol is bound to proteins in plasma. Being lipophilic metoprolol readily penetrates blood-brain barrier, 78% can be found in cerebrospinal fluid. It is also found in amniotic fluid and in breast milk. Metoprolol is almost completely metabolized in the liver. Only 5% is excreted unchanged renally. There are three major routs of metabolism – alfa-hydroxylation, O-demethylation and oxidative deamination. O-desmethylmetoprolol and alfa-hydroxymetoprolol possess very little beta-blocking effects.

Figure 4. Scheme of metoprolol metabolism




Studies show that there is no alternative way of metoprolol metabolism via alfa-hydroxylation since in case of enzymatic inactivity 40% are excreted unchanged[83]. Metoprolol is a racemic mixture where S-metabolite is considered to be clinically active[84]. At the same time R enantiomer is primarily metabolized[85]. Studies indicate that metoprolol metabolism is performed in the following way: 10% of primarily S-enantiomer is metabolized into alfa-hydroxy-metoprolol, 65% of primarily R-enantiomer is metabolized into O-desmethylmetoprolol, and less than 10% of metabolism leads to formation of dealkylmetoprolol[86–88]. Major enzyme involved in the metoprolol metabolism is cytochrome P450 (CYP) 2D6. It is responsible for formation of the whole alfa-hydroxy-metabolite, and partly for O-desmethyl-metabolite[89]. There have been discussions about the possible role of CYP3A4 as another enzyme that can participate in the metabolism. However there is evidence of that alfa-hyroxy metabolism is performed completely via CYP2D6, which is therefore the most important enzyme since alfa-hydroxylation is a rout of metabolism of the active S-enantiomer. The shortest T-half-life observed in the studies was 2.1 hours and the longest was 9.5 with the average range of 3 to 7 hours[83,90]. Half-life is usually independent of the dose. There is a strong correlation between metoprolol plasma concentrations and effects expressed in heart rate (HR)[91]. Its effects are clearly dose-dependent[92]. The effect is increased proportionally to the logarithm of concentration and afterwards it reaches the plato phase. In long term use some increase of the effects may be observed due to accumulation of S-enantiomer and possible accumulation of the drug in nervous endings[93]. There is no clear evidence of sex dependence of the metabolism[94,95], only one study demonstrated slower metabolism in women[96]. According to the early investigations metoprolol pharmacokinetics is not changed with age[83], there are scarce data showing that metoprolol metabolism is slower in elderly when taken long-term[97]. Generally there is evidence that elderly patients can tolerate normal metoprolol concentration of 85-203 nM without adverse events[98]. Renal diseases are not

expected to influence metoprolol pharmacokinetics[99]. According to the studies glomerular filtration rate of 5-55 ml/minute did not change metoprolol half-life and bioavailability leading to similar decrease of HR in patients compared to controls. Accumulation of inactive metabolites was observed[100,101]. In patients with liver chirrosis bioavailability of metoprolol was shown to be increased up to 83% as compared to 50% in the controls[102]. General clearance was decreased and half life increased compared to controls. These data show that severe liver disease may have some effect on pharmacokinetics.

### 1.7.4 Metoprolol adverse effects

Close relation of metoprolol plasma concentrations and effects may also mean relation of plasma concentrations and adverse effects. When metoprolol was released possible adverse effects listed were variable and not always clearly related to the drug. Central Nervous system adverse effects included fatigue and dizziness in 10%, depression in 5%, other effects like nightmares, insomnia, somnolence were only in single reports. Cardiovascular adverse effects included shortness of breath and bradycaridia (3%), cold extremities and peripheral vascular insufficiency, peripheral edema, hypotension (1%), gastrointestinal adverse effects reported were diarrhea (5%), nausea, dry mouth, pain in stomach, constipation (1%). Hypersensitivity reactions were registered in 5% of patients receiving metoprolol[90]. In the real clinical practice studies prevailing adverse effects were fatigue, headache, dizziness [103], it was also demonstrated that these adverse effects are concentration-dependent[104]. In healthy volunteers sleep disturbances and worsened libido were reported[105]. Also impaired quality of life was reported[106]. Metorpolol intake was associated with increased free fatty acids with concomitant decrease of total cholesterol and triglycerids[107]. In atherosclerotic patients even reverse effects on plaques formation in carotid arteries was observed[108,109]. Since with increased concentrations beta1-selectie blockers may lose their selectivity several studies investigated the effects of metoprolol on bronchial tone, which was also considered to be dose-dependent[110,111].
Since most adverse effects seem to be concentration dependent, they may be considered also potentially preventable. Therefore several studies were addressing the possibility to define predisposing factors. Wuttke et al in a retrospective study demonstrated five times higher frequency of genetically defined poor CYP2D6 activity among patients who experienced adverse effects of metoprolol[112]. This logical proposal was not, however, confirmed in later prospective studies[113,114]. In one study researchers found higher frequency of adverse effects in women, but it was a naturalistic uncontrolled observation and was not replicated anywhere else[115].

## 1.8 CYP2D6

Cytochromes P450 is a term defining a group of enzymes localized mainly in hepatic endoplasmic reticulum, but also found in many other tissues (intestines, lungs, kidneys, lymphocytes, placenta, brain etc). These enzymes are responsible for metabolism of many drugs, other exogenous and endogenous compounds. Cytochromes P450 are categorized into families based on 40% sequence homology, and subfamilies based on 55% or more of homologous sequence. Currently 18 families are recognized in humans with 44 subfamilies[116]. There are many genes encoding cytochromes that are functionally inactive – pseudogenes. There are currently 57 sequenced human genes and 58 pseudogenes[117,118]. The system of cytochromes is a system of xenobiotic transformation, and the vast majority of drugs, which are xenobiotics for the body, are structurally changed by the CYP system. Five CYPs – CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A – are involved in 95% of drug metabolism, while the remaining enzymes are involved in steroidogenesis, fatty acid metabolism and other endogenous processes. In the process of drug pharmacokinetics these enzymes are involved in several processes – they participate in the first-pass metabolism in the

liver and determine drugs' bioavailability, for some medications that are initially inactive prodrugs cytochromes may be a factor of their activation and prerequisite of their effects, and for most of them cytochromes represent the major system of phase I oxidative metabolism. The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction – insertion of one atom of oxygen into an organic substrate with the other atom of oxygen being reduced to water:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

***Cytochrome P4502D6*** is one of the most widely investigated human cytochrome P450 subfamilies - phase I metabolism enzyme[119]. Its polymorphic expression was the first one described on the molecular level, which was a cornerstone in the history of pharmacogenetics. Currently there are over 50 drug substrates of this enzyme known. Its primary function is metabolism of xenobiotics in the human body including about 16% of all clinically used medications[116]. Polymorphic activity of this enzyme was independently discovered in three laboratories: Sjöqvist's group in Sweden showed significant variability in concentrations of antidepressants in the 60s [120], Smith's in London later demonstrated polymorphic hydroxylation of debrisoquin[121] and Eichelbaum in Bonn showed polymorphic metabolism of spartein[122]. Genetic bases was described 10-15 years later by Gonzalez et al.[123]. The gene location was defined to be 22q 13.1. Currently there are over 80 polymorphic alleles described in this gene. The most common mutation is a single nucleotide polymorphism (SNP) resulting in amino acid substitutions, which leads to changed protein catalytic function, instability, and/or substrate specificity[124]. There are 9 exons in the gene[125]. Neighboring to the active CYP2D6 gene there are 2 pseudogenes – CYP2D7 and CYP2D8P. Different polymorphisms, including SNPs, insertions, deletions or gene duplications are related to increased or reduced enzyme activity (Table 1). Increased number of the active gene copies leads to proportional increase of the enzyme activity[126].

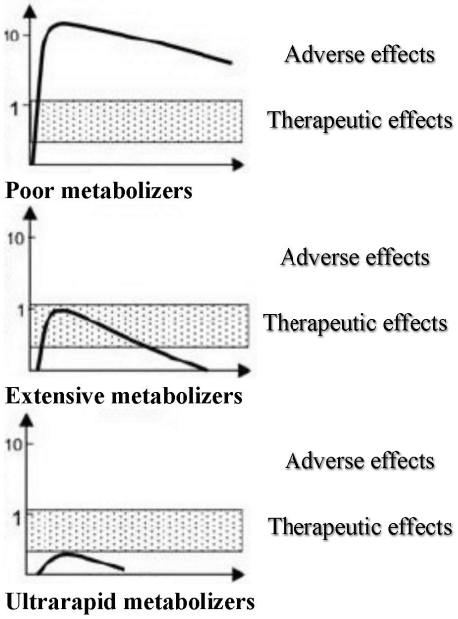
Table 1. CYP2D6 alleles in relation to enzyme activity[124]

| Enzyme activity | Alleles | possible copy number increase |
|---|---|---|
| normal or increased | *1,*2,*27,*33,*35,*48,*53 | *1,*2,*35 |
| Reduced | *9,*10,*17,*29,*41,*49,*50,*54,*55,*59,*72 | *9,*10,*17 |
| nonfunctional | *3–*8;*11–*16;*18-*21;*31,*36,*38*40,*42,*44,*47,*51,*56,*62 | *4,*36 |
| undetermined | *22–*26;*28,*30,*32,*34,*37,*39,*43,*45,*46,*52,*68,*70,*71,*73–*75,*82 | |

This genetic polymorphism results in four major phenotypes – carriers of 2 functional alleles are called phenotypically Extensive Metabolizers (EM), subjects carrying 2 nonfunctional alleles are called Poor Metabolizers (PM), subjects with increased copy number of functional alleles are called Ultrarapid Metabolizers (UM), and those with one active and one inactive allele, or two reduced activity alleles represent the group of Intermediate Metablizers (IM). These are phenotypes of drug metabolism, EMs represent the typical pharmacokinetics, while PMs are characterized by higher bioavailability due to lack of presystemic metabolism, higher maximal concentrations and longer half-lives; UMs are extensively metabolized with the lowest bioavailability, lowest maximal concentrations and shortest half-lives. In clinical use of

medications these phenotypic differences may result in variable response to similar drug doses – PMs are presumably more prone to experience adverse effects from average therapeutic doses, while UMs may be lacking clinical effects. In case of prodrugs the relations will be the opposite – lack of effect in PMs and risk of ADRs in UMs (Figure 5).

Figure5. Pharmacokinetics of active drugs resulting from PM, EM and UM phenotypes (see explanation in the text)




There are significant differences in ethnic distribution of CYP2D6 alleles[116,124]. The most common allelic variants in Caucasians are *2,*3,*4,*5 and *41; *2 and *17 are mostly observed in Africans; *10 and *36 are the most prevalent alleles in Asians[124]. Phenotypes are therefore also differentially distributed. In Asians CYP2D6 activity in average population is lower than in Caucasians due to high prevalence of CYP2D6*10 encoding the unstable enzyme, but at the same time nonfunctional alleles are more rare[127]. In European population the overall prevalence of PM phenotype is 7-10% and is rather stable in different European regions. The most common null alleles are *3 and *4. The UM phenotype frequency varies across different regions being the lowest in the North (1-2% in Swedish Europeans) and highest in the South (10% in Spaniards, 29% in Ethiopians)[128,129]. In the study performed in Russian population the frequency of alleles is not different from other Europeans[130].

***Endogenous role of CYP2D6***

CYP2D6 endogenous substrates are not known, though findings of its expression in different organs and tissues indicates possible substrates other than xenobiotics[131]. There have been a number of experimental studies demonstrating the role of CYP2D6 in dopamine formation from tyramine[132,133], and also formation and metabolism of serotonin [134,135]. These findings were preceded by clinical studies, which showed association of CYP2D6 phenotype with personality traits[136,137]. Later this association was not confirmed in Asian studies[138,139],but reproduced in southern European studies[140,141]. Relations were shown between CYP2D6 genotype and harm avoidance[142], eating disorders[143] and suicide[144,145].

## 1.9 METOPROLOL PHARMACOGENETICS

Pharmacogenetics is defined as the science of interactions between genetic differences and variable response to medications[146]. These genetic differences may refer to the drugs pharmacokinetics – polymorphic activity of metabolizing enzymes and transporters, or pharmacodynamics – polymorphic activity of drug targets. The most studied genetic polymorphisms in targets of metoprolol action are polymorphisms in genes encoding beta-adrenergic receptors[147]. It was shown in the in vitro studies that beta-1 adrenergic receptors are more active in presence of the following genetic variants: Ser49Gly and/or Arg389Gly [148,149]. Several studies demonstrated the role of this polymorphism in metorpolol effects on blood pressure [150–152]. There have also been studies on these polymorphisms in relation to effectiveness of metoprolol in heart failure[153]. However negative studies have also been published[154].

Relation of differential ability to metabolize debrisoquin and metoprolol pharmacokinetics was first described by Lennard in 8 healthy volunteers, two of whom developed higher areas under the concentration time curves (AUC) and were later described as poor metabolizers [155]. This was further reproduced in other smaller and bigger studies[156,157], PMs were characterized by longer half-lives and prolonged beta-blockade[158]. EMs however always produced more pronounced beta-blockade compared to PMs even in case of similar plasma drug concentrations, which may be explained by stereoselective metabolism. Rau et al. showed in a population study that metoprolol plasma concentrations in patients taking metoprolol differ in people with 0, 1 and 2 active alleles[159]. Similar results were shown in Malaysian patients by Ismail[160]. Influence of long-term intake of metoprolol was studied in 2005 by Nozava in 72 patients receiving metoprolol or bisoprolol. Since the population was Asian only the *10 allele was addressed in that study[161]. Influence of CYP2D6*10 allele on metoprolol concentrations after single oral intake were also demonstrated in the study by Jin et al[162].

In 1993 Bertilsson et al described a case of ultrarapid metabolism of nortriptiline and explained the molecular basis for this – CYP2D6 gene duplication[163]. This mutation is usually not obvious since it is mainly not related to adverse effects, but rather to lack of effects. The study by Kirchheiner was the only one to show in 29 healthy volunteers that those who were carriers of gene duplication developed lowest metoprolol concentrations and had lowest effects in terms of HR reduction[164]. In the latest studies relation of CYP2D6 genotype and metoprolol concentrations were reproduced, but not the relation of CYP2D6 genotype and beta-adrenergic receptors polymorphism to clinical effects in patients with chronic heart failure[165,166]. In the large population study in the Netherlands it was demonstrated on the population of over 6000 beta-blocker users that *4 allele is significantly related to the HR, but not blood pressure in patients on metoprolol[167]. In another prospective study from Germany, however, investigators found significantly more pronounced effects of metopolol among PMs both in terms of HRs and blood pressure[168].

## 1.10 METOPROLOL DRUG INTERACTIONS

Other factors that may lead to unexpectedly increased metoprolol concentrations are drug-drug interactions. They very typically lead to adverse effects of drugs and may be observed on different levels. Some drugs that have higher affinity to the enzyme than others may inhibit the metabolism of the latter, some other agents may not only be substrates for CYP2D6, but inhibitors. In contrast to other enzymes CYP2D6 does not seem to be induced[124]. Therefore one could expect drug-drug interactions in EMs resulting in PM phenotype due to the enzyme inhibition and in UMs potentially turning to EMs in presence of inhibitors. No interactions are expected then in genetically PMs.

Major substrates for CYP2D6 are lipophilic agents – mainly antidepressants, neuroleptics, beta-blockers and antiarrhythmics. Strong inhibitors among drugs are fluoxetin, levomepromazine, lobelin, methadone, paroxetine, quinidine, trifluperidol[119].
First interactions described for metoprolol were related to H2-blockers used for gastrointestinal diseases. It was shown that cimetidine and ranitidine may lead to 70% increased metoprolol concentrations[169]. Increased metoprolol concentrations were also observed in administration with verapamil[170] and propafenone[171]. In vitro data demonstrate possible increase of metoprolol effects in co-treatment with an antihistamine agent diphenhydramine[172], and celecoxib[173]. A publication in Lancet 1993 described interaction of metoprolol and Selective Serotonin Reuptake Inhibitor (SSRI) fluoxetin with severe clinical consequencies[174]. This appeared to be an important issue, since combination of beta-blockers and SSRIs is possible in AMI IHD patients due to high prevalence of depression among them and necessity to treat in order to decrease potential risk of sudden cardiac death[63,64]. SSRIs are considered the safest group of antidepressants for use in IHD patients with possible cardiovascular protection in terms of their antiplatelet effects and endothelial function [175]. They were among the most widely metoprolol co-prescribed group of drugs in a Norwegian epidemiologic study[176]. Experimental studies demonstrated in vitro inhibition of stereoselective metabolism of metoprolol immediate and extended release by paroxetine[177,178] and in healthy volunteers[179–181]. Clinical report of complete atrioventricular block in a patient treated concomitantly with metoprolol and paroxetine was published in 2008[182].

## 1.11 CARDIOVASCULAR PHARMACOGENETICS IN CLINICAL ROUTINE

Cardiovascular medications are metabolized by all major drug metabolizing enzymes[116] and many drug transporters[183]. During the past 20 years great volume of experimental data was accumulated in the area of pharmacogenetics, and researchers started to look for its clinical application. Major aim of clinical pharmacogenetics is to improve quality of treatment by providing maximal safety and efficacy for each patient, which generally corresponds to the WHO definition of rational use of drugs: “rational use of drugs requires that patients receive medications appropriate to their clinical needs, in doses that meet their own individual requirements for an adequate period of time, and the lowest cost to them and their community”[184]. Oral anticoagulants represent probably the best example of close approximation of pharmacogenetics to clinical practice. Warfarin effects are largely determined by genetic activity of drug metabolizing enzymes CYP2C9, CYP4F2 and the target VKORC gene[185]. The US Food and Drug Administration label was changed in 2007 to include information about genetic factors of individual response. Randomized trials showed that pharmacogenetic test guiding pharmacotherapy provides better maintenance of target INR, but do not provide stable target effects[186]. One of possible reasons could various environmental factors (drug-drug, drug-herb interactions, and food constituents) that may influence warfarin effects. Further prospective clinical studies are ongoing. Antiplatetel medications have also been thoroughly studied with regard to pharmacogenetic variability. Genetic variability in genes encoding target – COX-1 – have been related to clinical effects in some studies, but this was not replicated in others[187]. Another antiplatelet agent clopidogrel is a prodrug undergoing activation via CYP2C19 and CYP3A enzymes. Loss of function mutations in CYP2C19 gene were related to clinically unfavorable outcomes – increased cardiac death, acute coronary syndrome and stent thrombosis[185]. FDA reacted to these data by issuing a “black box” warning recommending switching to alternative medications in CYP2C19 PMs[183]. Clopidogrel has also been involved in a clinically important drug-drug interaction with omeprazol [188]. Clinical difficulty in monitoring these individual effects is absence of clinically available methods to evaluate antiplatelet effects. Several genetic

polymorphisms have been involved in individual variability of effects of statins – cholesterol ester transfer protein, apolipoprotein E, target HMG-CoA gene have been related to differential response to statins[183], and polymorphisms in the organic anion transporter protein gene (OATP1B1) was related in a prospective trial to statin-induced myopathy[189]. Clinical dosing guidelines have been suggested, but not evaluated in clinical trials. Based on current data one could conclude that despite quite a number of clinically oriented studies there is not enough information to incorporate pharmacogenetics into clinical routine. Genome-wide association studies produced thousands of associations, tens of which get tested phenotypically, and only some – clinically[190]. More prospective clinical studies are required and it is quite obvious that drug-drug interactions should be considered together with pharmacogenetic background when guiding clinical personalized treatment.

# 2 AIMS

The overall aim of this dissertation is to increase knowledge of the factors that may affect quality of hospital care in the treatment of general infections and acute myocardial infarction and suggest methods to minimize their negative influence.

## 2.1 STUDY-WISE AIMS

(I) To evaluate occurrence and clinical importance of drug-drug interaction of a beta-blocker metoprolol and an antidepressant paroxetine (study 1);

(II) To evaluate clinical importance of genetic polymorphism of CYP2D6 in metoprolol plasma concentrations and therapeutic effects in hospitalised patients with acute myocardial infarction (study 2);

(III) To test a method of combined presentation of antimicrobial utilization data and cumulative resistance as a quality of care indicator in treatment of general infections (study 3);

(IV) To describe patterns of antimicrobial use and key hospital microbes' resistance in two Russian and one Swedish hospitals, and prospectively evaluate benefits of a method of combined presentation of antimicrobial use and cumulative resistance. (study 4);

(V) To test a hypothesis of association between higher number of active CYP2D6 genes and ventricular arrhythmias in early period after acute myocardial infarction (supplementary unpublished material);

(VI) To evaluate in a pilot study attitudes of prescribing physicians to antimicrobials efficacy and infection control. (supplementary unpublished material)

# 3 MATERIALS AND METHODS

## 3.1 SETTINGS

(I) Studies (I) an (II) were performed in the departments of cardiology of two city hospitals admitting patients with acute myocardial infarction mostly from north-eastern parts of the city of St Petersburg. These hospitals are working in collaboration with several teaching centers and have well controlled local treatment guidelines;

(II) Study (III) was performed in a 1300 bed-city hospital with all major departments excluding bone marrow transplant unit, haematology, psychiatry and infectious diseases, although general infections are treated in different therapeutic and surgical departments;

(III) Study (IV) was performed in three different hospitals. The study university hospital 1 in St Petersburg, Russia is a 1,300 bed tertiary care hospital with all the general departments excluding pediatrics, neurosurgery, transplantation, haematology, infectious diseases and psychiatry. A Russian comparison hospital (2) was a tertiary care hospital in St Petersburg This hospital has 1,050 beds and the same structure and patient categories as the study hospital. The Swedish hospital – Karolinska University Hospital, Solna has 800 beds and is also a referral hospital. Pediatric departments were excluded from the analysis. Other departments were the same with the exception of neurosurgery, infectious disease1, hematology and transplantation that are absent in the study hospital in Russia.

## 3.2 STUDY MATERIALS AND INFORMATION SOURCES

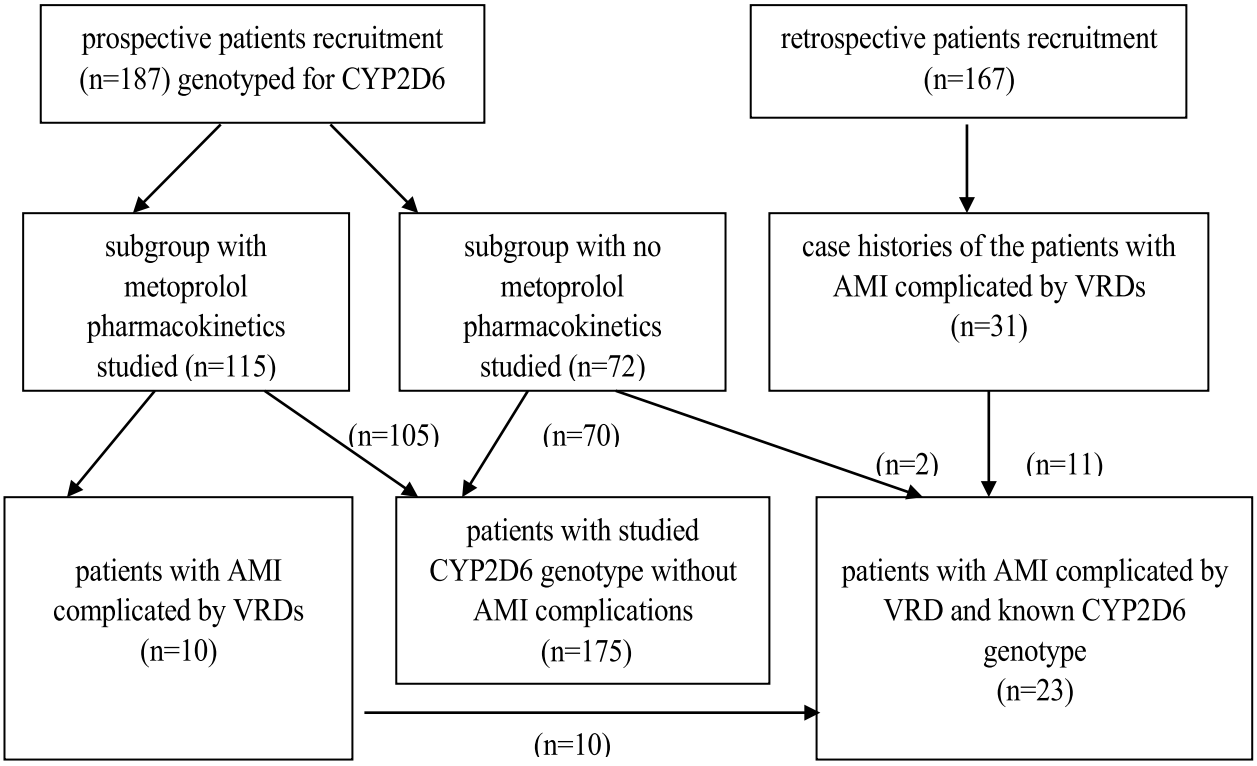
**Studies I and II.** Prior to the prospective study a pilot retrospective analysis of beta-blockers utilization was performed using electronic case-histories from the cardiology clinic for the period of September, 2004 – July, 2005. A total number of 167 case histories were analyzed retrospectively. Number of patients receiving beta-blockers was analyzed instead of the intensity of use expressed in DDDs as it was a more precise measure in terms of clinical relevance of the following studies. The 187 prospective patients with confirmed acute myocardial infarction, admitted to the cardiology clinics, in whom metoprolol treatment was initiated for clinical reasons, were recruited for genotyping. Of those 115 patients were included into the study of metoprolol pharmacokinetics. The same 115 patients were screened for mood disorders using a screening self-questionnaire Hospital Anxiety and Depression Scale (HADS)[191] and interview questionnaire Hamilton Depression Rating Scale (HamDRS)[192] on the 7$^{th}$ day of hospitalization. 26 (23%) patients presented with symptoms of mild to moderate depression. 17 patients received paroxetine as a routinely used antidepressant. These 17 patients comprised a natural group for the study I. Another subgroup of patients from the metoprolol pharmacokinetics group was selected from non-depressed patients on metoprolol, who received a stable dose of the drug during a week. Changes of heart rates were monitored in a natural course of the disease. Patients not receiving metoprolol or receiving other drugs with antiarrhythmic activity were excluded as well as patients with clinically relevant thyroid dysfunction, severe diabetes mellitus, liver or kidney insufficiency and intake of other CYP2D6 substrates.

Substudy of association of CYP2D6 activity and ventricular rhythm disturbances (VRD).

Another subgroup of patients was selected for the analyses of a chance finding – increased frequency of CYP2D6 active alleles in patients with VRDs. This substudy was planned after the major data were analyzed. From all the patients recruited for the study those with VRDs complicating AMI were identified (12 in total). At the same time we identified such patients from

the retrospective data set (31 from 167 retrospective case histories September, 2004 – July 2005). Only eleven patients of those could be reached on the phone and agreed to participate. These patients were merged with the prospective subgroup making a total of 23 patients with VRDs after AMI. The total scheme of patients selection is presented in the Figure 6.

Figure 6. Scheme of patients recruitment for studies I and II.




**Study III.** The material for this study was hospital routine data on consumption of antimicrobials and microbiology data on bacterial resistance. The data for antimicrobials use were obtained from the hospital pharmacy. Routine microbiology data represented the second source of information. All microbiology results are stored in the format of electronic laboratory using the software WHONET - a free Windows-based database software developed for the management and analysis of microbiology laboratory data with a special focus on the analysis of antimicrobial susceptibility test results and available for download from the WHO website [30]. Susceptibility interpretation rules corresponded to the national guidelines.

**Study IV.** The same sources of information were used as in the previous study, though from other hospitals. As a development from the previous study hospital specific microorganisms were selected and resistance profiles were analyzed for each of them as a detailed confirmation of cumulative resistance data. In the Swedish hospital the data were collected in a similar way, although different resistance interpretation rules were used corresponding to the local guidelines.

**Supplemantary non-published data.**

(I) In order to test the hypothesis of association of CYP2D6 gene product activity and VRDs in patients after AMI we performed a prospective analysis. Similarly to studies I and II all patients with confirmed AMI admitted to the cardiology clinics were screened. Patients with VRDs in early period (within 10 days) after AMI were selected for CYP2D6 genotyping. Patients with similar characteristics in terms of age, sex, time of admission and type of AMI were recruited as controls and also genotyped.

(II) As a continuation of the study IV we created a questionnaire for prescribers that evaluated their attitudes towards effectiveness of antibiotics and local situation with resistance. The questionnaire was presented to prescribers in the study hospital – a total of 40 physicians from departments of surgery, intensive care units and general therapy filled it in.

## 3.3 METHODS

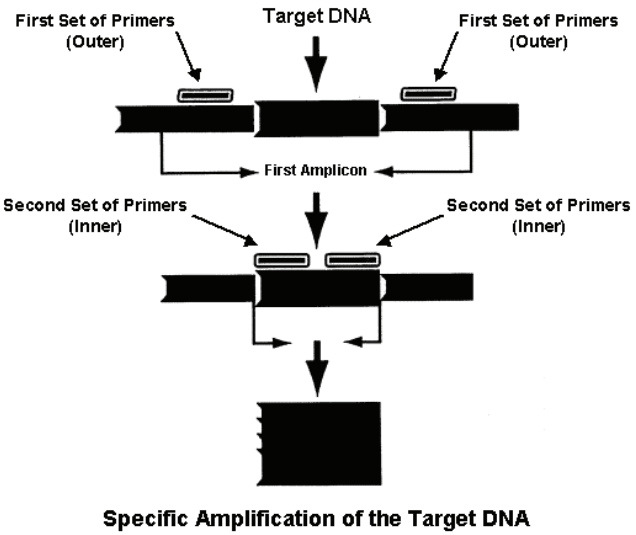
### 3.3.1 Clinical methods

Acute myocardial infarction was confirmed in the patients screened according to the guidelines of the national cardiology society[193], that are harmonized with the European guidelines[60,62]. It was generally performed by the hospital staff and reanalyzed by us at the time of screening. Acute myocardial infarction was diagnosed if two of the three criteria were present: specific pain syndrome, specific ECG changes in two or more neighboring leads, creatine phosphokinase-MB and/or troponine T diagnostic elevation. VRDs were registered either on routine or urgent ECG records. They were classified according to Lown classification, utilized in the hospital as class I – rare monomorphic premature ventricular complexes (<30 per hour), class II – frequent monomorphic premature ventricular complexes (>30 complexes per hour), class III – polymorphic premature ventricular complexes, class IVa – coupled premature ventricular complexes, class IVb – short-term ventricular tachycardia (3 complexes or more together), and class V – early premature ventricular complexes R/T.
Mood disorders diagnosing in AMI patients was a part of a hospital routine at the time of investigation. Self-questionnaire for depression (HADS[191]) and an interview questionnaire (HamDRS[192]), both validated in Russia, were used. The diagnosing was performed by us for the screened patients under the guidance of a certified specialist in psychiatry.

### 3.3.2 CYP2D6 genotyping

DNA was isolated from the thawed whole blood using the QIAamp DNA mini kit (QIAGEN, USA) according to the procedure described by the manufacturer. The patients were genotyped for CYP2D6 *3, *4, *10 and gene duplication. These alleles were chosen for two reasons – known importance for metoprolol disposition and representation in the study population[130]. *3 and *4 genotyping was performed with allele-specific 5′ nuclease assay using pre-developed reagents of TaqMan (Applied Biosystems, Foster City, CA, United States) on ABI PRISM® 7700 Sequence Detection System (PE Applied Biosystems, UK)[194]. *10 was analyzed with allele-specific nested polymerase chain reaction (PCR)[195]. Gene duplication was detected by long PCR as described by Lundqvist et al[196].
CYP2D6 gene is localized on the chromosome 22. It consists of an active gene CYP2D6 and two pseudogenes CYP2D7P and CYP2D8P[197]. In the process of long PCR the whole CYP2D6 gene is amplified; at the same time primers for the CYP2D6 3'flanking areas and the CYP2D7P sequence are used.
Nested PCR is a modification used to specify amplification products and delete unwanted amplification fragments. It is performed in two steps – first – amplification of the target site, and the second step – amplification of the final target product (Figure 7).

Figure 7. Scheme of nested polymerase chain reaction




### 3.3.3 Metoprolol pharmacokinetics

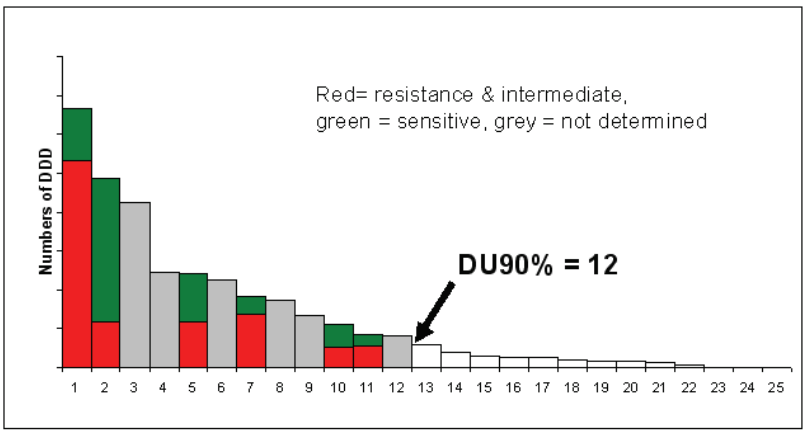
All patients were receiving two types of metoprolol – metoprolol tartrate salt (Egilok, "Egis"Hungary) or succinate salt (Betalok, AstraZeneka). No other generics were used. All patients with minor exclusion were receiving immediate release formulations giving the necessity of 2 times' daily intake. Metoprolol concentrations were measured on the 7$^{th}$ day of treatment in order to achieve both – initial dose titration and stabilized concentration on this titrated dose. The four points were defined to create area under the concentration time curve – 0h (before the tablet intake), 3 h (corresponding to average time of maximal concentration (Cmax) achievement), 6h and 12h – time covering range prior to the next tablet intake. For metoprolol pharmacokinetics analysis we used a method of High Performance Liquid Chromatography (HPLC) with fluorescence detection for metoprolol and ultraviolet detector for paroxetine. 5 ml of blood was collected in heparinized tubes. Samples were centrifuged; plasma was separated and stored at -20 until analysis. Metoprolol and its metabolite were separated by isocratic reverse phase HPLC. Analytic column eclipse XDB-phenyl 15x4 mm 5 μm particle diameter with guard column ("Zorbax" Agilent technologies, USA) was used. Mobile phase consisted of 50 mM potassium phosphate buffer (pH 3.0):acetonitrile:tetrahydrofurane (85:13:2 [vol:vol:vol]). Fluorescent detector was set at 216 and 312 nm excitation and emission wavelengths respectively. Retention times were 2.7, 8.4 and 10.1 min for α-hydroxy metoprolol, metoprolol and dextrorphan (internal standard) respectively with the flow rate 1ml/min. Extraction procedure in brief was as follows: 500 μl of plasma with 20 μl of internal standard (5μM dextrorphan water-methanol solution) was alkalinized with 200 μl of 0.1M sodium hydroxide. Substances were further extracted with 3 ml dichloromethane : 1-butanol (85:15 [vol:vol]). After the extraction, and evaporation of the organic phase under nitrogen flow, the samples were reconstituted in 50 μl of mobile phase. 20 μl was injected into the chromatographic system. Calibration curves were constructed over the range from

12.5 to 400 nM and were linear in that range. Lower quantification limits were 6 nM for metoprolol and 3 nM for α-hydroxy metoprolol. Intraday and interday variations were less than 10% and 15%, respectively.
*Paroxetine plasma analysis*: 5 mL of plasma with 50 μl of internal standard (doxepin 200 μg/mL) were alkalinized with 0.5 mL 0.5M sodium hydroxide and extracted into 1.5 mL 3% isoamyl alcohol-heptane. After centrifugation and freezing the organic phase was separated and substances back extracted into 75 μL of 25mM acetic acid. After centrifugation the organic phase was discarded and 30 μL of the solution was injected into the chromatographic system. A Waters XTerra RP 18 3,3 μm (100 x 3 mm) was used. Paroxetine was detected at 293 nm with UV detector with gradient elution with 20-80% acetonitrile with addition of 20 mM ammonia and 6mM acetic acid. Time of analysis was 18 minutes with the flow rate 0.6 mL/min. Range of quantification was 20-200 nM.

### 3.3.4 Antimicrobials utilization and microbial resistance analysis

At the time of analysis the data were available in numbers of packages of all antibiotics delivered to each department for the years 2003, 2004 and 2005. We classified all the drugs manually according to the Anatomic Therapeutic Chemical (ATC) classification and analyzed the group belonging to the classification code J01 – antibacterials for systemic use[34]. The amount of the consumed antibacterials in grams was calculated in numbers of DDDs – a unit recommended by the WHO for drug consumption studies[198]. The units used for calculation corresponded to the DDD index versions of the years when the data were collected. This was done in order to control for changing strategies of dosing. Number of beds was received from the statistics unit of the hospital in order to transfer the DDDs into the unit of intensity of use – DDD/100 bed-days. All the antibiotics (ATC group J01) were then ranged according to the number of DDD/100 bed-days. From the total volume the 90% of utilization segment was defined[41]. Cumulative resistance was calculated for the microbes naturally susceptible to each antibiotic from the DU90% segment as percentage of resistant, intermediate or susceptible strains from the total number of strains analyzed. Then resistant and intermediate were combined and corresponding part of the bar representing utilization of each antibiotic in the DU90% segment was colored red. The rest part of the bar corresponding to percentage of sensitive strains was colored green. Costs for each DDD were also calculated as one of the possible methods of complex drug utilization data presentation[31]. This was the main methodological tool (Figure 8). All sources of the microbes (pus, sputum, wounds, blood, urine etc.) were analyzed together since it was supposed to represent the volume of resistant pathogens circulating in the hospital.

Figure 8. Intervention tool for studies III and IV




| | SUBSTANCE | (DDD) | DDD | % TOT | DDD/100 BED-DAYS | COST/DDD (rubles) | % RESIST |
|---|---|---|---|---|---|---|---|
| 1 | Gentamicin | 0.24 g | 6 687 | 20% | 3,1 | 5 | 80% |
| 2 | Cefazolin | 3 g | 4 865 | 15% | 2,3 | 41 | 23% |
| 3 | Amoxicillin + clav. acid | 1 g | 4 242 | 13% | 2,0 | 105 | nd |
| 4 | Ampicillin + Oxacillin | 2UD (2g) | 2 438 | 7% | 1,1 | 13 | nd |
| 5 | Benzylpenicillin | 3.6 g | 2 435 | 7% | 1,1 | 129 | 49% |
| 6 | Pefloxacin | 0.8 g | 2 245 | 7% | 1,1 | 6 | nd |
| 7 | Ampicillin | 2 g | 1 838 | 6% | 0,9 | 10 | 74% |
| 8 | Metronidazole | 1.5 g | 1 726 | 5% | 0,8 | 50 | nd |
| 9 | Lincomycin | 1.8 g | 1 357 | 4% | 0,6 | 5 | nd |
| 10 | Amikacin | 1 g | 1 099 | 3% | 0,5 | 143 | 49% |
| 11 | Ciprofloxacin | 1 g | 845 | 3% | 0,4 | 400 | 66% |
| 12 | Pipemidic acid | 0.8 g | 828 | 2% | 0,4 | 22 | nd |
| **DU 90%** | **1 - 12** | | 30 603 | 92% | 14,4 | | |
| 13 - 25 | | | 2 477 | 8% | 1,2 | | |
| **TOTAL** | **1 - 25** | | **33 080** | **100%** | **15,5** | | |

## 3.4 STUDY DESIGNS

**Studies I and II.** The studies were based on the natural course of clinical routine treatment of patients admitted to cardiology clinics with the diagnosis of acute myocardial infarction. We did not intervene, rather re-evaluated all the diagnoses and followed the treatment details to ensure the correct description of the target population, and include patients in whom the data retrieved could be interpreted in an appropriate way. Procedure of the patients' selection is described above in the Study materials section and presented in the Figure 6. For the preliminary part evaluating frequency of metoprolol use we retrospectively evaluated electronic case histories. For the prospective part after the eligible patients were defined and informed consent obtained the intravenous catheter was introduced into the cubital vein for 12 hours and blood samples were taken: 5 ml into the EDTA containing vacuum tube and 5 ml into the heparinized vacuum tubes for pharmacokinetics analyses during the time corresponding to the metoprolol dosing interval. A subset of patients with VRDs was defined retrospectively and consisted of patients who have suffered VRDs on different periods of time. For paroxetine interaction study we did not utilize any intervention either, rather followed clinical routine during the study period. The same type of an

antidepressant was used in all patients – namely paroxetin generic "Rexetin" (Gedeon Richter, Hungary) because this agent was available at the hospital at the time of our study.

**Studies III and IV.** In the studies III and IV we as in the whole theses followed the generally naturalistic design. In all studies we used the data routinely collected in each clinical setting – data from the hospital pharmacies and data from the microbiology units. For the study III the data were obtained for 2003 in the beginning of 2004. The figure containing combined information on antibiotics use and resistance was created and presented to the hospital authorities on the annual conference. The clinical pharmacologist of the hospital was during the year discussing the findings with the prescribers and the epidemiologists responsible for the infection control in the hospital. The data for 2004 and 2005 were collected and handled in the same manner. Costs per DDD were calculated using local data on expenditures for each antibiotic. In the study IV we tested the same method using more hospitals. Generally the procedures were similar for the study hospital. We added retrospective data collection from the two other hospitals – one in Russia with major similar characteristics and one in Sweden. This addition had multiple aims – first aim is to have a sort of control hospitals where the data were not shown to the hospital authorities and/or prescribers; second – to see the applicability of the method in a higher number of clinical settings with probably different local situation in terms of antibiotic use, microbial resistance and infection control; the third – to describe on the example of three hospitals (one in Study III and two in Study IV) general patterns of antimicrobial utilization and microbial resistance in Russian hospitals and compare it to a Swedish hospital for the same years.

## 3.5 ETHICAL CONSIDERATIONS

For studies I an II ethical permits were received from both – ethics committee of St Petersburg State Medical Academy n.a.I.I.Mechnikov (currently North-Western State University n.a. I.I.Mechnikov), Russia (protocol №10, 23 September, 2004) and of Karolinska Institutet, Sweden (№ 2004-580/3).

For studies III and IV ethical committees were approached, but the permission was not considered necessary since the studies did not collect any individual patients' data and the surveillance was a part of hospital routine quality of care assurance.

# 4 RESULTS AND DISCUSSION

## 4.1 STUDIES I AND II

In these studies we were aiming to find out the clinical importance of pharmacogenetics and drug-drug interactions and answer the question whether these factors may be important to improve quality of treatment of hospitalised patients as it is known that successful treatment initiation in the hospital is a prerequisite of overall success[74]. Beta-adrenergic blockers are among the leading antiischemic agents, with metoprolol having the best evidence in patients who have suffered AMI[62]. This was very well reflected in our findings concerning epidemiology of beta-blockers prescription. Retrospective analysis of β-blockers use in 167 patients treated for AMI in cardiology clinic from September, 2004 till July, 2005 showed, that they were prescribed to 97% of patients (n=162); of those 77% (n=127) received metoprolol tartrate immediate release, 8% (n=14) received metoprolol tartrate or succinate extended release, 5% (n=9) – received carvedilol, 4% (n=7) – bisoprolol, 2% (n=3) – atenolol, and 1% (n=2) – nebivolol. If no β -blocker was prescribed patients received other drugs with antianginal properties – amiodarone (2%, n=3) or dilthiazem (1%, n=2). Metoprolol was the most prevalent beta-blocker in our study population, it is also the first line agent recommended for patients after AMI in the Swedish "Wise list" [40]. It is at the same time an agent with active metabolism via CYP2D6 – an enzyme with widely distributed genetic polymorphism[88]. This indicates clinical relevance of our studies.

**CYP2D6 genotype and its impact on metoprolol effects in 187 consecutive patients**

The AMI patients on metoprolol recruited for our study were receiving the following medications (Table 2).

Table 2 Drugs other than β-blockers taken by patients with AMI (n=187)

| Drug group | number of patients | Percentage |
|---|---|---|
| antiaggregants (aspirin/clopidogrel) | 173 (139/44) | 93% |
| ACE inhibitors (perindopril, enalapril, fosinopril, lisinopril, quinapril) | 162 (78/64/2/8/10) | 87% |
| statins (simvastatin, atorvastatin, rozuvastatin) | 78 (62/11/5) | 42% |
| diuretics (indapamide, hydrochlorthiazide, furosemide, spironolactone) | 62 (33/26/3/12) | 34% |
| Mononitrates | 55 | 29% |
| dihydropiridine calcium channel blockers (nifedipine, amlodipine, felodipine, nimodipine) | 31 (16/12/2/1) | 17% |
| anticoagulants (warfarin) | 14 | 8% |
| metabolic drugs (trimetazidin) | 11 | 6% |
| iron preparations | 5 | 3% |
| hypoglycemic drugs (glibenclamide, metformin, repaglinide, gliclazide) | 6 (4/2/1/1) | 3% |
| proton pump inhibitors (omeprazole) | 6 | 3% |
| angiotensin receptor antagonists (losartan) | 3 | 2% |
| Molsidomine | 2 | 1% |

| | | |
|---|---|---|
| bronchodilators, mucolytics | 2 | 1% |

These data indicate good correspondence of treatment strategies to international guidelines[62,74] in antiaggregants and ACE inhibitors, but not in lipid lowering agents, prescribed in only 42% of patients.

In 6 patients no DNA could be obtained due to technical problems, in the remaining 181 prospective patients genotypes were distributed as predicted from Hardy-Weinberg equilibrium (Table 3).

Table 3 Observed frequency of CYP2D6 genotypes in patients with AMI (n=181)

| CYP2D6 genotype | *4/*4 | *10/*10 | *3/*10 | *4/*10 | *1/*1 | *1/*3 | *1/*4 | *1/*10 | *1/*4 x n | *1/*1 x n |
|---|---|---|---|---|---|---|---|---|---|---|
| Expected phenotype | PM | IM | IM | IM | EM | EM | EM | EM | EM/ UM | UM |
| N | 3 | 1 | 1 | 4 | 110 | 2 | 49 | 3 | 1 | 7 |
| Observed % | 1,7 | 0,6 | 0,6 | 2 | 61 | 1 | 27 | 1,7 | 0,6 | 4 |

IM – intermediate metabolizer phenotype

The frequency of CYP2D6 alleles was similar to other Caucasian populations and another Russian population[130] (Table 4).

Table 4. CYP2D6 allele distribution in 181 AMI patients (n of alleles=362) compared to a publication from Russian Voronez population study[130] – only the alleles measured in our study are given

| CYP2D6 allele | *1 | *3 | *4 | *10 |
|---|---|---|---|---|
| N | 289 | 3 | 60 | 10 |
| Observed frequency | 0,80 | 0,008 | 0,17 | 0,03 |
| frequency in Voronez Russian population | 0,71 | 0,01 | 0,18 | 0,04 |

The patients with different genotypes were comparable with regard to major demographic and clinical parameters (Table 5) and also mean resting HRs were not different at admission with a general mean of 78±14 beats/min.

Table 5. Patient characteristics in CYP2D6 genotype-based phenotype groups (n=115)

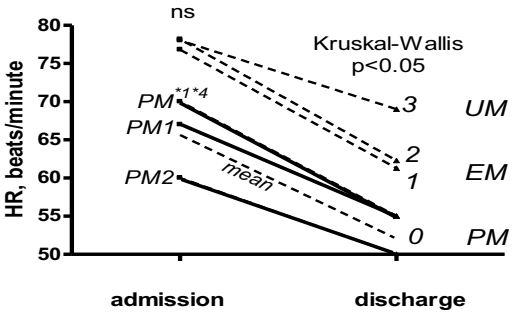
| CYP2D6 phenotype | PM | EM | | UM | p |
|---|---|---|---|---|---|
| CYP2D6 genotype | 0 functional genes (*4/*4) | 1 functional gene (*1/*3, *1/*4, *1/*4xn, *10/*4) | 2 functional genes (*1/*1, *1/*10) | >2 functional agenes (*1/*1xn) | |
| number (n) | 2 | 34 | 74 | 5 | – |
| Men/Women | 1/1 | 20/14 | 46/28 | 3/2 | ns |
| Age (mean±SD) | 54; 80 | 60±10 (39-78) | 62±12 (43-80) | 53±11 (43-70) | ns |
| Anterior AMI | 0 | 18 | 35 | 3 | ns |
| Repeated AMI | 0 | 7 | 24 | 0 | ns |
| Early postinfarction angina (n) | 2 | 23 | 36 | 3 | ns |
| LVEF % mean±SD (n[1]) | 51 (1) | 58±7 (6) | 56±9 (13) | 53±15 (1) | ns |

[1] Number of patients whom no LVEF determined

| | | | | | |
|---|---|---|---|---|---|
| metoprolol dose, mg/kg (mean±SD) | 0,5; 0,7 | 0,9±0,5 | 0,9±0,4 | 0,9±0,4 | ns |

We observed, however, that HRs reached at discharge (15-20$^{th}$ days after admission) varied in patients with different CYP2D6 genotypes (Kruskal-Wallis $p<0.05$). The lowest HRs were achieved by metoprolol PMs (50 and 55 beats/min), while the HR was higher in metoprolol UMs (69±8 beats/min). HRs at discharge were 61±8 and 62±8 beats/min in carriers of 1 and 2 functional CYP2D6 genes respectively (Fig.9).

Figure 9. HR changes at admission to the general ward (approximately 2$^{nd}$-3$^{rd}$ day after admission to the hospital) and after final metoprolol dose adjustment in different genotypes




This finding corresponds to the later published populational study by Rau, where HRs were lower in patients receiving metoprolol for different indications[168]. In our study we did not observe adverse effects in PMs, most probably because beta-blockers are commonly up-titrated to the maximal tolerated dose. In contrast to other studies addressing mostly null alleles we show lack of therapeutic effects in carriers of active gene duplication, which may be more clinically important for AMI patients in whom metoprolol is frequently underdosed and do not provide target effects [80,199] while heart rate is a known prognostic factor with increased HR related to cardiac death[81].

**Metoprolol Pharmacokinetics/Pharmacodynamics in relation to CYP2D6 genotype**

The first 115 of all prospectively recruited patients made up a subgroup for metoprolol disposition analysis. Mean daily metoprolol dose was 75±38 (range 25-150) mg; 1.0±0.5 (range 0.3-2.3) mg/kg.

Wide variation of metoprolol concentrations was observed in plasma. Before metoprolol dose intake in the morning, trough concentrations were low in most patients and unquantifiable in 41%, after 6 hours post dose metoprolol plasma concentrations were lower than those considered therapeutically effective according to the experimental data for metoprolol in healthy volunteers [30-540 nM][90] in 36% of patients, in 15% the concentrations were below the level of quantification. After 12 hours post dose metoprolol concentrations were below the level of quantification in 33% of patients. Inability to reach effective concentrations was observed not only at the trough, but also at the peak levels. This finding may reflect underdosing of metoprolol in the clinical setting studied. In the recent study by Herman et al., however, low hospital doses of metoprolol among the patients after acute coronary syndrome were demonstrated and the lack of clinical effect[80], which means that the problem of low doses of the drugs is common worldwide and not restricted to our study setting.

The patients were divided into groups corresponding to the genotype-based assumed phenotype: PM, EM, and UM. Plasma concentrations of metoprolol and α-hydroxy metoprolol varied widely among groups with different CYP2D6 genotypes (Table 6). Metabolic ratios differed significantly in groups with different genotypes (Figure 10).

Figure 10. Metoprolol/α-hydroxy metorpolol plasma metabolic ratio (MR) distribution in genotype groups. Mosaic parts if the bars correspond to exactly quantified concentrations, while the rest was limited by the method sensitivity in some of the measured points, so that exact data were replaced by the lower quantification limit (6 nM). Upper figure represents the whole group of patients.

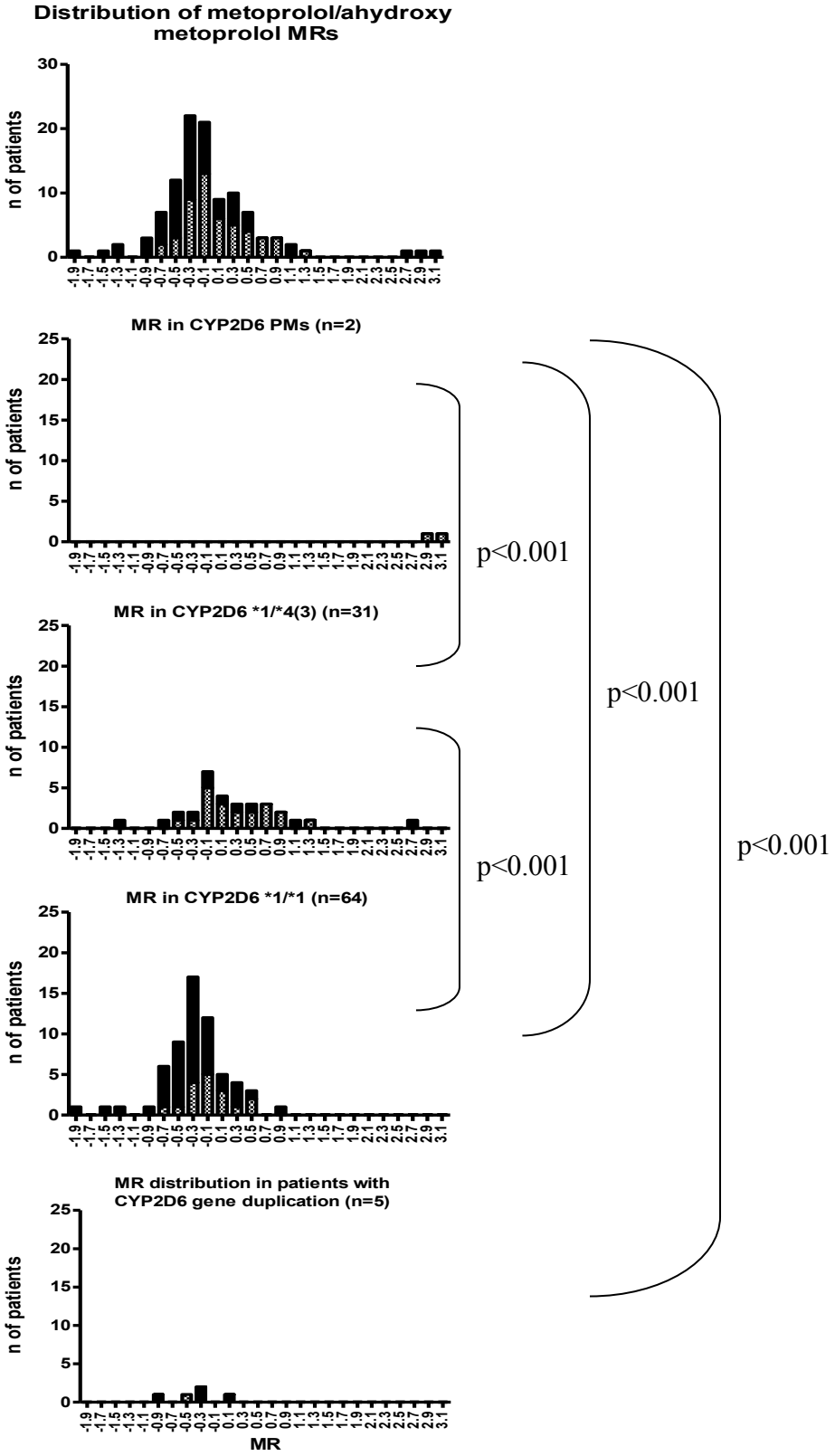


Table 6. Prameters of metoprolol pharmacokinetics and pharmacodynamics in patients with different CYP2D6 genotypes.

| parameter | CYP2D6 *4/*4, n=2 | CYP2D6*1/*3 (*4), n=32 | CYP2D6*1/*1, n=71 | CYP2D6 dupl, n=5 | P |
|---|---|---|---|---|---|
| metoprolol concentrations AUC (nM*h; normalized for dose in mg/kg) | 5167; 5202 | 905 [705-1809] *(PM $^{*1/*4}$) 2814* | 559 [285-1063] | 336 | 0.0002[1] |
| α-hydroxy metoprolol concentrations AUC (nM*h; normalized for dose in mg/kg) | 4; 6 | 811 [359-1413] *(PM $^{*1/*4}$) 5* | 1215 [814-1836] | 762 | 0.003[1] |
| MR | 1196; 867 | 1.3 [0.6-3.5] *(PM $^{*1/*4}$) 531* | 0.5 [0.3-0.7] | 0.4 | <0.0001 |
| metoprolol steady state (before dose) concentrations (nM/mg/kg) min-max [median] | 169; 276 | 33 [1-277] *(PM $^{*1/*4}$) 123* | 11 [2-295] | 18 [3-46] | 0.002[1] |
| AUEC beats*h/min | 344; 342 | 394 [372-438] *(PM $^{*1/*4}$) 360* | 403 [367-432] | 439 | 0,1[1] |
| HR, beats/min | 54; 61 | 68 [65-70] *(PM $^{*1/*4}$) 61* | 70 [66-71] | 73 [68-75] | 0,01[1] |

mean±SD, p – ANOVA of log10 values; [1] median [25%-75% percentile], p- Kruskal-Wallis

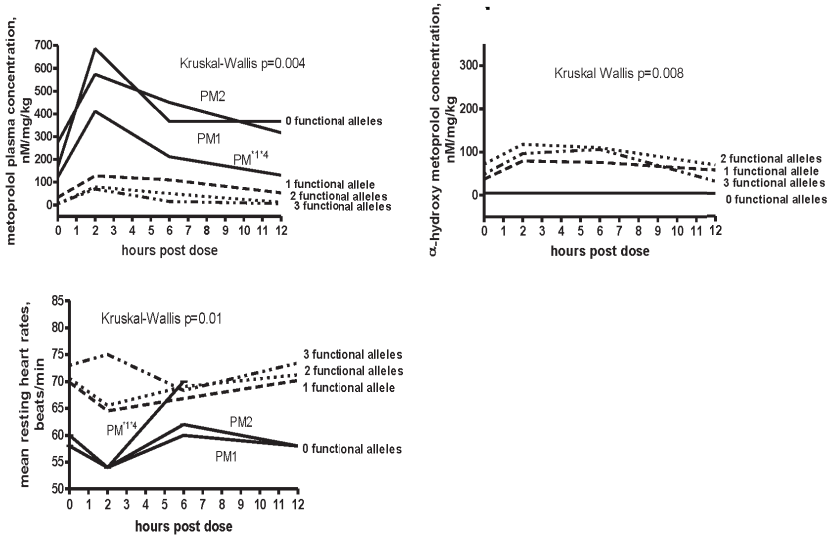
Metoprolol dose and patient weight adjusted concentrations were highest in CYP2D6 PM, while α-hydroxy metoprolol concentrations were lowest. Metabolic ratio (MR) distribution was as expected from the genotypes in all patients but one, who followed the group of PM (Fig. 9). This patient was genotyped as CYP2D6*1*4, but exhibited all the features of the PM phenotype, we designated him separately in the tables and figures as PM$^{*1*4}$.

Relation of CYP2D6 genotype and metoprolol disposition has been shown previously in vitro and in vivo [88,159,160]. The alleles that we chose for analysis reflected the most relevant with regard to the enzyme activity and most expected in the studied population [117]. One should not forget, however, that other less common allelic variants may be present in some patients. This is reflected by the presence of the patient genotyped as CYP2D6*1*4, but being phenotypically a PM. It is always a problem with any genotyping that is supposed to be used clinically. One should carefully chose alleles to include into the genotyping panel, and this should primarily be based on the supposed ethnicity of the population as allele distribution is different in different ethnic populations[200]. In our population we included major European alleles with an addition of one Asian allele *10 since presence of Asian ancestors is very possible in Russian population because of its history. However we observed generally European distribution of alleles, which was in accordance with Hardy-Weinberg equilibrium. There were less homozygote carriers of mutated CYP2D6*4 alleles and more CYP2D6 gene duplications than reported for Swedish population and slightly higher number of *10 allele carriers[200], but generally the distribution corresponded to the previously reported for Southern Russia[130].

Since early morning hours are considered the most dangerous with regard to the risk of coronary events occurrence, metoprolol concentrations before the dose intake in the morning were assessed separately. Metoprolol could be detected only in plasma of patients carrying one or two mutations in CYP2D6-encoding gene (Table 6, Fig.11 a,b).

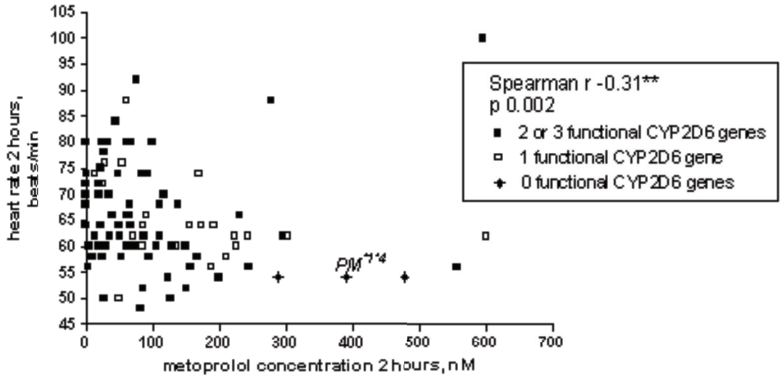
Figure 11 Variable median values of metoprolol (a) and α -hydroxy metoprolol (b) concentration AUCs, and mean resting heart rates (c) in AMI patients (n=115) with 0, 1, 2 and

more (designated as a minimal number of 3) functional CYP2D6-encoding genes on the 7-10th day of treatment.




Correspondingly, lower HRs during the day were observed in patients with lower CYP2D6 genetically predefined activity, though differences were not statistically significant (Fig. 11c). Heart rates at the time of presumed metoprolol highest plasma concentrations – 2 hours after intake correlated with metoprolol plasma concentrations (Figure 12).

**Figure 12. Correlation of patients resting heart rates and metoprolol plasma concentrations 2 hours after metoprolol intake.**




***Metoprolol pharmacokinetics in relation to non-genetic individual variables***

Experimental background allows for assumption that some non-genetic factors, namely hepatic blood flow, patient's sex and age, may influence metoprolol disposition. We evaluated these differences in the studied group of patients.

First, patients were divided into two groups with different left ventricular ejection fractions (LVEF). A group with unchanged LVEF (>50%) (n=73), and a group with impaired LVEF

(≤50%) (n=20) (we did not have LVEF measured in the remaining 22 patients of the group). Metoprolol concentration AUCs were not different in analysis of all the patients, and a trend was observed to higher metoprolol and α-hydroxy metoprolol concentrations in patients with lower LVEF, when only patients with both functional CYP2D6 alleles were analyzed. Metoprolol concentration AUCs did not correlate with patients' age in the group with no mutations in CYP2D6 encoding gene despite a slight trend observed towards higher metoprolol plasma concentrations in older patients (Spearman r=0.23; p 0.06). Similarly, sex did not explain any differences in either metoprolol and α -hydroxy metoprolol concentrations, or effects, though slightly higher concentrations were observed in women (data not shown). These findings generally replicate experimental early observations, but do not seem to be significant enough for clinical consideration.

***Metoprolol-paroxetine interaction***

As drug-drug interactions represent another factor with potential clinical importance in the study I we included a subset of patients on natural clinical grounds receiving potentially interacting metoprolol and paroxetine.

The 17 study patients included in this subgroup were in the age range of 47-80 years, mean 66±10 years; seven were males; eight had anterior myocardial damage; seven had a history of ischemic heart disease (IHD). Left ventricular ejection fraction (LVEF) was 40-68%, mean 55.6±8%. All patients were compliant and completed the time of observation. Metoprolol doses ranged from 25 to 125 mg/day (0.3 to 1.8 mg/kg), with the mean of 75±39 mg/day (0.8±0.4 mg/kg). Nine patients were identified as CYP2D6*1/*1, three patients were CYP2D6*1/*3, and five patients - CYP2D6*1/*4. No gene duplication was found. Metoprolol doses tended to be slightly lower in carriers of a mutated allele, but the difference was not statistically significant.

The 17 controls were 44-73 years old, mean 58±8; 12 were males, eight had anterior myocardial damage, four – previous history of IHD. LVEF was 47-71%, mean 59.9±2%. Metoprolol doses ranged from 50 to 150 mg/day (0.5 to 2 mg/kg), with the mean of 90±35 mg/day (1.2±0.5 mg/kg).

***Pharmacokinetics:***

Baseline metoprolol trough concentrations ranged from below the limit of quantification (6 nM) to 130 nM (163 nM/mg/kg). The highest detected concentration during the study was 600 nM (545 nM/mg/kg). Baseline metoprolol/α-hydroxy metoprolol ratios (metabolic ratio - MR) ranged between 0,05 and 4,6.

Paroxetine trough plasma concentrations on day 8 ranged from 20 to 82 nM (80 to 287 nM/mg/kg). During paroxetine co-treatment metoprolol concentrations AUC increased significantly (p<0,0001) and the metabolite levels decreased (Fig.13; Table 7). Mean MRs increased from 0,92±1,3 to 25,9±28.5 (p < 0,0001) as a reflection of CYP2D6 inhibition.

Figure 13. Mean ±SD plasma metoprolol (left) and α-hydroxymetoprolol (right) concentrations (nM/mg/kg) in patients before and on the 8th day of paroxetine administration in 17 patients. ** p< 0.01; *** p<0.001.

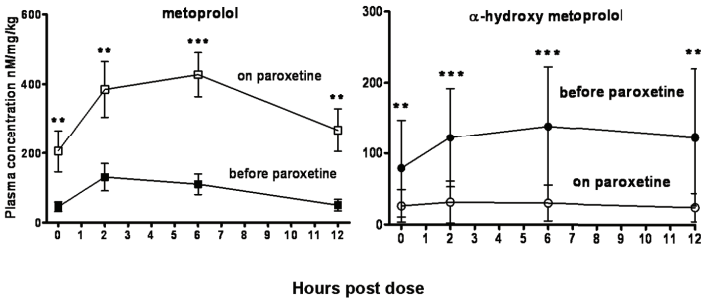


Table 7. Metoprolol and metabolite pharmacokinetics (AUC nM*h/mg/kg) and pharmacodynamics (area under heart rate versus time curve – AUEC beats*h/min) before and after 7 days of paroxetine co-administration (Mean ±SD, paired t-test of log values)

| Parameter | Paroxetine treatment | | P value |
|---|---|---|---|
| | no | Yes | |
| AUC 0-12 metoprolol | 1064±1213 | 4476±2821 | < 0.0001 |
| AUC 0-12 α hydroxy metoprolol | 1492±872 | 348±272 | < 0.0001 |
| AUC ratio | 0.9±1.3 | 26±29 | < 0.0001 |
| AUEC | 835±88 | 728±84 | 0.0007 |

***Pharmacodynamics***

Plasma metoprolol concentration AUCs significantly correlated with patients' HRs (AUEC) at the baseline with Spearman r = -0,64, p<0,01, whereas no significant correlation was observed on day 8 of the study (Figure 14). The 9 patients with the CYP2D6 *1/*1 genotype had higher metoprolol AUC, than the 8 heterozygote patients before paroxetine treatment (Fig.14, left, p=0.06), but there was only a tendency for a difference during paroxetine treatment (Fig.14, right). Significant decrease in HRs was observed when paroxetine was added to the unchanged dose of metoprolol in study patients, but not in controls (Fig.15). Areas under metoprolol effect curves decreased in the majority of the study patients (p= 0,0007) (Table 7).

Figure 14. Correlation of metoprolol AUC (nM*h/mg/kg) and AUEC (Area Under Effect Curve, beats*h/min) before (left) and on the 8th day of paroxetine administration (right). Numbers designate individual patients. Open circles – CYP2D6 *1/*3(*4) genotype. Closed circles – CYP2D6 *1/*1 genotype.

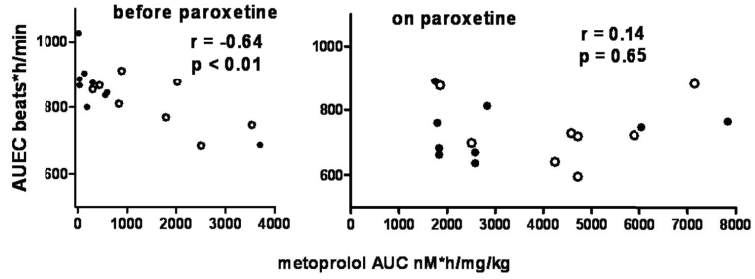
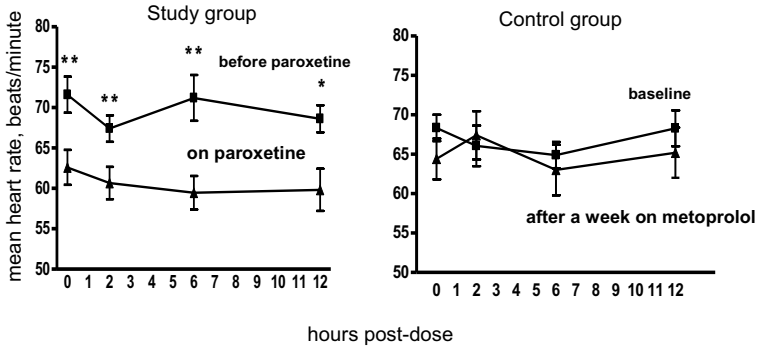


Figure 15. Mean heart rates (±SD) before and on the 8th day of paroxetine administration in study group (left) and control group receiving no paroxetine (right). ** p< 0.01; * p<0.05.




***Treament tolerance***

Mean systolic blood pressure was 125 (98-147) mm Hg at the baseline and 118 (105-135) mm Hg after a week of paroxetine treatment (p=0.2). Two patients required reduction of initial metoprolol dose after the study week with paroxetine**,** one due to severe postural hypotension, and another – due to bad tolerance of bradycardia (<45 beats per min). Both patients were carriers of 1 non-functional CYP2D6 allele. Excessive hypotension (<100 mm Hg) was observed in four patients with initially low systolic blood pressure, this could not be exclusively assigned to metoprolol action, rather than combined medication (β-blockers, mononitrates, ACE-inhibitors). Since it was well tolerated, reduction of medication dosages was not required. No other adverse effects of either of the studied drugs could be clearly distinguished.

A study from Norway reported the combination of CYP2D6 substrates codeine and metoprolol with a CYP2D6 inhibitor paroxetine as one of the most frequently prescribed according to the data from pharmacies[11]**.** The authors did not report any clinical consequences of these combination prescriptions. The magnitude of metoprolol metabolism inhibition by paroxetine co-administration needs to be assessed with clinically used metoprolol doses to establish whether the currently widely used combination is safe or not. In this study we investigated an interaction of routinely prescribed metoprolol and paroxetine. We observed resting HR reduction, we avoided physical exertion tests for it did not correspond to the clinical routine at this time period after AMI. The changes were not observed in 17 patients who didn't receive paroxetine, which let us assign them to the interaction. In contrast to the findings form study II, where no adverse effects were registered and the doses were successfully adjusted, initiation of the CYP2D6 inhibitor lead to unpredicted increase of effects. This did not lead to any serious adverse effects. The only case of poorly tolerated bradycardia was observed, which was not accompanied by PQ-interval prolongation on ECG, and the dose reduction was based merely on the patient's request. Another case of postural hypotension was in a woman with type 2 diabetes mellitus, which could have been the main predisposing factor for this reaction. We can see that an inhibited EM requires more clinical attention than a genetic PM because changes in substrate metabolism occur on the dose that has already been adjusted. The fact that was left beyond the observation is termination of an antidepressant, which may lead to sudden lack of effects and even possible rebound syndrome.

Significant negative correlation of patients' metoprolol concentrations and HR dynamics at the baseline (r=-0.64, p<0.01), disappeared with larger metoprolol concentrations after a week of paroxetine co-administration (r=0.14, ns; Fig.14). If not an accidental finding, this could reflect a blockade of β1-adrenergic receptors at metoprolol concentration AUCs up to 4000 nM*h/mg/kg with concomitant decrease of HRs. The HRs increase at higher metoprolol concentration AUCs could either be the plateau of concentration-effect curve or possible recovery of β-receptors

sensitivity under significant β-blockade (Fig.12 right, patient with metoprolol AUC above 6 000). These findings, that might be a link from metoprolol concentrations to its specific effects in AMI patients, need further investigation on a larger cohort.

***CYP2D6 activity in relation to complications in early period after AMI, namely VRDs***

During the data analysis we found that AMI complications differed in patients with different genotype-based CYP2D6 activity (See Figure 6 for patient group formation). Among 115 patients with studied metoprolol pharmacokinetics 62 had complications in early AMI –VRD were seen in 10 patients, early postinfarction angina (EPA) in 47 patients. Mean CYP2D6 activity (calculated as mean number of active genes in relation to the whole gene number, minimal number of active genes (n=3) was considered in gene duplication) was higher in patients with VRD than in those with EPA and patients with no complications. Metoprolol and α -hydroxy metoprolol concentrations were not different in these groups (Table 8).

Table 8. CYP2D6 activity and metoprolol and α-hydroxy metoprolol concentrations in patients with complications in early period after AMI (n=115)

| parameter | ventricular rhythm disturbances (VRD) | | early postinfarction angina | |
|---|---|---|---|---|
| complication | present | Absent | present | absent |
| number of patients, n | 10 | 105 | 47 | 68 |
| mean±SD (median and range) CYP2D6 activity | 2,2±0,8** ([2]*;1-2) | 1,7±0,5** ([2]*;1.5-3) | 1,6±0,6 ([2];1-2) | 1,7±0,5 ([2];1-2) |
| metoprolol concentrations AUC nM*h | [694] 250-1271 | [538] 342-734 | [700] 231-1423 | [645] 270-1110 |
| α-hydroxy metoprolol concentrations AUC nM*h | [448] 293-1056 | [924] 463-1315 | [818] 444-1727 | [901] 412-1275 |
| MR | [0.6] 0.4-1.9 | [0.6] 0.3-1.4 | [0.6] 0.4-1.8 | [0.5] 0.3-1.4 |

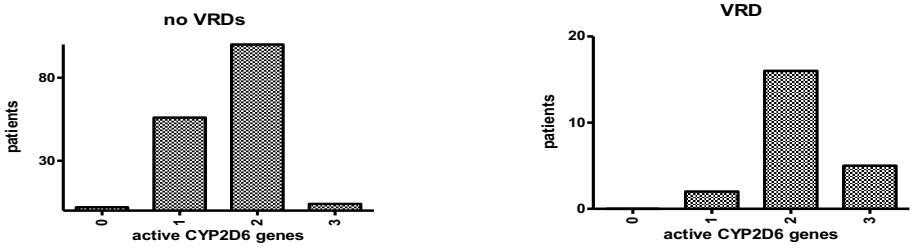
mean±SD, p – t-test; [median] 25%-75% percentile, Mann-Witney test; MR difference was calculated with parametric comparison of log values

** p <0,01 * p <0,05

Thirty one patients with VRD after AMI were identified retrospectively. Eleven of them were available and recruited for the study (see Fig.6). Another twelve patients were selected from the whole group (187 patients), thus altogether 23 VRD patients were included.

Of these 23 patients five carried CYP2D6 gene duplication, two were genotyped as CYP2D6*1/*4 and another 18 did not have mutations in the studied gene. The time of VRD occurrence varied from 2nd to 10th day of AMI. We observed that patients with VRD class II and higher had higher CYP2D6 activity (2.1±0.5 vs 1.6±0.6, p 0.0002) due to higher prevalence of CYP2D6 duplication genotype (5 out of 18 (22%) vs 4 out of 173 (2%), p 0.0002) (Fig. 16).

**Figure 16. Frequency distribution of active CYP2D6 genes in AMI patients without VRDs (n=177) and with VRDs (n=23)**

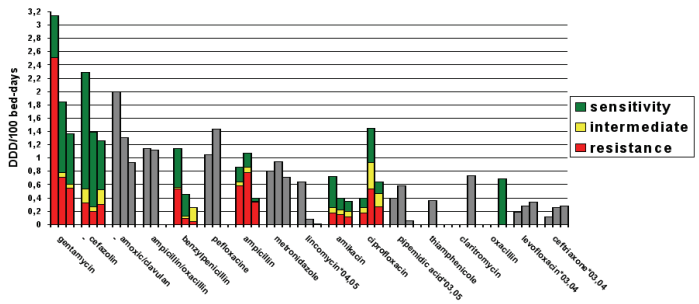


Observed in our study frequency of complications (ventricular rhythm disturbances (VRDs) and early postinfarction angina (EPA)) was not different from the commonly known[62]. Patients were comparable with regard to common parameters, but the total number of active CYP2D6 genes was higher in patients with VRDs. This complication is different from EPA by its relation to adrenergic activity while EPA is demonstrative for the insufficient coronary vessels diameter. Since the relation between the number of active genes and the enzyme amount is proportional, the reason for higher complications rate could be in the differences in metoprolol concentrations, which is a known potent drug in suppression of ventricular premature complexes[201]. There was a trend towards different concentrations of metoprolol, which is what one could expect, however, it did not reach statistical significance. Another evidence against the relation of observed differences in AMI complications to metoprolol concentrations is that metoprolol concentration shown experimentally to be protective against ventricular premature complexes [72±34 ng/ml][202] was hardly reachable at the very early time after AMI when most VRDs were registered. However, higher number of CYP2D6 genes was still observed in a larger group of patients with VRDs complicating AMI. This was mainly attributable to the higher (almost 6 times) prevalence of patients carrying additional copies of the CYP2D6-encoding gene.
Together with the need to prove whether this was not a chance-finding, both, clinical importance and reasons for this require further investigation. A trend to higher frequency of ST-elevation AMI as a debut of the ischemic heart disease in these patients and their younger age lets assume a more severe degree of initial myocardial damage in them, which may be due to increased adrenergic activity. Another hypothesis may be related to higher platelet activation, which has been shown to be an independent predictor of myocardial damage[203] and is directly related to serotonin metabolism[204]. No specific endogenous substrates for CYP2D6 have been identified yet, though it is expressed in different organs and tissues, like brain, intestines, right cardiac ventricle[131]. Some studies suggested its' involvement in catecholamine formation[135].

## 4.2 STUDIES III AND IV

In 2003, the 25 different antibiotic agents (including combinations) made up 15.5 DDD/100 bed-days and 12 of those constituted the DU90% segment (Figure 7). There was a wide expected variation between different departments (from 2,7 DDD/100 bed-days in the 2nd neurological department, to 567 DDD/100 bed-days in the 1st intensive care unit (ICU)). The most prevalent antibiotics were "second-generation" aminoglycosides(J01GB03), penicillins (with and without beta-lactamase inhibitors)(J01C), "first generation" fluoroquinolones (J01MA02-03) and cephalosporins (J01DB).
The total number of antibiotics used increased from 25 in 2003 to 36 in 2005 mainly due to the introduction of macrolides and third generation cephalosporins. The DU90% segment included mostly the same antibiotics, though in a different range and amounts (Figure 17).

Figure 17. DU90% antibiotic use (J01) and resistance change in the years 2003, 2004, and 2005



The total amount of DDDs increased from 33 080 in 2003 to 34 011 in 2005. However, with the increasing bed occupancy rate, the number of DDD/100 bed-days decreased by 57% from 15.5 to 8.8 for the whole hospital in 2005. Antibiotic resistance data were still only available for 6 of 13 antibiotics in the DU90% segment (14 of the 36 overall). Resistant micro-organisms generally changed according to the change of utilization of respective antibiotics. Total expenditures for antibiotic drug purchases decreased by 64% from 2003 to 2005 (Table 9).

Table 9. Range of all antibacterial expenditures in rubles and percentage for the years 2003 and 2005*.

| Range of antibacterial expenditures 2003 | | | Range of antibacterial expenditures 2005 | | |
|---|---|---|---|---|---|
| Antibacterial | Costs (rubles) | Costs (percentage) | Antibacterial | Costs (rubles) | Costs (percentage) |
| meropenem | 873,267 | 23 | ceftazidim | 480,752 | 35 |
| cefepime | 517,563 | 14 | levofloxacin | 153,998 | 11 |
| amoxicillin/clavulanate | 513,025 | 14 | cefazolin | 134,606 | 10 |
| imipenem/cilastatin | 449,806 | 12 | cefepim | 94,835 | 7 |
| cefazolin | 201,388 | 5 | amoxicillin/clavulanate | 84,538 | 6 |
| amikacin | 178,764 | 5 | meropenem | 72,656 | 5 |
| vancomycin | 159,021 | 4 | imipenem/cilastatin | 65,031 | 5 |
| cefoperazone/sulbactam | 138,818 | 4 | metronidazole | 49,705 | 4 |
| ciprofloxacin | 134,751 | 4 | moxifloxacin | 48,965 | 4 |
| pefloxacin | 125,987 | 3 | vancomycin | 29,660 | 2 |
| ceftriaxone | 122,364 | 3 | pefloxacin | 28,642 | 2 |
| levofloxacin | 83,740 | 2 | linezolid | 21,441 | 2 |
| metronidazole | 57,364 | 1 | claritromycin | 17,892 | 1 |
| cefuroxime | 53,840 | 1 | ciprofloxacin | 16,402 | 1 |
| ampicillin+oxacillin | 32,680 | 1 | ampicillin | 15,262 | 1 |
| penicillin | 29,073 | 1 | amikacin | 12,599 | 1 |
| gentamicin | 27,458 | 1 | amoxicillin | 5,620 | 0. |
| ampicillin | 18,670 | 0,5 | erytromycin | 5,489 | 0. |
| moxifloxacin | 16,877 | 0,5 | ceftriaxone | 5,027 | 0. |
| pipemidic acid | 16,276.53 | 0. | ofloxacin | 4,840 | 0. |
| ticarcillin/clavulanate | 6,226 | 0. | azithromycin | 3,960 | 0. |
| lincomycin | 5,523 | 0. | ampicillin+oxacillin | 3,930 | 0. |
| nitroxoline | 1,359 | 0. | pipemidic acid | 3,536 | 0. |
| ampicillin/suilbactam | 846 | 0. | gentamicin | 2,829 | 0. |
| ofloxacin | 167 | 0. | oxacillin | 1,079 | 0. |
| | | | lincomycin | 956 | 0. |
| | | | norfloxacin | 604 | 0. |
| | | | canamycin | 411 | 0. |
| | | | doxycycline | 272 | 0. |
| | | | benzylpenicillin | 338 | 0. |
| | | | tetracycline | 169 | 0. |
| TOTAL | 3,765,266 | 100 | TOTAL | 1,366,044 | 100 |

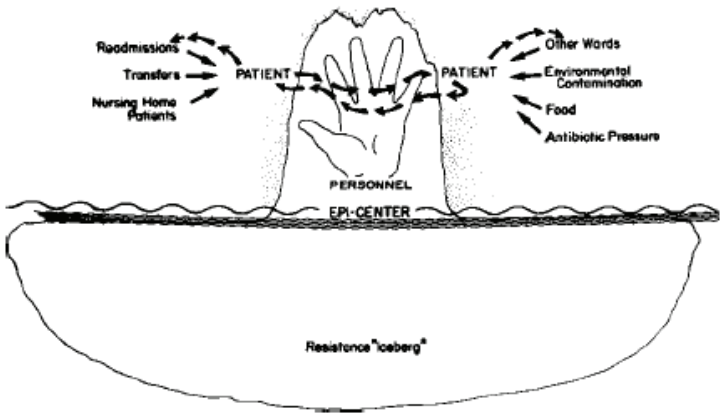
* for the sake of space the 2004 data were omitted. (Ruble = 1/35 Euro and 1/30 USD in 2003, Ruble = 1/36 Euro and 1/28 USD in 2005)

A detailed analysis showed that total costs per DDD also decreased. This was partially explained by the higher use of generics as we found out during the detailed analysis. These savings and the decrease in use of the most widely utilized drugs (gentamicin, penicillin, first generation cephalosporins and fluoroquinolones etc.) allowed increased purchase of more expensive newer fluoroquinolones and cephalosporins with a wider spectrum.

This study was the first to test the methodology of combined presentation of antibiotic utilization and cumulative microbial resistance. This attempt seems generally to be relevant for quite obvious reasons. It is based on the scientifically proven background - mechanism of selection of resistant strains under antibiotics' pressure. The link between antibiotics use and microbial resistance was demonstrated in numerous naturalistic studies[21,23,205,206] and also in some controlled studies

[207]. At the same time it is clear that the relations are not always straight forward[25] because use of some antibacterials may stimulate certain resistance mechanisms that will lead to resistance to a class of antibiotics or even to several classes, which is called multidrug resistance [12,13,208]. The link of antibacterial use and resistance may also be disturbed by the influence of another factor – uncontrolled spread of resistant strains, which is especially relevant in hospitals and is dependent on hospital infection control strategies[209]. All these factors lead to increases in overall resistance of hospital pathogens (Figure 18).

Figure 18. Resistance iceberg (adapted from Weinstein et al[209])




Consequently monitoring of microbial resistance, antibacterial use and establishment of infection control programs is emphasized in the WHO global strategy for containment of antimicrobial resistance [210]. It is therefore clear that implementation of any isolated measure – either antibiotic utilization surveillance, or microbial resistance surveillance, or infection control strategies implementation would not have the same potency in resistance containment as possible implementation of all strategies in combination. One should also pay attention to another factor emphasized in the WHO global strategy – namely a factor of education of prescribers. It is therefore essential not only to monitor antimicrobial use and microbial resistance, but also to bring this information to prescribers. In many hospitals antibiotic utilization and microbial resistance are monitored, but it may frequently be done by different services, and close relations of these factors are therefore not stressed. Here we attempted to combine these two important issues in one. Study III was mainly methodological and pilot. It was not designed to evaluate prescribers' reactions, or to intervene. In this study we only described routine data in a specific format of a diagram. The message, however, seems quite obvious to us – prevalence of older antibiotics – aminoglycosides, aminopenicillins, fluoroquinolones – and all cumulative resistance levels being very high (Figure 8). It gave a simplistic impression that most widely used antimicrobials are not able to be effective against most widely detected pathogens. From this point of view the methodology may be used as a quality of treatment metric being a development of another quality of use evaluation methodology – namely adherence to guidelines within the 90% of utilization segment (DU90%)[41,43]. In Sweden DU90% segment is evaluated for adherence to the expert summary of recommended first-line medications – the "Wise list" [211]. The "Wise list" includes recommendations on preferable antibiotics as well, which are also based on resistance. This is an alternative way to improve quality of use and contain resistance, but it does not give understanding

and responsibility from prescribers. Our method, keeping the idea of the 90% segment as the most influential part of medicinal treatment, suggests another quality metrics, which is less easy to control due to lack of any strict parameter to adhere, but which is more understandable for people with different levels of responsibility – prescribers, and administrative workers.

We repeated observations during two consecutive years and observed significant changes among which the most prominent was the decrease of aminoglycosides use. Expenditures for antibiotics were also significantly decreased. Besides being naturalistic and not controlled by its design this study had a particular limitation of changes in the hospital structure. This factor could much more significantly influence the changes observed. In order to evaluate the methodology in a more controlled manner we performed our Study IV, where we applied this methodology for a longer period of time incorporating it into routine in a Russian hospital (SPH1), and comparing changes with a control Russian hospital (SPH2) with similar characteristics, where the data were evaluated retrospectively, and a Swedish hospital (SWH) that is different mainly with regard to infection control strategies, there the calculations were also performed in a retrospective manner.

*Antibiotic utilization profiles in the three study hospitals*

Total antimicrobial use in the Russian hospital was 22.8; 31.2; 25.1; 24.5 and 28.7 DDD/100 bed-days in 2007; 08; 09; 10 and 2011 respectively (Figures 19,20). The number of beds was not changed during the study period. The number of antibiotics within the DU90% segment varied from 11 to 14, and the total number of antibiotics used varied from 31 to 43 during the observation period. Quinolone antibacterials (J01M), penicillins with extended spectrum (J01CA), and extended-spectrum cephalosporins (J01DD) prevailed in the beginning of observation in 2007, with only minor use of potent antibiotics active against multi-resistant microorganisms.

Prevalence of antimicrobials was comparable although slightly higher in the comparison Russian hospital: 38; 43 and 43 DDDs/100 bed-days in 2009; 10 and 2011 respectively (Figure 20a). The list of antibiotics used comprised 36; 32 and 33 different agents, while the DU90% segment consisted of eight antibiotics: ciprofloxacin, ceftriaxone, cefazolin, metronidazole, ampicillin, ampicillin with enzyme inhibitor, clarithromycin and an aminoglycoside (gentamicin/amikacin).

In the Swedish hospital (SWH) antimicrobial exposure was 58 DDD/100 bed-days in 2009; 57 in 2010 and 58 in 2011 (Figure 20a). The total number of antibiotics used was 49; 47 and 49 in 2009; 2010 and 2011 respectively with 19 antibiotics present in the DU90% segment each year. The segment contained beta-lactamase sensitive penicillins (J01CE), beta-lactamase resistant penicillins (J01CF), 2$^{nd}$ and 3$^{rd}$ generation cephalosporins (J01DC; J01DD), carbapenems (J01DH) and vancomycin. We also compared distributions of major antibiotic classes for each hospital, which is presented in Figures 21, 22.

After the general agreement summarized in the Copenhagen recommendations on “The microbial threat” in 1998 [28] where the need for antibiotic utilization was stressed, numerous publications were released describing patterns of antimicrobial consumption, and European program of surveillance of antimicrobial consumption (ESAC-net) was launched. This gave a general overview of significant differences in antimicrobial consumption in different geographic areas, which was first clearly demonstrated for the year 1997 in the work by Cars et al[37]. These data were reproduced in the following studies[212] and then publications from the ESAC group showed similar differences for 1998-2005[213] and then up to 2009[214]. In the former ESAC study Russian data for outpatient antibiotic consumption were presented and revealed the lowest antibiotic use compared to other European countries. Since hospitals are reservoirs for specific sometimes multiple resistant flora publications also appeared presenting hospital antibiotic utilization patterns[46,47,215]. Also the ESAC group initiated surveillance of hospital antibiotic consumption[216]. According to these data differences in the intensity of antibiotic use revealed in outpatient setting remained in the hospital settings. This could probably let us suppose that Russian hospital antibiotic consumption, which was not present in these publications, would also be low if we extrapolated the outpatient data. According to the data from our observation antibiotic

consumption was lower in both Russian hospitals compared to the Swedish hospital, though it was higher than that reported for Lithuanian hospital in 1997[215]. We cannot compare the data with those published by the ESAC group since population denominator (DDD/1000 inhabitants per day) was used by them for the sake of international comparisons. The data published in the study by Dumpis et al. [46] gives us a possible explanation for differences in terms of variable duration of hospital stay, which is much shorter in Sweden than in Baltic countries and Russia, which would increase overall density of use in the former hospital. Classes of antibiotics used differed too - in Sweden fluoroquinolones and cephalosporins were used less often, but higher use of penicillins and carbapenems was observed. Higher use of penicillins and carbapenems is characteristic for Scandinavian countries in comparison to other European countries [216]. Our observation is the first to our knowledge comparison of hospital consumption of antibiotics in Sweden and Russia.

*Microbial resistance in the three study hospitals*

High cumulative resistance levels were observed in both Russian hospitals during the whole observation period (Figure 20, 20a), which was confirmed by the detailed resistance pattern (Table 11 a,b) comprising cumulative resistance. Presence of the *mecA* gene was not tested, nor was the cefoxitin disc method used for MRSA detection in Russian hospitals. In the Swedish hospital cumulative resistance levels were low (Table 11c). This finding is one of the most interesting in the study, because in contrast to expected lower resistance in settings with lower antibiotic density use we observed very high resistance levels in both Russian hospitals and low resistance in a Swedish hospital. The hospitals were generally similar in structures, the only difference was the absence of oncology and hematology departments in the Russian hospitals, but still these departments together were responsible only for 5% of the consumption in the Swedish hospital in 2011 (oncology 3% and hematology 2%). We found correlation of aminopenicillins use and corresponding *E.coli* resistance (Spearman r 1, p=0.02), but no correlation of *E.coli* resistance and use of extended-spectrum cephalosporins, fluoroquinolones or aminoglycosides. There was also no correlation between *K. pneumoniae* and *P.aeruginosa* resistance and use of any group of antibacterials. We did not study correlations for the other two hospitals due to a shorter observation period.

Generally lack of correlation between antibacterial use and microbial resistance may indicate that other factors than bare antimicrobial pressure are important in these settings. There have been good studies demonstrating that infection control may decrease infections and microbial resistance in health-care settings[217,218]. These strategies are not fully implemented in the study hospitals (no infection control team or controlled strategy) which may explain high resistance and lack of its direct association with utilization of antimicrobials. With regard to microbial data there are some certain limitations that should be noted – namely low number of species analyzed in Russian hospitals, which may have influenced interpretation of the results. There are certain limitations that should be remembered that are common for all aggregate microbiology data. One refers to random or selected sampling for analysis. It is mostly true that species form most severe cases are taken more often and analyzed for different antibiotics susceptibility, while more predictable species in uncomplicated patients especially with community-acquired infections would require just formal screening for confirmation of the pathogen. This could explain slightly higher than reported officially[219] resistance levels to some pathogens observed in the Swedish hospital (SWH). In the nature review in 2004[220] another important aspect was emphasized regarding microbiology data – it was stressed that hospital resistance data should not be interpreted as a measure of burden of disease.

*Cumulative utilization/resistance figure*

The figure showing antibiotic use and cumulative resistance of the key microorganisms (Figure 19) was presented to the study hospital prescribers and authorities in early 2008. It revealed a general problem of high levels of resistance in the whole segment of agents used. Prospective annual surveillance of antibiotic use and resistance in combination was established in the hospital after the first intervention (Table 1). Next year figure showed that the use of ciprofloxacin decreased from 5.2 DDD/100 bed-days to 3.5 DDD/100 bed-days ($p<0.0001$); ampicillin use increased from 3.1 to

6.3 DDD/100 bed-days ($p<0.0001$). There was also an increase in cephalosporins use: ceftriaxone+cefotaxime use increased from 2.4 DDD/100 bed-days to 4.5 DDD/100 bed-days ($p<0.0001$).

The next year, 2009 showed return of fluoroquinolones to the top position, and the whole segment of DU90% was changing each year. General trends were in increase of beta-lactams with enzyme inhibitors use (amoxicillin/clavulanate + amlicillin/sulbactam use was 0.4 DDD/100 bed-days (1.6%) in 2007, 1.3 DDD/100 bed-days (4%) in 2008, 2.2 DDD/100 bed-days (8.6%) in 2009, 3.2 DDD/100 bed-days (13%) in 2010 and 4.8 DDD/100 bed-days (16.8%) in 2011). Despite utilization changes we did not observe any significant changes in the susceptibility levels of microorganisms (Table 11a).

In the comparator Russian hospital (SPH2) the DU90% segment was stable during all three years of observation (Figure 20a). Cumulative resistance rates were also generally stable with slight increase of resistance to fluoroquinolones (53-64%). Oxacillin resistant *Staphylococcus aureus* rates were 48%-55%-44% in 2009, 2010 and 2011 respectively. Tendency to increased resistance of *Pseudomonas aeruginosa* to imipenem was observed (46%, 54% and 66% in 2009, 2010 and 2011 respectively).

Antibiotics utilization profiles in the Swedish hospital was stable without significant changes in 2009-2011. The only tendency to gradually decreased cefuroxime use was not statistically significant. Cumulative resistance rates were also generally unchanged.

We tested the methodology of combined presentation of utilization and resistance. In our previous study [221] the figure was created and revealed clear problems in the rationality of antimicrobials use – prevalence of a limited number of antibiotics, and high levels of microbial resistance. In the current study we improved the methodology to make it more informative and setting-specific. We defined the key-microorganisms for each setting, which would help to evaluate figures in terms of practical use of antibiotics. There have been numerous attempts to combine antibiotic utilization and resistance in one indicator[222,223], since these relationships are obviously important in long-term perspective[224–226]. A «drug resistance index», which is based on an advanced calculation and composite evaluation of antibacterial use and a certain microbial resistance was recently proposed [223]. This index shows trends of resistance related to use for each drug-bacteria combination, which makes it unfeasible for prescribers to grasp general situation. Another indicator used is correlation of percentage of patients receiving antibiotic and rate of infections caused by resistant flora[23]. This approach requires specific selection of a pair antibiotic-resistant microbe and does not allow seeing the whole picture of rational antimicrobials use. The figure combining key microorganisms susceptibility data and prevalence of corresponding antibiotic utilization has a benefit of highlighting the problem in a way easily interpreted by both prescribing physicians and hospital management. We used a concept of DU90%, based on the fact that this segment is the most influential and could be used for quality evaluation[41,211]. In these studies we are developing the concept further using the assumption that resistance might be looked at as inappropriate quality of antibiotic use. The differences observed between the figures from the Russian hospitals and the Swedish hospital indicate differences in potential effectiveness of the most widely prescribed antibiotics against microbes, i.e. differences in rationality of use.

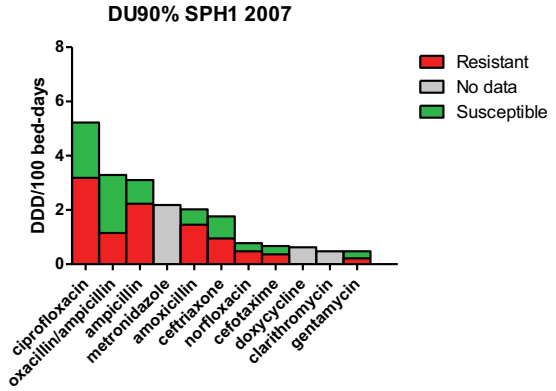
In our Study I we observed changes following the presentation of the figure of combined utilization-resistance profile to the hospital authorities. In the current study we could also observe significant changes in the utilization profile – decrease of fluoroqhinolones consumption increase of aminopenicillins, and general increase of the number of various antibiotics the next year after the intervention. The changes, however, were unstable and resulted in return of fluoroquinolones and extended-spectrum cephalosporins, and increase of aminopenicillins combined with enzyme inhibitor use. The assumption that these changes could be mostly due to the figure presentation is supported by the fact that there were no changes in our control hospital (SPH2) with a similar structure of drug utilization and infection control despite similar problems in microbial resistance observed. There were also no changes in the Swedish hospital. In local perspective it could be seen from the figure that resistance is present for extended-spectrum cephalosporins and trimetoprim-sulfamethoxazole, which is most obvious from separate microbial resistance data (Table 11a).

An unexpected finding was that lower overall antibiotic use in Russian hospitals was accompanied by much higher resistance levels. These high levels were not changed after the changes in utilization profile. At the same time in the Swedish hospital resistance levels were generally low despite high exposure. This may indicate that infection control strategies besides antimicrobial use are important to combat microbial resistance. Since many years Sweden has had an aggressive strategy of combating spread of antimicrobial resistance in health care institutions. Active screening, high focus on hand hygiene and contact precautions, as well as using cohort care or patient isolation, have all been part of the strategy to limit nosocomial spread[227].

*Limitations.*

Our study was based on a natural observation with some elements of intervention. Since the periods of time for the data collection was different during 2007-2008 the study cannot be considered as controlled. The DDD concept has certain methodological limitations that have been widely discussed and that can also be referred to the current study. These are the problems of differences in DDDs and PDD (Prescribed Daily Doses) that may vary in time and lead to certain variability in the final data. Number of admitted patients may also vary and influence the results which has been repeatedly addressed in the literature[228]. With regard to microbiology data one limitation was the very low number of strains analyzed that were used to produce percentage of resistance in the Russian hospitals. We relied upon internal quality control in the bacteriology laboratory and did not perform external quality check of the results, There was also a possible bias in the selection of species for susceptibility testing. Some of the resistance rates reported for Sweden in this paper are significantly higher than those reported in the EARS-Net surveillance system[219]. The main bias is that some bacteria are only tested against some compounds in specific situation, e.g. when the isolate is derived from specific important departments or when the isolate is resistant to a lot of first-line agents. This is a likely explanation for the high rates of resistance to extended-spectrum cephalosporins in *E. coli* and methicillin-resistance in *S. aureus*. One way to come around this problem is to focus the susceptibility data on blood culture isolates only, as they are normally tested against all antimicrobial agents. However, trends of resistance development within one institution will not be affected by this bias, so it will mainly be a problem when trying to extract information on resistance levels of specific bacteria-antimicrobial combinations. For better quality of cumulative resistance data a stable number of isolates and definite sources are both important, and the data observed in the current naturalistic study should not be interpreted in a more global perspective, but rather should be a screening tool for local or regional use [220].

Figure 19. Method of combined presentation of DU90% volume of antibiotic use and cumulative resistance (intervention tool)




| | SUBSTANCE (DDD) | TOTAL DDD | % | DDD/100 BED-DAYS | % RESISTANCE |
|---|---|---|---|---|---|
| 1 | ciprofloxacin (0.5 g P*; 1 g O*) | 15827 | 22,91 | 5,22 | 61 |
| 2 | oxacillin/ampicillin (2 g) | 9980 | 14,45 | 3,29 | 35 |
| 3 | ampicillin (2 g) | 9399 | 13,61 | 3,10 | 72 |
| 4 | metronidazole (1.5 g P) | 6610 | 9,57 | 2,18 | no data |
| 5 | amoxicillin (1g) | 6132 | 8,88 | 2,02 | 72 |
| 6 | ceftriaxone (2g) | 5335 | 7,72 | 1,76 | 54 |
| 7 | norfloxacin (0.8g) | 2371 | 3,43 | 0,78 | 61 |
| 8 | cefotaxime (4g) | 2038 | 2,95 | 0,67 | 54 |
| 9 | doxycycline (0.1g) | 1871 | 2,71 | 0,62 | no data |
| 10 | clarythromycin (1g P; 0.5g O) | 1460 | 2,11 | 0,48 | no data |
| 11 | gentamicin (0.24g) | 1454 | 2,10 | 0,48 | 45 |
| **DU90%** | **1-11** | **62476** | **91** | **20.6** | |
| 12 | amoxicillin/clavulanate (3g P; 1g O) | 1120 | 1,62 | 0,37 | |
| 13 | pipemidic acid (0.8g) | 820 | 1,19 | 0,27 | |
| 14 | cefuroxime (3g P; 0.5g O) | 788 | 1,14 | 0,26 | |
| 15 | lincomycin (1.8g) | 665 | 0,96 | 0,22 | |
| 16 | cefoperazone (4g) | 649 | 0,94 | 0,21 | |
| 17 | benzylpenicillin (3.6g) | 635 | 0,92 | 0,21 | |
| 18 | amikacin (1g) | 426 | 0,62 | 0,14 | |
| 19 | vancomycin (2g) | 282 | 0,41 | 0,09 | |
| 20 | pefloxacin (0.8g) | 220 | 0,32 | 0,07 | |
| 21 | ceftazidime (4g) | 208 | 0,30 | 0,07 | |
| 22 | azithromycin (0.5g P; 0.3g O) | 198 | 0,29 | 0,07 | |
| 23 | nitroxoline (1g) | 125 | 0,18 | 0,04 | |
| 24 | trimethoprim/sulfamethoxazole (2g**) | 116 | 0,17 | 0,04 | |
| 25 | cefazolin (3g) | 108 | 0,16 | 0,04 | |
| 26 | levofloxacin (0.5g) | 85 | 0,12 | 0,03 | |
| 27 | fosfomycin (8g P; 3g O) | 53 | 0,08 | 0,02 | |
| 28 | meropenem (2g) | 40 | 0,06 | 0,01 | |
| 29 | cefoperazone/sulbactam (4g) | 30 | 0,04 | 0,01 | |
| 30 | cefepime (2g) | 25 | 0,04 | 0,01 | |
| 31 | imipenem/cilastatin (2g) | 13 | 0,02 | 0,00 | |
| | **12-31** | **6606** | **9** | **2.2** | |
| **TOTAL** | **1-31** | **69082** | **100** | **22.8** | |

* P- DDD for parenteral use, O – DDD for oral use

* for sulfamethoxazole

Figure 20. Drug utilization 90% - cumulative resistance profiles in the Russian study hospital (SPH1) in 2007-2011

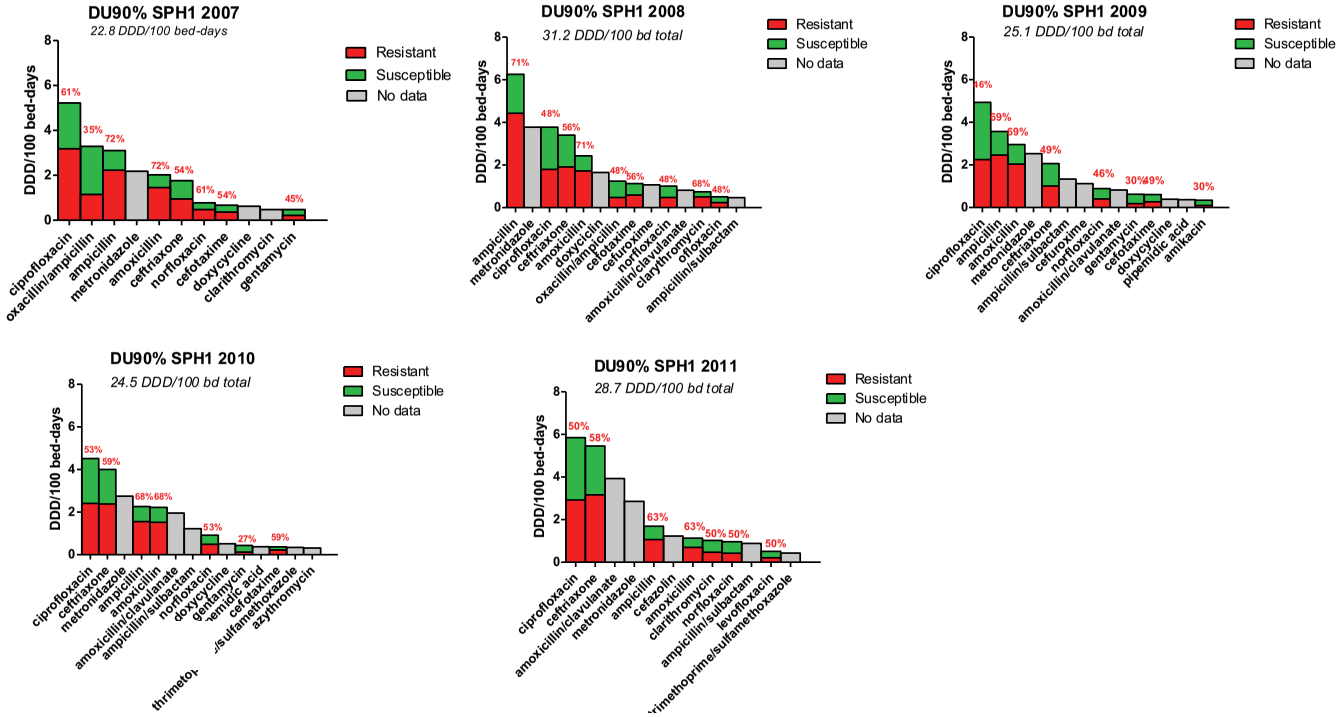


Figure 20a. Drug utilization 90% - cumulative resistance profiles in the Russian control hospital (SPH2) – upper row, and the Swedish hospital (SWH) – lower row in 2009-2011

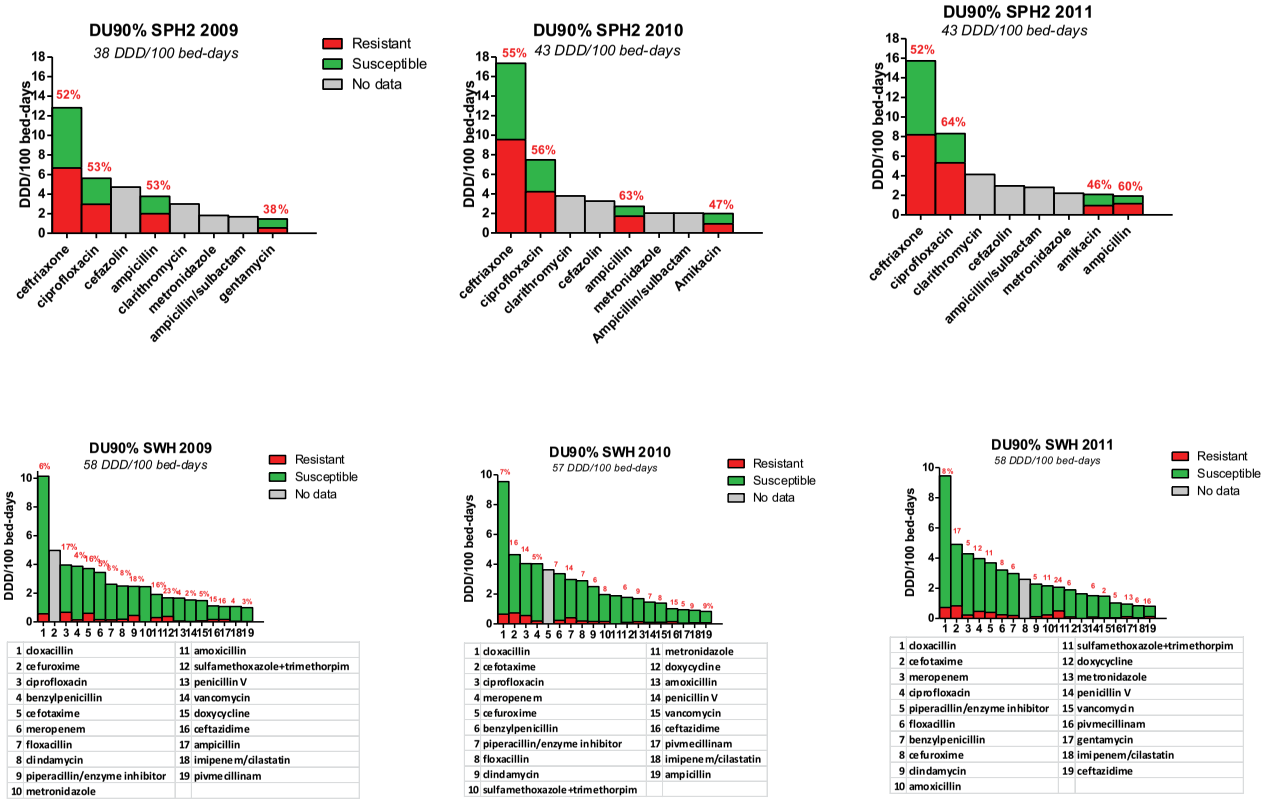


Figure 21. Antibiotic use and distribution of main classes in three study hospitals during the observation years (2007-2011 or 2009-2011)

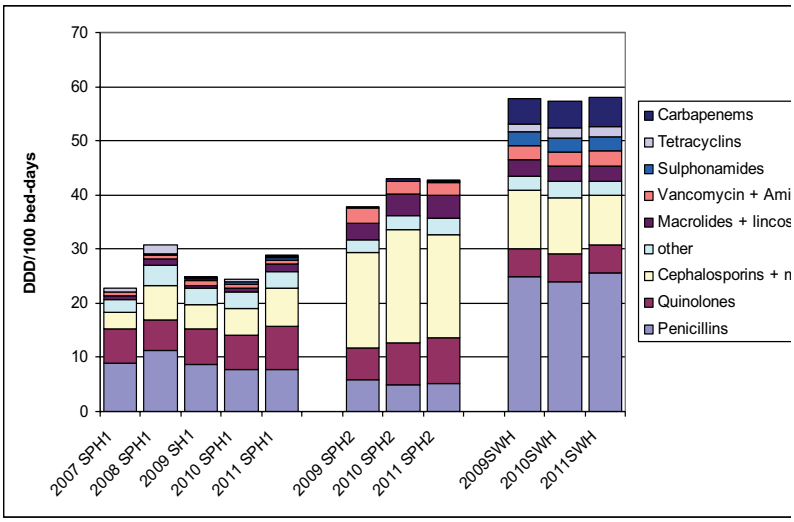



Figure 22. Distribution of major classes of antibiotics in three hospitals (SPH1, S SWH) during the observation period

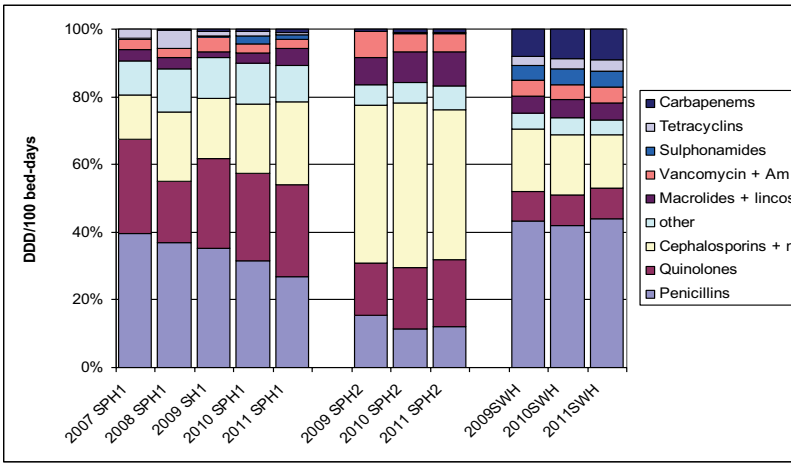


Table 10.

Key microorganisms in Russian and Swedish hospitals

| Antibiotic | Key microorganisms in Swedish hospitals | Key microorganisms in Russian hospitals | Notes |
|---|---|---|---|
| **Penicillins** | | | |
| benzylpenicillin | S. pneumoniae, S.pyogenes | S. pneumoniae, group A streptococcus | |
| amoxicillin | H.influenzae | E.coli, Enterococcus faecalis, | H.influenzae was rarely detected in Russian study hospitals |
| ampicillin | H.influenzae | E.coli, Enterococcus faecalis, streptococci | H.influenzae was rarely detected in Russian study hospitals |
| cloxacillin | S. aureus | No | Not in use in Russia |
| flucloxacillin | S. aureus | No | Not in use in Russia |
| fenoximethylpenicillin | S. pneumoniae, group A streptococcus | No | Not in use in Russia |
| oxacillin/ampicillin | No | Staphylococci | An outdated combination never used in Sweden |
| amoxicillin/clavulanate | | Enterobacteriaceae, streptococci | |
| ampicillin/sulbactam | | Enterobacteriaceae, streptococci | |
| piperacillin/tazobactam | Enterobacteriaceae, P. Aeruginosa, Acinetobacter, B. fragilis group | No | The drug was not in use during the test period in Russia |
| **Cephalosporins** | | | |
| cefazolin | No | No | |
| cefuroxime | No | Staphylococci | No testing is routinely performed in Sweden, |
| ceftriaxone | Enterobacteriaceae | Enterobacteriaceae | |
| cefotaxime | Enterobacteriaceae | Enterobacteriaceae | |
| cefoperazone | | Enterobacteriaceae, P. aeruginosa | these agents are used in Russia mostly where pseudomonas is suspected |
| ceftazidime | Enterobacteriaceae | Enterobacteriaceae, P. aeruginosa | these agents are used in Russia only where pseudomonas is suspected |
| cefoperazone/sulbactam | | Enterobacteriaceae, P. aeruginosa | these agents are used in Russia mostly where pseudomonas is suspected |
| **Carbapenems** | | | |
| meropenem | Enterobacteriaceae, P. Aeruginosa, Acinetobacter, B. fragilis group | Enterobacteriaceae, P. aeruginosa, Acinetobacter | No anaerobe testing is routinely performed in Russia |
| doripenem | | Enterobacteriaceae, P. aeruginosa, Acinetobacter | Was not in use during the study period in Sweden |
| imipenem/cilastatin | Enterobacteriaceae, P. Aeruginosa, Acinetobacter, B. fragilis group, E. Faecalis | Enterobacteriaceae, P. aeruginosa, Acinetobacter, E. faecalis | No anaerobe testing is routinely performed in Russia |
| **Lincosamides** | | | |
| clindamycin | S. aureus, group A S.pyogenes | S. aureus, group A streptococcus | |
| lincomycin | S. aureus, group A streptococcus | S. aureus, group A streptococcus | |
| **Fluoroquinolones** | | | |
| ciprofloxacin | Enterbacteriaceae, P. aeruginosa, Acinetobacter | Enterbacteriaceae, P. aeruginosa, Acinetobacter | |
| norfloxacin/ofloxacin /pefloxacin | | Similar to ciprofloxacin | |
| levofloxacin | | Enterobacteriaceae, S.pneumoniae | |
| moxifloxacin | | Enterobacteriaceae, S.pneumoniae | |
| **Quinolones** | | | |
| pipemidic acid | | No | |
| **Tetracyclines** | | | |
| doxycycline | H.influenzae, S. pneumoniae | H.influenzae, S. pneumonia | |
| **Aminoglycosides** | | | |
| gentamycin | E. coli, K. Pneumoniae, S. aureus | E. coli, K. pneumoniae, S. aureus | |
| amikacin | E. coli, K. Pneumoniae, S. aureus | E. coli, K. pneumoniae, S. aureus | |
| **Macrolides** | | | |
| clarithromycin | | No | Hospital use is mostly for H.pylori in Russian hospitals |
| azythromycin | | No | |
| **Various** | | | |
| metronidazole | B.fragilis group | No | No anaerobes were cultured in Russian hospitals |
| trimethoprim-sulfamethoxazole | Enterobacteriaceae, H. influenzae, S. Pneumoniae | | |
| vancomycin | S. aureus, E. Faecium | S. aureus, E. faecium | |

Table 11a. Rates of resistance of selected bacteria to antimicrobial agents during 5 consecutive years SPH1l

| Antibiotic | Resistant isolates, % (n of strains) | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | | | *K.pneumoniae* | | | | | *P.aeruginosa* | | | | | *S.aureus* | | | | | *Enterococcus spp.* | | | | |
| | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 |
| Ampicillin | 73 (96) | 67 (40) | 63 (90) | 46 (26) | 66 (345) | 100 (48) | 100 (7) | 93 (15) | 100 (2) | 90 (95) | | | | | | | | | | | 42 (76) | 54 (46) | 50 (66) | 60 (5) | 33 (255) |
| Oxacillin | | | | | | | | | | | | | | | | 40 (158) | 36 (63) | 30 (76) | 17 (97) | 32 (452) | | | | | |
| Ciprofloxacin | 62 (97) | 27 (15) | 45 (87) | 46 (93) | 48 (352) | 77 (48) | 71 (7) | 73 (15) | 59 (17) | 61 (113) | 58 (48) | 60 (10) | 57 (14) | 42 (19) | 62 (55) | 43 (160) | 42 (36) | 38 (86) | 20 (97) | 30 (356) | | | | | |
| Ceftriaxone | 55 (95) | 41 (17) | 42 (86) | 45 (49) | 45 (497) | 72 (47) | 83 (6) | 78 (14) | 58 (12) | 71 (138) | | | | | | | | | | | | | | | |
| Gentamicin | 58 (96) | 36 (39) | 34 (90) | 24 (58) | 27 (422) | 77 (48) | 71 (7) | 60 (15) | 63 (8) | 46 (117) | 61 (49) | 50 (14) | 33 (15) | No data | 50 (104) | | | | | | | | | | |
| Vancomycin | | | | | | | | | | | | | | | | | | | | | 7 (80) | 9 (46) | 3 (67) | 0 (26) | 0.3 (358) |
| Ceftazidime | | | | | | | | | | | 60.4 (48) | 71 (14) | 40 (15) | 21 (14) | 35 (84) | | | | | | | | | | |
| *N of isolates* | *97* | *40* | *91* | *115* | *610* | *48* | *7* | *15* | *18* | *147* | *49* | *15* | *15* | *23* | *129* | *160* | *66* | *90* | *98* | *524* | *81* | *49* | *67* | *39* | *495* |

Table 11b. Rates of resistance of selected bacteria to antimicrobial agents during 3 consecutive years SPH2

| Antibiotic | Resistant isolates, % (n of strains) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | *K.pneumoniae* | | | *P.aeruginosa* | | | *S.aureus* | | | *Enterococcus spp.* | | |
| | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 |
| Ampicillin/amoxicillin | 58 (426) | 66 (341) | 81 (304) | 99 (195) | 100 (182) | 99 (136) | | | | | | | 26 (288) | 38 (291) | 46 (197) |
| Oxacillin | | | | | | | | | | 45 (282) | 52 (272) | 42 (234) | | | |
| Ciprofloxacin | 36 (425) | 44 (149) | 17 (12) | 82 (195) | 75 (88) | | 52 (98) | 54 (67) | 65 (99) | 52 (273) | 58 (256) | 52 (229) | | | |
| Ceftriaxone | 30 (431) | 41 (420) | 42 (595) | 82 (197) | | 77 (290) | | | | | | | | | |
| Amikacin | | | | | | | 6 (48) | 47 (61) | 15 (101) | | | | | | |
| Gentamicin | 22 (424) | 31 (421) | 35 (562) | 70 (191) | | | 26 (60) | 24 (42) | | | | | | | |
| Vancomycin | | | | | | | | | | 0 (231) | 0 (272) | 1 (227) | 2.6 (267) | 1 (347) | 6 (387) |
| Imipenem | 2 (427) | 4 (422) | 3 (562) | 17 (196) | | 8 (269) | 45 (97) | 43 (82) | 57 (114) | | | | | | |
| Ceftazidime | | | | | | | 46 (54) | 37 (60) | 29 (100) | | | | | | |

Table 11c. Rates of resistance of selected bacteria to antimicrobial agents during 3 consecutive years (SWH)

| Antibiotic | Resistant isolates, % (n of strains) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | *K.pneumoniae* | | | *P.aeruginosa* | | | *S.aureus* | | | *Enterococcus spp.* | | |
| | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 |
| Ampicillin | | | | | | | | | | | | | 0.3 (1541) | 0.2 (3739) | 0.1 (2282) |
| Pivmecillinam | 2 (14436) | 5 (244347) | 4 (29739) | 16 (1579) | 10 (1852) | 9 (2514) | | | | | | | | | |
| Cloxacillin | | | | | | | | | | 5.6 (12962) | 6.8 (21214) | 7.7 (26749) | | | |
| Ciprofloxacin | 17 (16234) | 14 (27738) | 12 (34166) | 17 (2065) | 9.6 (2395) | 7.5 (4044) | 18 (1681) | 19 (3059) | 17 (3814) | | | | | | |
| Cefotaxime | 17 (3581) | 17 (8559) | 19.5 (11191) | 6 (994) | 9.5 (2389) | 7.5 (2705) | | | | | | | | | |
| Trimethoprim/sulfamethoxazole | 24 (14675) | 26 (27502) | 26 (33819) | 23 (992) | 20 (3292) | 18 (3919) | | | | | | | | | |
| Clindamycin | | | | | | | | | | 7.7 (4623) | 6.6 (11237) | 5.5 (14576) | | | |
| Vancomycin | | | | | | | | | | 0 (262) | 0 (54) | no data | 2.6 (660) | 8.6 (938) | 2.3 (1148) |
| Meropenem | 0.08 (3504) | 0.01 (6264) | 0.09 (7960) | 0.3 (961) | 1.3 (1577) | 1.3 (1715) | 20 (1679) | 19 (3058) | 19 (3783) | | | | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Piperacillin/tazobactam | | | | | | | 12 (1684) | 13 (3064) | 13.5 (3774) | | | | | | |
| Ceftazidime | | | | 6 (989) | 10 (2386) | 8 (2690) | 13 (1683) | 14 (3059) | 13 (3777) | | | | | | |

## 4.3 PRELIMINARY UNPUBLISHED DATA

### 4.3.1 Prospective evaluation of relation of CYP2D6 genotype and VRDs in patients with AMI.

This study was a follow up from the previous study (paper II) where we found that the prevalence of CYP2D6 gene duplication was higher than expected in patients with VRDs in early period after AMI. The hypothesis was formulated about possible involvement of CYP2D6 in catecholamine metabolism leading to adrenergic overload in early AMI leading to VRDs. In this study we were aiming to test this hypothesis in a prospective way.

***Patients and procedures***

We utilized all the similar procedures for the patients recruitment as in the previous one. All patients admitted to the cardiology clinic with confirmed AMI were screened. Blood samples were taken from the cubital vein for future genotyping. Patients with VRDs in early period (1-7 days) after AMI were treated as a separate group. Similar patients admitted to the clinics were analyzed as a control group. Blood samples were used in these patients for DNA extraction and genotyping. Real-time Polymerase Chain Reaction (RT-PCR) was used for genotyping. CYP2D6 alleles *4 and gene duplication were analyzed.

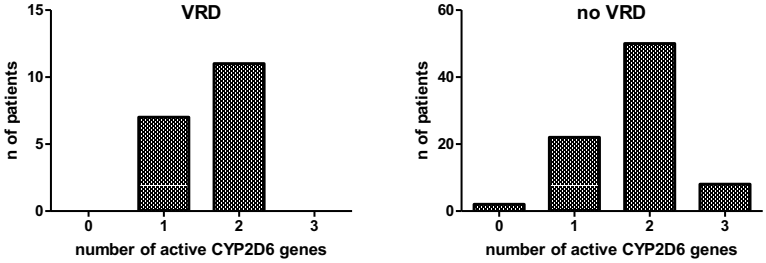
Total number of the patients screened was 93. 34 (36%) were women. Mean age of the patients was 62±11 (min 32 max 79). CYP2D6 *4 allele frequency was 13%. VRDs in early period after AMI were observed in 18 patients. We observed higher frequency of mutant heterozygotes in the group of patients with VRDs (23% allele frequency vs 11% allele frequency in the control group). CYP2D6 gene duplication was detected in 9% of cases. All mutations were present in the patients without VRDs and not in the VRD group. Patient details in both groups are presented in Table 12.

Table 12 Patient characteristics in groups with and without VRDs

| Parameters | Patients with VRDs | Patients without VRDs | $X^2$ test |
|---|---|---|---|
| n of patients | 18 | 76 | |
| women, n (%) | 5 (28%) | 29 (38%) | Ns |
| mean age | 61±12 | 61±11 | Ns |
| ST-elevation AMI, n (%) | 18 (100%) | 69 (91%) | Ns |
| mean enzyme activity | 0.8±0.3 | 0.9±0.3 | Ns |
| *4 allele frequency | 23% | 18% | Ns |
| CYP2D6 gene duplication frequency | 0% | 10% | Ns |
| concomitant antiarrhythmics | no | no | - |
| beta blockers use, n (%) | 17 (94%) | 74 (97%) | ns |

Similarly to the previous study we created figures summarizing CYP2D6 gene activity in both groups – with and without VRDs (Figure 23). It seems that in both groups the allele distribution corresponds to the expected in the general population and was not different.

Figure 23. CYP2D6 activity in patients with and without VRDs after AMI




In this study we were unable to confirm the hypothesis of higher representation of CYP2D6 gene duplication in patients with VRDs in early period after AMI. This study however had limitations and differed from the previous one with regard to the way the patients were treated and the way VRDs were diagnosed. Major difference in this patient cohort was principal change in treatment strategies. In our first study the patients were recruited during 2006-2008, when no vascular interventions were routinely available, while current cohort consisted of patients all of whom had undergone percutaneous vascular intervention and blood flow restoration by angioplasty or stenting, and some – coronary artery bypass grafting. This led to significant decrease of VRDs registered generally, and most probably led to the change of their origin. Most probably observed VRDs were due to structural changes of myocardial tissue, and not due to catecholamine overload. Decreased frequency of VRDs led also to smaller than initially expected number of patients recruited, which could not provide statistical power for the negative finding observed.

### 4.3.2 Prescribers' attitudes to antimicrobial use and infection control in their hospital

After the findings from the study IV that demonstrated lack of association between antibiotics use and microbial resistance, which led to assumption of both irrational medications use and lack of infection control. Therefore we decided to perform a small pilot study evaluating prescribers attitude to their view on antibiotics efficacy and need to improve infection control.

The questionnaire consisted of 19 questions related to physicians' habits in prescribing, their attitude to hospital resistance (Figure 24). The questionnaire was distributed via the service of clinical pharmacology in the departments of surgery and general therapeutic departments. Senior nurses were instructed to provide the questionnaire to physicians and collect their response. The questionnaire was anonymous in the end, only the responsible nurse could identify physicians.

Figure 24. Questionnaire for prescribers on their attitude to antibiotics use and hospital resistance.

**Antibiotics and resistance**

Department

Length of experience years

**1 How often do you prescribe antibiotics in your practice:**

To every patient staying long in the hospital,

To every somatically weak patient independently of infection signs,

Only if clear evidence of infection is present,

In case of high risk of infection prior to its development

**2 What antibiotics do prescribe more often?**

1.

2.

3.

**3 How many different antibiotics do you use in practice?**

1-2 3-4 5-7 8-10

**4 How long do you continue antibiotics in case of surgery with no infection (for surgeons)**

1-3 days, 5-7 days, 10-14 days

**5 How long do you continue antibiotics in case of bacterial infection**

3-5 days, 5-7 days, 10-14 days, 20-30 days, up to the complete resolution of signs of infection with no specific time

**6 How often do you meet bacterial infection resistant to antibiotics?**

-every day –Every week –Every month –Rarely –Never –I do not know

**7 Did you treat bacterial infections, for which available antibacterial treatment was inadequate or incompletely adequate, and which resulted in patient's death?**

Yes

No

**8 What was the most possible causative agent in the last of these cases? _____**

**9 What antibiotic was not effective? _____**

**10 What do you predominantly base your decision when you prescribe antibiotics?**

Availability in the hospital pharmacy

Most possible causative agent

I always start with the same antibiotic and change when needed

Other (explain)

**11 How do you think what is the main reason for emergence of resistant flora in the department?**

Insufficient use of antibiotics,

Excessive use of antibiotics,

Insufficient diagnostics (explain),

Insufficient cleansing of instruments (equipment) and furniture at the department,

Insufficient use of gloves and lack of hand disinfection of the personnel,

Natural mutation of microorganisms, not depending on actions of physicians and hospital conditions

**12 What in your opinion is the main reason for ineffective treatment with antibiotics**

Lack of the necessary antibiotics in the hospital

Resistant infections

Insufficient diagnostics (explain)

Other

**13 Do you think that the less often antibiotics are prescribed the lower the risk of resistant flora development in the department?**

Yes I don't know no it is vice versa

**14 Do you think that it is possible to cure some patients with "traditional" antibiotics (penicillins)?**

Yes No

**15 How often do you choose antibiotics based on the bacteriology results?**

Always only in severe cases sometimes seldom never

**16 Do you know resistance profile in your department?**

Yes No I was never told about it

**17 Would you like to know resistance profile at your department?**

Yes No It will not change anything

**18 Will knowledge of local resistance profile influence your choice of antibiotic?**

Yes No

**19 Will you replace a potent antibiotic with a traditional one if you find out that the bacteria are sensitive to it?**

Yes No I do not trust bacteriology results

25 physicians completed the questionnaire from therapeutic departments, surgery, urology and intensive care units.

| Question number | Common answers |
|---|---|
| **1 How often do you prescribe antibiotics in your practice** | In high risk patients and in patients with signs of infection - 25 (100 %) |
| **2 What antibiotics do prescribe more often** | Extended spectrum cephalosporins – 25 (100%), fluoroquinolones – 20 (80%), metronidazole – 10 (40%) |
| **3 How many different antibiotics do you use in practice?** | 3-4 antibiotics – 10 (40%); 5-7 antibiotics – 15 (60%) |
| **4 How long do you continue antibiotics in case of surgery with no infection (for surgeons)** | 1-3 days – 10 (40%); 5-7 days – 10 (40%); no answer (not surgeons) – 5 (20%) |
| **5 How long do you continue antibiotics in case of bacterial infection** | 5-7 days – 10 (40%); 7-10 days – 5 (20%); up to recovery – 10 (40%) |
| **6 How often do you meet resistant bacterial infection resistant to antibiotics?** | Rarely – 10 (40%); every month – 10 (40%); do not know – 5 (20%) |
| **7 Did you treat bacterial infections, for which available antibacterial treatment was inadequate or incompletely adequate, and which resulted in patient's death?** | No – 20 (80%); yes – 5 (20%) |
| **8 What was the most possible causative agent in the latest of these cases?** | Do not know – 10 (40%), various pathogens were listed |
| **9 What antibiotic was not effective?** | Various antibiotics were listed |
| **10 What do you predominantly base your decision when you prescribe antibiotics?** | Antibiotic availability in the hospital pharmacy – 13 (52%); start with one agent and change when needed – 7 (28%); most possible causative agent – 5 (20%) |
| **11 What is the main reason for emergence of resistant flora in the department?** | Spontaneous mutations of microbes – 23 (92%); excessive antibiotic use – 2 (8%) |
| **12 What in your opinion is the main reason for ineffective treatment with antibiotics** | Lack of necessary antibiotics in the hospital – 10 (40%); microbial resistance – 15 (60%) |
| **13 Do you think that the less often antibiotics are prescribed the lower the risk of resistant flora development in the department?** | yes – 18 (70%); no – 7 (30%) |
| **14 Do you think that it is possible to cure patients with "traditional" antibiotics (penicillins)?** | yes – 13 (52%); no – 12 (48%) |
| **15 How often do you choose antibiotics based on the bacteriology results?** | only in severe cases – 18 (72%); always – 3 (12%); sometimes – 4 (16%) |
| **16 Do you know resistance profile in your department?** | yes – 10 (40%); no – 15 (60%) |
| **17 Would you like to know resistance profile at your department?** | yes – 25 (100%) |
| **18 Will knowledge of local resistance profile influence your choice of antibiotic?** | yes – 25 (100%) |
| **19 Will you replace a potent antibiotic with a traditional one if you find out that the bacteria are sensitive to it?** | yes – 25 (100%) |

Almost half of respondents answered that they meet resistant infection every month, and another half – rarely. Most respondents consider resistant infection as the main reason of ineffective treatment. None of them think that insufficient hygiene stimulates emergence of resistance, 90% find that resistance is a cause of natural mutation of microorganisms independent of physicians actions or hospital conditions, at the same time 70% of respondents told that the risk of resistant flora emergence is less if antibiotic use is less. Half of respondents considered that penicillins can not be ever curative in our days. Most were not aware of local resistance profile and showed to be in need of timely getting bacteriology results. Our preliminary results indicate that changes in the antibiotic utilization profile should be supported by a complex of measures, among which constant surveillance, active work of specialists in microbiology and drug utilization and most of all educative work are crucial.

# 5 CONCLUSIONS, PRACTICAL INTERPRETATION AND PERSPECTIVES

The conclusions of the relevant findings from these studies may be formulated in the following way:

1. CYP2D6 genotype is a factor that influences major pharmacodynamic parameters and quality of use of metoprolol when it is used according to routine clinical standards; especially gene duplication is important since it is a prerequisite of increased heart rate in post AMI patients, which is a known negative prognostic factor;
2. Paroxetine, prescribed concomitantly with metoprolol due to clinical reasons, may be a more important factor than genetically defined phenotype of poor metabolism, because increased effects are observed on the already adjusted dose, which may lead to unexpected adverse effects of metoprolol, and lack of its effects after paroxetine withdrawal;
3. Quality of antimicrobial use in hospitals is dependent on a number of factors including antimicrobial use patterns, infection control and physicians awareness, these factors need to be screened and controlled concomitantly;
4. A graphic tool of presentation of antimicrobial use and resistance in combination is a promising instrument to change prescribing pattern, but is not capable of changing resistance patterns in settings with high overall resistance and poor infection control;
5. As an additional finding our data showed that CYP2D6 genotype may be involved in factors predisposing to ventricular rhythm disorders in patients after AMI, but not in patients in whom percutaneous coronary interventions were performed.

All of the studies in this thesis were placed in the real clinical settings and were directed towards studying factors that may not be covered by any universal guidelines but are important for the qualified hospital care. Studies I and II identified presence and clinical importance of two major factors of individual susceptibility to cardiovascular medications – drug-drug interactions and polymorphic activity of drug metabolizing enzymes; while studies III and IV investigated known factors of rational hospital use of antibiotics and a novel methodology for its surveillance and improvement. The two different parts of the studies are combined in this dissertation under the overall idea of quality of care in the two clinically relevant areas – treatment of AMI and general infections; it shows applicability and importance of two different methodological approaches – one based on aggregate hospital data and another based on individual patient data. Probably the major limitation of both parts is also lack of longer-term observation and evaluation of clinical outcomes for the AMI studies and lack of detailed patient-wise evaluation of the aggregate data in the antibiotics part. The binding of these approaches is for sure an important aspect and corresponds to the strategies of translational research.

These studies may have clear further developments. Studies in AMI patients emphasize the potential importance of CYP2D6 gene duplication in contrast to most previous studies addressing non-functional alleles. It is a matter of further outcome studies to

show if genotyping may help to improve quality of beta-blockers use. Clinical importance of drug-drug interactions have been demonstrated in studies repeatedly[188] and it is probably a reason of failure to develop pharmacogenetics based dosing guidelines. Our study confirmed clinical importance of another interaction of agents very naturally prescribed together. A further question to answer is again in longer term consequencies, especially whether metoprolol effects get significantly lower after withdrawal of an interacting agent, since in contrast to hospital setting where patients are supposed to be under constant control, further events will take place in out-patient care. It is very straight forward to suggest inclusion of drug-drug interaction check into clinical routine of physicians in hospitals and out-patient care centers, which will help to provide treatment of better quality.

Antibiotic studies have direct practical implication. Our method of combined presentation of antibiotic use and resistance is a good screening tool for practice. The information provided by the figure is not a replacement for a detailed scrutiny of microbial resistance, but it has a benefit of giving a clear message that can be understood by both – prescribers and authorities, which may help to increase personal awareness. This methodology does not seem to be suitable for direct international comparisons, but it is useful as a component of hospital quality assurance. Further development would be in incorporating clinical outcomes - usually the best appreciated quality of infections care metrics.

Quality of hospital care is a complex issue, but translation of experimental research into this process is highly relevant. We think that our studies could be regarded as another step towards this process.

# 6 ACKNOWLEDGEMENTS

There are many beautiful people behind this thesis, whom I want to acknowledge with all my deep gratitude.

Above all I want to express my gratitude to all my supervisors for having very big, warm and enormously patient hearts for me.

In person I would like to acknowledge:

Ulf Bergman, my main supervisor, for introducing me into the field of clinical pharmacology in the world, and life in Sweden; for your attitude to life, your never-ending inspiration, creativity and fantastic ability to enjoy science, music, nature, family – everything what is really beautiful around us. For making me know your family, which is a real treasure. For supporting me through all these years and teaching to believe that everything is possible if we really want. For being able to make something out of me despite me being a "newborn blind puppy" in clinical pharmacology and research when we met.

Leif Bertilsson, my co-supervisor, for changing my life and making me fall in love with fundamental research. For being a researcher and a person, whose openness, confidence and truth in both - science and life - is something I will keep weighing my own life and professional work against. For being a guide who opens my eyes, but at the same time the one whom I'd follow with my eyes closed.

Aleksandra Burbello – my Russian supervisor and much more than this. You have been a wise teacher and a supportive person; you have been preserving me from all life challenges. You are the sun in our solar system of the team of your students keeping us all around. I will always keep praying for you.

Svetlana Boldueva – my Russian co-supervisor. For your energy and ability to create enthusiastic atmosphere around. For your being on the front line and bringing everyone with you.

Aleksandr Shabrov – rector of St Petersburg State Medical Academy n.a.I.I.Mechnikov and former head of the department of Hospital therapy with the course of clinical pharmacology – for providing a six-year education where I started the never-ending process of medical education, for giving me possibility to be introduced into science and this collaboration, for being aware of my progress and supporting all collaborative initiatives, and for your personal supportive attitude.

Tommy Linne – an energetic, enthusiastic and diplomatic coordinator of Swedish-Russian (and many other) collaborations! Thank you for bringing me to Sweden, for bringing Swedish research to Russia, and for your invaluable support in difficult relations different people sometimes experience! Thank you for pushing me to climb to the end of this thesis.

Natalia Mazzhuhina, the Russian coordinator of the collaborative KIRT project for providing hospitable reception for every visitor and making all negotiations within the project more fruitful;

Anders Rane, the former head of the department of clinical pharmacology, for the kind, tactful and welcome atmosphere and for being a very supportive person;

Georgios Panagiotidis, the current head of the department of clinical pharmacology, for your openness and smile, for being a welcome chef and very nice person;

Folke Sjöqvist, professor emeritus, father and preacher of clinical pharmacology, for teaching with fanastic inspiration, for erasing borders, for your unique energy and width of scientific thinking;

Lars L. Gustafsson, professor, for being a great example of a passionate researcher, for being a creator of the overall unique atmosphere in the pepartment;

Marja-Liisa Dahl, professor, for being a source of energy and enthusiasm, for your brightness and hospitality;

Margit Ekström, Yvonne Sjölind, Geannette Grünstein and Catarina Cleveson, secretaries at the division of clinical pharmacology, for being the heart and lungs of the division, for rescuing in the most unsolvable situations, for surviving and helping survive;

Lilleba Bohman, biomedical analyst, for being a patient and helpful teacher in the laboratory techniques, for teaching me to visualize invisible genetics, for being a real friend with very warm heart;

Jolanta Widen, biomedical analyst, for teaching me HPLC techniques and making me feel that we can always do valuable things out of scrap if we do it well, for being a beautiful lady and surrounding me with your warm care, for unforgettable women's day celebrations;

Eleni Aklillu, the chief of the research laboratory, for being a real researcher and supportive educator, and for being a beautiful person, with whom it was a real pleasure to share the office and discuss so many different things about research, life, politics and culture;

Ylva Böttiger for being a wise person, making big things and giving thoughtful advice;

Anders Hellden for being a great example of a clinician, researcher, and freandly person; for your sence of humor and passion for music;

Annika Asplund, Marine Andersson and Åsa Jansson, the pharmacists in the Drug Information Center, for taking care of me when I just came to Huddinge and had hard times of adaptation, for teaching me how brilliant can the information search be, for being my first educators here, great professionals and lovely people;

Erik Eliasson and Lena Ekström and other researchers for being inspiring, friendly and always ready to give any advice whenever needed;

Olof Beck and routine lab staff for possibility to get any advice and support, and for possibility to share your experience and equipment for my research;

Björn Wettermark, Vera Vlachovic-Palcevski, Christian Giske, Natalia Zaharova and Annika Hahlin – my co-authors in the antibiotic papers and co-fighters for qualified use of medicines – for your kind help and friendly valuable critisism that helped me a lot, for your warm welcome whenever we meet or communicate;

Birgitta Ask, Margarita Rais Mahindi, Roza Ghotbi, Sara Karlsson, Jenny Jakobsson Shultze and other people in the research lab for being friendly, helpful and supportive, for the great atmosphere in the lab;

Filip Josephson, Jonatan Lindh, Erik Sparve, Staffan Rosenborg, Jaran Eriksen, Chistine Skogastierna and other members of the team of former PhD students and current researchers and educators in the division of clinical pharmacology and outside, for your personalities, friendly advice, valuable criticism, welcome and support;

Raquel, Natasa, Milan, Takashi, Takaki, Jackson, Minzi and all other guest researchers and PhD student with whom we shared lab evenings and weekends, nice shopping, food, concerts and had a lot of fun together;

Svetlana Babak, Nastya Fedorenko, Nonna Dobrynina, Katya Myasnikova, Masha Pokladova, Masha Kosticyna – my Russian family of clinical pharmacology! It would never be possible without your help, energy, personal and professional support and great sense of humor. I have never in my life met such people who would really do everything for each other first, and then will think of themselves. Clinical pharmacologists in Russia are not numerous, but they are very much distinguishable from others. You are very special! And we will win!

Marina V. Samokhvalova, head of clinical division of cardiology in St Petersburg hospital n.a.Peter the Great for teaching me to be a cardiologist, and a clinician, for your personal support and for being a person I'd like to keep in touch independently of locations and positions;

Natalia Ersh, Julia, Viktoria, Irina, Natalia and Aleksandra – nurses at the cardiology department who were my teachers in vein catheterization and my support;

All my friends from the KIRT project for sharing first impressions, walks, talks, food and all other aspects of life and your help whenever and wherever it is needed;

Anya Sidorchuk – for your welcome and warmth, for cozy evenings with a cup of tea, for your invaluable help and for your very special personality which I really enjoy;

Zainab and Tatiana – for long discussions, for sharing your feelings and thoughts, for being my support and my real friends, to know you is something that has real value in my life;

Anton – for being my company, support and friend in many beautiful and different moments of my life in Sweden;

Maj Bergman – for being a beautiful, patient and helpful person with big heart and beautiful soul;

Larissa Koukel – for being a fantastic person, my dear friend and collaborator, the best of Russia in Sweden, for your beautiful Katya and Nadya;

Sergey, my dear husband, for your patience, support and love. This is your thesis too;

My parents, grandparents and sister for you gave me love and education; you found teachers for me and let me go wherever I needed to go, for believing in me;

Nadezhda Bondar – my Russian teacher of English, for you opened many borders for me;

My friends in Vologda and St Petersburg - for making my life brighter, for believing in me;

I would also like to acknowledge the KIRT program supported by the Swedish Institute for giving me the ability to come to Stockholm, and be a part of the collaborative program;

The Swedish Heart and Lung foundation and Swedish Research Councile, Medicine (3902) for giving additional financial support;

The administration of the North-Western State Medical University n.a.I.I.Mechnikov and the department of Therapeutics and Clinical Pharmacology for supporting this collaboration and the final stages of my doctoral education.

# 7 REFERENCES


1. WHO | Infectious diseases. World Health Organization; [cited 2013 Mar 8]; Available from: http://www.who.int/topics/infectious_diseases/en/

2. Perry R, Fetherston J. Yersinia pestis--etiologic agent of plague. Clin. Microbiol. Rev. 1997 Jan 1;10(1):35–66.

3. Lowis GW. Epidemiology of puerperal fever: the contributions of Alexander Gordon. Medical history. Cambridge University Press; 1993 Oct 1;37(4):399–410.

4. Gordon AK. NOTES ON THE TREATMENT OF PUERPERAL FEVER. British medical journal. BMJ Group; 1908 Apr 25;1(2469):970–2.

5. WHO | World Health Statistics. World Health Organization; [cited 2013 Mar 8]; Available from: http://www.who.int/gho/publications/world_health_statistics/en/index.html

6. World Health Organization. Ten leading causes of deaths in 2008 [Internet]. 2011. Available from: http://gamapserver.who.int/gho/interactive_charts/mbd/cod_2008/graph.html

7. ECDC. Annual epidemiological report on communicable diseases. [Internet]. 2010. Available from: http://www.ecdc.europa.eu/en/publications/Publications/1011_SUR_Annual_Epidemiological_Report_on_Communicable_Diseases_in_Europe.pdf

8. Corrales-Medina VF, Musher DM, Shachkina S, Chirinos JA. Acute pneumonia and the cardiovascular system. Lancet. 2013 Feb 9;381(9865):496–505.

9. ABRAHAM EP, CALLOW D, GILLIVER K. Adaptation of Staphylococcus aureus to growth in the presence of certain antibiotics. Nature. 1946 Dec 7;158(4023):818–21.

10. Wright GD. The origins of antibiotic resistance. Handbook of experimental pharmacology. 2012 Jan;(211):13–30.

11. Rennie RP. Current and future challenges in the development of antimicrobial agents. Handbook of experimental pharmacology. 2012 Jan;(211):45–65.

12. Trecarichi EM, Cauda R, Tumbarello M. Detecting risk and predicting patient mortality in patients with extended-spectrum β-lactamase-producing Enterobacteriaceae bloodstream infections. Future microbiology. Future Medicine Ltd London, UK; 2012 Oct 3;7(10):1173–89.

13. Pitout JDD. Infections with extended-spectrum beta-lactamase-producing enterobacteriaceae: changing epidemiology and drug treatment choices. Drugs. 2010 Mar 12;70(3):313–33.

14. Livermore DM. Current epidemiology and growing resistance of gram-negative pathogens. The Korean journal of internal medicine. 2012 Jul;27(2):128–42.

15. Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrobial agents and chemotherapy. American Society for Microbiology (ASM); 2008 Mar 1;52(3):813–21.

16. EARS-net. Antimicrobial resistance surveillance in Europe 2010. 2010.

17. Calfee DP. Methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci, and other Gram-positives in healthcare. Current opinion in infectious diseases. 2012 Aug;25(4):385–94.

18. Gould IM, Cauda R, Esposito S, Gudiol F, Mazzei T, Garau J. Management of serious meticillin-resistant Staphylococcus aureus infections: what are the limits? International journal of antimicrobial agents. 2011 Mar;37(3):202–9.

19. Benedict KM, Gow SP, Reid-Smith RJ, Booker CW, Morley PS. Metrics for quantifying antimicrobial use in beef feedlots. The Canadian veterinary journal. La revue vétérinaire canadienne. Canadian Veterinary Medical Association; 2012 Aug 1;53(8):841–8.

20. Marchaim D, Chopra T, Bhargava A, Bogan C, Dhar S, Hayakawa K, et al. Recent exposure to antimicrobials and carbapenem-resistant Enterobacteriaceae: the role of antimicrobial stewardship. Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America. 2012 Aug;33(8):817–30.

21. Miyawaki K, Miwa Y, Seki M, Asari S, Tomono K, Kurokawa N. Correlation between the consumption of meropenem or doripenem and meropenem susceptibility of Pseudomonas aeruginosa in a university hospital in Japan. Biological & pharmaceutical bulletin. 2012 Jan;35(6):946–9.

22. Skjøt-Rasmussen L, Olsen SS, Jensen US, Hammerum AM. Increasing consumption of antimicrobial agents in Denmark parallels increasing resistance in Escherichia coli bloodstream isolates. International journal of antimicrobial agents. 2012 Jul;40(1):86–8.

23. team EC for DP and C (ECDC)-HCU-E editorial. Trends in yearly prevalence of third-generation cephalosporin and fluoroquinolone resistant Enterobacteriaceae infections and antimicrobial use in Spanish hospitals, Spain, 1999 to 2010 [Internet]. European Centre for Disease Prevention and Control (ECDC) - Health Comunication Unit; 2011 [cited 2013 Apr 2]. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19983

24. Kritsotakis EI, Tsioutis C, Roumbelaki M, Christidou A, Gikas A. Antibiotic use and the risk of carbapenem-resistant extended-spectrum-{beta}-lactamase-producing Klebsiella pneumoniae infection in hospitalized patients: results of a

double case-control study. The Journal of antimicrobial chemotherapy. 2011 Jun;66(6):1383–91.

25. Ababneh M, Harpe S, Oinonen M, Polk RE. Trends in aminoglycoside use and gentamicin-resistant gram-negative clinical isolates in US academic medical centers: implications for antimicrobial stewardship. Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America. 2012 Jun;33(6):594–601.

26. Jankovic S, Djordjevic Z, Matovic M. Resistance rates of Pseudomonas aeruginosa and Acinetobacter species causing ventilator-associated pneumonia do not always correlate with utilisation of antibiotics. The Journal of hospital infection. 2011 Jan;77(1):77–8.

27. Control of antibiotic-resistant bacteria: memorandum from a WHO meeting. Bulletin of the World Health Organization. 1983 Jan;61(3):423–33.

28. Rosdahl VT, Pedersen KB. The Copenhagen Recommendations The Microbial Threat. Copenhagen; 1998.

29. PR-99-33/ WHO issues. World Health Organization; [cited 2013 Apr 4]; Available from: http://www.who.int/inf-pr-1999/en/pr99-33.html

30. World Health Organization. WHONET software. World Health Organization; [cited 2013 Apr 4]; Available from: http://www.who.int/drugresistance/WHO_Global_Strategy.htm/en/

31. World Health Organization. Introduction to drug utilization research. World Health Organization; 2003 [cited 2013 Apr 3]; Available from: http://www.who.int/medicines/areas/quality_safety/safety_efficacy/utilization/en/

32. Andersen AH. [European Symposium on the Consumption of Drugs. Lysebu, Oslo, 3-7 November 1969]. Ugeskrift for laeger. 1970 Feb 19;132(8):399–400.

33. Bergman U. The history of the Drug Utilization Research Group in Europe. Pharmacoepidemiology and drug safety. 2006 Feb;15(2):95–8.

34. WHOCC - Home [Internet]. Available from: http://www.whocc.no/

35. Teng L, Xin H, Blix HS, Tsutani K. Review of the use of defined daily dose concept in drug utilisation research in China. Pharmacoepidemiology and drug safety. 2012 Oct;21(10):1118–24.

36. European Surveillance of Antimicrobial Consumption Network (ESAC-Net) [Internet]. [cited 2013 Apr 3]. Available from: http://www.ecdc.europa.eu/en/activities/surveillance/esac-net/pages/index.aspx

37. Cars O, Mölstad S, Melander A. Variation in antibiotic use in the European Union For personal use . Only reproduce with permission from The Lancet Publishing Group . Lancet. 2001;357(62):1851–3.

38. Muraki Y, Kitamura M, Maeda Y, Kitahara T, Mori T, Ikeue H, et al. Nationwide surveillance of antimicrobial consumption and resistance to Pseudomonas aeruginosa isolates at 203 Japanese hospitals in 2010. Infection. 2013 Apr;41(2):415–23.

39. Jacob JT, Gaynes RP. Emerging trends in antibiotic use in US hospitals: quality, quantification and stewardship. Expert review of anti-infective therapy. 2010 Aug;8(8):893–902.

40. Kloka Listan - Janusinfo [Internet]. [cited 2013 Apr 5]. Available from: http://www.janusinfo.se/Beslutsstod/Kloka-Listan/Kloka-listan-2013/

41. Bergman U, Popa C, Tomson Y, Wettermark B, Einarson TR, Aberg H, et al. Drug utilization 90%--a simple method for assessing the quality of drug prescribing. European journal of clinical pharmacology. 1998 Apr;54(2):113–8.

42. Vlahovic-Palcevski V, Wettermark B, Bergman U. Quality of non-steroidal anti-inflammatory drug prescribing in Croatia (Rijeka) and Sweden (Stockholm). European journal of clinical pharmacology. 2002 Jun;58(3):209–14.

43. Wettermark B, Pehrsson A, Jinnerot D, Bergman U. Drug utilisation 90% profiles--a useful tool for quality assessment of prescribing in primary health care in Stockholm. Pharmacoepidemiology and drug safety. 2003 Sep;12(6):499–510.

44. Vlahović-Palcevski V, Wettermark B, Prpić I, Bergman U. Attitudes to feedback with drug utilisation 90% (DU90%) profiles among prescribers in Rijeka, Croatia. Pharmacoepidemiology and drug safety. 2004 Oct;13(10):725–7.

45. Bergman U, Risinggård H, Vlahović-Palcevski V, Ericsson O. Use of antibiotics at hospitals in Stockholm: a benchmarking project using internet. Pharmacoepidemiology and drug safety. 2004 Jul;13(7):465–71.

46. Dumpis U, Gulbinovic J, Struwe J, Lagergren A, Griskevicius L, Bergman U. Differences in antibiotic prescribing in three university hospitals in the Baltic region revealed by a simple protocol for quality assessment of therapeutic indications. International journal of clinical pharmacology and therapeutics. 2007 Oct;45(10):568–76.

47. Vlahović-Palcevski V, Dumpis U, Mitt P, Gulbinovic J, Struwe J, Palcevski G, et al. Benchmarking antimicrobial drug use at university hospitals in five European countries. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2007 Mar;13(3):277–83.

48. Coenen S, Ferech M, Haaijer-Ruskamp FM, Butler CC, Vander Stichele RH, Verheij TJM, et al. European Surveillance of Antimicrobial Consumption (ESAC): quality indicators for outpatient antibiotic use in Europe. Quality & safety in health care. 2007 Dec;16(6):440–5.

49. Adriaenssens N, Coenen S, Versporten A, Muller A, Vankerckhoven V, Goossens H. European Surveillance of Antimicrobial Consumption (ESAC): quality appraisal of antibiotic use in Europe. The Journal of antimicrobial chemotherapy. 2011 Dec;66 Suppl 6:vi71–77.

50. Ibrahim OM, Polk RE. Benchmarking antimicrobial drug use in hospitals. Expert review of anti-infective therapy. 2012 Apr;10(4):445–57.

51. Fraser GL, Stogsdill P, Dickens JD, Wennberg DE, Smith RP, Prato BS. Antibiotic optimization. An evaluation of patient safety and economic outcomes. Archives of internal medicine. 157(15):1689–94.

52. Solomon DH, Van Houten L, Glynn RJ, Baden L, Curtis K, Schrager H, et al. Academic detailing to improve use of broad-spectrum antibiotics at an academic medical center. Archives of internal medicine. 161(15):1897–902.

53. Goossens H. Antibiotic resistance and policy in Belgium. Verhandelingen - Koninklijke Academie voor Geneeskunde van België. 2000 Jan;62(5):439–69.

54. Griffith M, Postelnick M, Scheetz M. Antimicrobial stewardship programs: methods of operation and suggested outcomes. Expert review of anti-infective therapy. 2012 Jan;10(1):63–73.

55. Dellit TH, Owens RC, McGowan JE, Gerding DN, Weinstein RA, Burke JP, et al. Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. Oxford University Press; 2007 Jan 15;44(2):159–77.

56. Rahal JJ, Urban C, Horn D, Freeman K, Segal-Maurer S, Maurer J, et al. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial Klebsiella. JAMA : the journal of the American Medical Association. 1998 Oct 14;280(14):1233–7.

57. Glowacki RC, Schwartz DN, Itokazu GS, Wisniewski MF, Kieszkowski P, Weinstein RA. Antibiotic combinations with redundant antimicrobial spectra: clinical epidemiology and pilot intervention of computer-assisted surveillance. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2003 Jul 1;37(1):59–64.

58. Camins BC, King MD, Wells JB, Googe HL, Patel M, Kourbatova E V, et al. Impact of an antimicrobial utilization program on antimicrobial use at a large teaching hospital: a randomized controlled trial. Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America. 2009 Oct;30(10):931–8.

59. Davey P, Brown E, Fenelon L, Finch R, Gould I, Hartman G, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. Cochrane database of systematic reviews (Online). 2005 Jan;(4):CD003543.

60. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. European heart journal. 2012 Oct 2;33(20):2551–67.

61. Bonaca MP, Wiviott SD, Braunwald E, Murphy SA, Ruff CT, Antman EM, et al. American College of Cardiology/American Heart Association/European Society of Cardiology/World Heart Federation universal definition of myocardial infarction classification system and the risk of cardiovascular death: observations from the TRITON-TIMI 38. Circulation. 2012 Jan 31;125(4):577–83.

62. Steg PG, James SK, Atar D, Badano LP, Blömstrom-Lundqvist C, Borger MA, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. European heart journal. 2012 Oct;33(20):2569–619.

63. Thombs BD, Bass EB, Ford DE, Stewart KJ, Tsilidis KK, Patel U, et al. Prevalence of depression in survivors of acute myocardial infarction. Journal of general internal medicine. 2006 Jan;21(1):30–8.

64. Sørensenf C, Friis-Hasché E, Haghfelt T, Bech P. Postmyocardial infarction mortality in relation to depression: a systematic critical review. Psychotherapy and psychosomatics. 2005 Jan;74(2):69–80.

65. Alboni P, Favaron E, Paparella N, Sciammarella M, Pedaci M. Is there an association between depression and cardiovascular mortality or sudden death? Journal of cardiovascular medicine (Hagerstown, Md.). 2008 Apr;9(4):356–62.

66. Opie LH, Gersh BJ. Drugs for the heart. 6th ed. Elsevier; 2005. p. 437.

67. Ellison KE, Gandhi G. Optimising the use of beta-adrenoceptor antagonists in coronary artery disease. Drugs. 2005 Jan;65(6):787–97.

68. Hjalmarson A, Elmfeldt D, Herlitz J, Holmberg S, Málek I, Nyberg G, et al. Effect on mortality of metoprolol in acute myocardial infarction. A double-blind randomised trial. Lancet. 1981 Oct 17;2(8251):823–7.

69. Hjalmarson A, Herlitz J, Holmberg S, Rydén L, Swedberg K, Vedin A, et al. The Göteborg metoprolol trial. Effects on mortality and morbidity in acute myocardial infarction. Circulation. 1983 Jun;67(6 Pt 2):I26–32.

70. Metoprolol in acute myocardial infarction (MIAMI). A randomised placebo-controlled international trial. The MIAMI Trial Research Group. European heart journal. 1985 Mar;6(3):199–226.

71. Nakatani D, Sakata Y, Suna S, Usami M, Matsumoto S, Shimizu M, et al. Impact of Beta Blockade Therapy on Long-Term Mortality After ST-Segment Elevation Acute Myocardial Infarction in the Percutaneous Coronary Intervention Era. The American journal of cardiology. 2013 Feb 15;111(4):457–64.

72. Yusuf S, Wittes J, Friedman L. Overview of results of randomized clinical trials in heart disease. II. Unstable angina, heart failure, primary prevention with aspirin, and risk factor modification. JAMA : the journal of the American Medical Association. 1988 Oct 21;260(15):2259–63.

73. Chen ZM, Pan HC, Chen YP, Peto R, Collins R, Jiang LX, et al. Early intravenous then oral metoprolol in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. Lancet. 2005 Nov 5;366(9497):1622–32.

74. Hamm CW, Bassand J-P, Agewall S, Bax J, Boersma E, Bueno H, et al. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevatio. European heart journal. 2011 Dec;32(23):2999–3054.

75. The Lopressor Intervention Trial: multicentre study of metoprolol in survivors of acute myocardial infarction. Lopressor Intervention Trial Research Group. European heart journal. 1987 Oct;8(10):1056–64.

76. Roberts R, Rogers WJ, Mueller HS, Lambrew CT, Diver DJ, Smith HC, et al. Immediate versus deferred beta-blockade following thrombolytic therapy in patients with acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI) II-B Study. Circulation. 1991 Feb;83(2):422–37.

77. Herlitz J, Karlson BW, Hjalmarson A. Ten year mortality in relation to original size of myocardial infarct: results from the Gothenburg metoprolol study. British heart journal. 1994 Mar;71(3):238–41.

78. DiNicolantonio JJ, Hackam DG. Carvedilol: a third-generation β-blocker should be a first-choice β-blocker. Expert review of cardiovascular therapy. 2012 Jan;10(1):13–25.

79. Ibanez B, Fuster V, Macaya C, Sánchez-Brunete V, Pizarro G, López-Romero P, et al. Study design for the “effect of METOprolol in CARDioproteCtioN during an acute myocardial InfarCtion” (METOCARD-CNIC): a randomized, controlled parallel-group, observer-blinded clinical trial of early pre-reperfusion metoprolol administration in ST-segmen. American heart journal. 2012 Oct;164(4):473–480.e5.

80. Herman M, Donovan J, Tran M, McKenna B, Gore JM, Goldberg RJ, et al. Use of beta-blockers and effects on heart rate and blood pressure post-acute coronary syndromes: are we on target? American heart journal. 2009 Sep;158(3):378–85.

81. Fox K, Borer JS, Camm AJ, Danchin N, Ferrari R, Lopez Sendon JL, et al. Resting heart rate in cardiovascular disease. Journal of the American College of Cardiology. 2007 Aug 28;50(9):823–30.

82. Plosker GL, Clissold SP. Controlled release metoprolol formulations. A review of their pharmacodynamic and pharmacokinetic properties, and therapeutic use in hypertension and ischaemic heart disease. Drugs. 1992 Mar;43(3):382–414.

83. Regårdh CG, Landahl S, Larsson M, Lundborg P, Steen B, Hoffmann KJ, et al. Pharmacokinetics of metoprolol and its metabolite alpha-OH-metoprolol in healthy, non-smoking, elderly individuals. European journal of clinical pharmacology. 1983 Jan;24(2):221–6.

84. Jonkers RE, Koopmans RP, Portier EJ, Van Boxtel CJ. Debrisoquine phenotype and the pharmacokinetics and beta-2 receptor pharmacodynamics of metoprolol and its enantiomers. The Journal of pharmacology and experimental therapeutics. 1991 Mar;256(3):959–66.

85. Cerqueira PM, Cesarino EJ, Bertucci C, Bonato PS, Lanchote VL. Stereoselective metabolism of metoprolol: enantioselectivity of alpha-hydroxymetoprolol in plasma and urine. Chirality. 2003 Jun;15(6):542–9.

86. Borg KO, Carlsson E, Hoffmann KJ, Jönsson TE, Thorin H, Wallin B. Metabolism of metoprolol-(3-h) in man, the dog and the rat. Acta pharmacologica et toxicologica. 1975 Jan;36(Suppl 5):125–35.

87. Murthy SS, Shetty HU, Nelson WL, Jackson PR, Lennard MS. Enantioselective and diastereoselective aspects of the oxidative metabolism of metoprolol. Biochemical pharmacology. 1990 Oct 1;40(7):1637–44.

88. Mautz DS, Nelson WL, Shen DD. Regioselective and stereoselective oxidation of metoprolol and bufuralol catalyzed by microsomes containing cDNA-expressed human P4502D6. Drug metabolism and disposition: the biological fate of chemicals. 1995 Apr;23(4):513–7.

89. McGourty JC, Silas JH, Lennard MS, Tucker GT, Woods HF. Metoprolol metabolism and debrisoquine oxidation polymorphism--population and family studies. British journal of clinical pharmacology. 1985 Dec;20(6):555–66.

90. Astra Zeneka. FDA report NDA 19962/S-027. 2005.

91. Ekbom T, Dahlöf B, Hansson L, Lindholm LH, Scherstén B, Wester PO. Antihypertensive efficacy and side effects of three beta-blockers and a diuretic in elderly hypertensives: a report from the STOP-Hypertension study. Journal of hypertension. 1992 Dec;10(12):1525–30.

92. Johnsson G, Regårdh CG, Sölvell L. Combined pharmacokinetic and pharmacodynammc studies in man of the adrenergic beta1-receptor antagonist metoprolol. Acta pharmacologica et toxicologica. 1975 Jan;36(Suppl 5):31–44.

93. Kendall MJ, John VA, Quarterman CP, Welling PG. A single and multiple dose pharmacokinetic and pharmacodynamic comparison of conventional and slow-release metroprolol. European journal of clinical pharmacology. 1980 Feb;17(2):87–92.

94. Schaaf LJ, Campbell SC, Mayersohn MB, Vagedes T, Perrier DG. Influence of smoking and gender on the disposition kinetics of metoprolol. European journal of clinical pharmacology. 1987 Jan;33(4):355–61.

95. Rochon PA, Anderson GM, Tu J V, Gurwitz JH, Clark JP, Shear NH, et al. Age- and gender-related use of low-dose drug therapy: the need to manufacture low-dose therapy and evaluate the minimum effective dose. Journal of the American Geriatrics Society. 1999 Aug;47(8):954–9.

96. Luzier AB, Killian A, Wilton JH, Wilson MF, Forrest A, Kazierad DJ. Gender-related effects on metoprolol pharmacokinetics and pharmacodynamics in healthy volunteers. Clinical pharmacology and therapeutics. 1999 Dec;66(6):594–601.

97. Larsson M, Landahl S, Lundborg P, Regårdh CG. Pharmacokinetics of metoprolol in healthy, elderly, non-smoking individuals after a single dose and two weeks of treatment. European journal of clinical pharmacology. 1984 Jan;27(2):217–22.

98. Dimenäs ES, Dahlöf CG, Heibel B, Moore RG, Olofsson BK, Westergren GE, et al. Subjective symptoms and pharmacokinetics/dynamics of metoprolol CR in elderly subjects--a comparison with atenolol. European journal of clinical pharmacology. 1990 Jan;38(6):571–8.

99. Jordö L, Attman PO, Aurell M, Johansson L, Johnsson G, Regårdh CG. Pharmacokinetic and pharmacodynamic properties of metoprolol in patients with impaired renal function. Clinical pharmacokinetics. 5(2):169–80.

100. Hoffmann KJ, Regårdh CG, Aurell M, Ervik M, Jordö L. The effect of impaired renal function on the plasma concentration and urinary excretion of metoprolol metabolites. Clinical pharmacokinetics. 5(2):181–91.

101. Seiler KU, Schuster KJ, Meyer GJ, Niedermayer W, Wassermann O. The pharmacokinetics of metoprolol and its metabolites in dialysis patients. Clinical pharmacokinetics. 5(2):192–8.

102. Regårdh CG, Jordö L, Ervik M, Lundborg P, Olsson R, Rönn O. Pharmacokinetics of metoprolol in patients with hepatic cirrhosis. Clinical pharmacokinetics. 6(5):375–88.

103. Benfield P, Clissold SP, Brogden RN. Metoprolol. An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in hypertension, ischaemic heart disease and related cardiovascular disorders. Drugs. 1986 May;31(5):376–429.

104. Gengo FM, Ermer JC, Carey C, Kalonaros GC, McHugh WB. The relationship between serum concentrations and central nervous system actions of metoprolol. Journal of neurology, neurosurgery, and psychiatry. 1985 Feb;48(2):101–6.

105. Cove-Smith JR, Kirk CA. CNS-related side-effects with metoprolol and atenolol. European journal of clinical pharmacology. 1985 Jan;28 Suppl:69–72.

106. Dahlöf C, Hedner T, Thulin T, Gustafsson S, Olsson SO. Effects of diltiazem and metoprolol on blood pressure, adverse symptoms and general well-being. The Swedish Diltiazem-Metoprolol Multi-Centre Study Group. European journal of clinical pharmacology. 1991 Jan;40(5):453–60.

107. Wiklund O, Hulthe J, Wikstrand J, Schmidt C, Olofsson S-O, Bondjers G. Effect of controlled release/extended release metoprolol on carotid intima-media thickness in patients with hypercholesterolemia: a 3-year randomized study. Stroke; a journal of cerebral circulation. 2002 Feb;33(2):572–7.

108. Wikstrand J, Berglund G, Hedblad B, Hulthe J. Antiatherosclerotic effects of beta-blockers. The American journal of cardiology. 2003 Jun 19;91(12A):25H–29H.

109. Hedblad B, Wikstrand J, Janzon L, Wedel H, Berglund G. Low-dose metoprolol CR/XL and fluvastatin slow progression of carotid intima-media thickness: Main results from the Beta-Blocker Cholesterol-Lowering Asymptomatic Plaque Study (BCAPS). Circulation. 2001 Apr 3;103(13):1721–6.

110. Hua AS, Assaykeen TA, Nyberg G, Kincaid-Smith PS. Results from a multicentre trial of metoprolol and a study of hypertensive patients with chronic obstructive lung disease. The Medical journal of Australia. 1978 Mar 11;1(5):281–6.

111. Wilcox PG, Ahmad D, Darke AC, Parsons J, Carruthers SG. Respiratory and cardiac effects of metoprolol and bevantolol in patients with asthma. Clinical pharmacology and therapeutics. 1986 Jan;39(1):29–34.

112. Wuttke H, Rau T, Heide R, Bergmann K, Böhm M, Weil J, et al. Increased frequency of cytochrome P450 2D6 poor metabolizers among patients with metoprolol-associated adverse effects. Clinical pharmacology and therapeutics. 2002 Oct;72(4):429–37.

113. Zineh I, Beitelshees AL, Gaedigk A, Walker JR, Pauly DF, Eberst K, et al. Pharmacokinetics and CYP2D6 genotypes do not predict metoprolol adverse events or efficacy in hypertension. Clinical pharmacology and therapeutics. 2004 Dec;76(6):536–44.

114. Fux R, Mörike K, Pröhmer AMT, Delabar U, Schwab M, Schaeffeler E, et al. Impact of CYP2D6 genotype on adverse effects during treatment with metoprolol: a prospective clinical study. Clinical pharmacology and therapeutics. 2005 Oct;78(4):378–87.

115. Thürmann PA, Haack S, Werner U, Szymanski J, Haase G, Drewelow B, et al. Tolerability of beta-blockers metabolized via cytochrome P450 2D6 is sex-dependent. Clinical pharmacology and therapeutics. 2006 Nov;80(5):551–3.

116. McGraw J, Waller D. Cytochrome P450 variations in different ethnic populations. Expert opinion on drug metabolism & toxicology. 2012 Mar;8(3):371–82.

117. Human Cytochrome P450 (CYP) Allele Nomenclature Committee [Internet]. Available from: http://www.cypalleles.ki.se/

118. Sim SC, Ingelman-Sundberg M. Update on allele nomenclature for human cytochromes P450 and the Human Cytochrome P450 Allele (CYP-allele) Nomenclature Database. Methods in molecular biology (Clifton, N.J.). 2013 Jan;987:251–9.

119. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. The pharmacogenomics journal. 2005 Jan;5(1):6–13.

120. Hammer W, Sjöqvist F. Plasma levels of monomethylated tricyclic antidepressants during treatment with imipramine-like compounds. Life sciences. 1967 Sep 1;6(17):1895–903.

121. Mahgoub A, Idle JR, Dring LG, Lancaster R, Smith RL. Polymorphic hydroxylation of Debrisoquine in man. Lancet. 1977 Sep 17;2(8038):584–6.

122. Eichelbaum M, Spannbrucker N, Dengler H. A probably genetic defect in the metabolism of sparteine in biological oxidation of nitrogen. In: Gorrow J, editor. Amsterdam: Elsevier North-Holland Biomedical Press; 1978. p. 113–8.

123. Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, et al. Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. Nature. 1988 Feb 4;331(6155):442–6.

124. Teh LK, Bertilsson L. Pharmacogenomics of CYP2D6: molecular genetics, interethnic differences and clinical importance. Drug metabolism and pharmacokinetics. 2012 Jan;27(1):55–67.

125. Kimura S, Umeno M, Skoda RC, Meyer UA, Gonzalez FJ. The human debrisoquine 4-hydroxylase (CYP2D) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. American journal of human genetics. 1989 Dec;45(6):889–904.

126. Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjöqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. Proceedings of the National Academy of Sciences of the United States of America. 1993 Dec 15;90(24):11825–9.

127. Bertilsson L, Lou YQ, Du YL, Liu Y, Kuang TY, Liao XM, et al. Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. Clinical pharmacology and therapeutics. 1992 Apr;51(4):388–97.

128. Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman-Sundberg M. Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. The Journal of pharmacology and experimental therapeutics. 1996 Jul;278(1):441–6.

129. Bernal ML, Sinues B, Johansson I, McLellan RA, Wennerholm A, Dahl ML, et al. Ten percent of North Spanish individuals carry duplicated or triplicated CYP2D6 genes associated with ultrarapid metabolism of debrisoquine. Pharmacogenetics. 1999 Oct;9(5):657–60.

130. Gaikovitch EA, Cascorbi I, Mrozikiewicz PM, Brockmöller J, Frötschl R, Köpke K, et al. Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. European journal of clinical pharmacology. 2003 Aug;59(4):303–12.

131. Elbekai RH, El-Kadi AOS. Cytochrome P450 enzymes: central players in cardiovascular health and disease. Pharmacology & therapeutics. 2006 Nov;112(2):564–87.

132. Martínez C, Agúndez JA, Gervasini G, Martín R, Benítez J. Tryptamine: a possible endogenous substrate for CYP2D6. Pharmacogenetics. 1997 Apr;7(2):85–93.

133. Schyman P, Lai W, Chen H, Wang Y, Shaik S. The directive of the protein: how does cytochrome P450 select the mechanism of dopamine formation? Journal of the American Chemical Society. 2011 May 25;133(20):7977–84.

134. Yu A-M, Idle JR, Byrd LG, Krausz KW, Küpfer A, Gonzalez FJ. Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. Pharmacogenetics. 2003 Mar;13(3):173–81.

135. Yu A-M, Idle JR, Herraiz T, Küpfer A, Gonzalez FJ. Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine O-demethylase. Pharmacogenetics. 2003 Jun;13(6):307–19.

136. Llerena A, Edman G, Cobaleda J, Benítez J, Schalling D, Bertilsson L. Relationship between personality and debrisoquine hydroxylation capacity. Suggestion of an endogenous neuroactive substrate or product of the cytochrome P4502D6. Acta psychiatrica Scandinavica. 1993 Jan;87(1):23–8.

137. Bertilsson L, Alm C, De Las Carreras C, Widen J, Edman G, Schalling D. Debrisoquine hydroxylation polymorphism and personality. Lancet. 1989 Mar 11;1(8637):555.

138. Iwashima K, Yasui-Furukori N, Kaneda A, Saito M, Nakagami T, Sato Y, et al. No association between CYP2D6 polymorphisms and personality trait in Japanese. British journal of clinical pharmacology. 2007 Jul;64(1):96–9.

139. Suzuki E, Kitao Y, Ono Y, Iijima Y, Inada T. Cytochrome P450 2D6 polymorphism and character traits. Psychiatric genetics. 2003 Jun;13(2):111–3.

140. Peñas-LLedó EM, Dorado P, Pacheco R, González I, LLerena A. Relation between CYP2D6 genotype, personality, neurocognition and overall psychopathology in healthy volunteers. Pharmacogenomics. 2009 Jul;10(7):1111–20.

141. González I, Peñas-Lledó EM, Pérez B, Dorado P, Alvarez M, LLerena A. Relation between CYP2D6 phenotype and genotype and personality in healthy volunteers. Pharmacogenomics. 2008 Jul;9(7):833–40.

142. Roberts RL, Luty SE, Mulder RT, Joyce PR, Kennedy MA. Association between cytochrome P450 2D6 genotype and harm avoidance. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics. 2004 May 15;127B(1):90–3.

143. Peñas-Lledó EM, Dorado P, Agüera Z, Gratacós M, Estivill X, Fernández-Aranda F, et al. CYP2D6 polymorphism in patients with eating disorders. The pharmacogenomics journal. 2012 Apr;12(2):173–5.

144. Ahlner J, Zackrisson A-L, Lindblom B, Bertilsson L. CYP2D6, serotonin and suicide. Pharmacogenomics. 2010 Jul;11(7):903–5.

145. Blasco-Fontecilla H, Peñas-Lledó E, Vaquero-Lorenzo C, Dorado P, Saiz-Ruiz J, Llerena A, et al. CYP2D6 Polymorphism and Mental and Personality Disorders in Suicide Attempters. Journal of personality disorders. 2013 Feb 11;

146. Roden DM, Altman RB, Benowitz NL, Flockhart DA, Giacomini KM, Johnson JA, et al. Pharmacogenomics: challenges and opportunities. Annals of internal medicine. 2006 Nov 21;145(10):749–57.

147. Shin J, Johnson JA. Pharmacogenetics of beta-blockers. Pharmacotherapy. 2007 Jun;27(6):874–87.

148. Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. The Journal of biological chemistry. 1999 Apr 30;274(18):12670–4.

149. Rathz DA, Brown KM, Kramer LA, Liggett SB. Amino acid 49 polymorphisms of the human beta1-adrenergic receptor affect agonist-promoted trafficking. Journal of cardiovascular pharmacology. 2002 Feb;39(2):155–60.

150. Johnson JA, Zineh I, Puckett BJ, McGorray SP, Yarandi HN, Pauly DF. Beta 1-adrenergic receptor polymorphisms and antihypertensive response to metoprolol. Clinical pharmacology and therapeutics. 2003 Jul;74(1):44–52.

151. Liu J. Gly389Arg polymorphism of β1-adrenergic receptor is associated with the cardiovascular response to metoprolol. Clinical Pharmacology & Therapeutics. 2003 Oct;74(4):372–9.

152. Liu J, Liu Z-Q, Yu B-N, Xu F-H, Mo W, Zhou G, et al. beta1-Adrenergic receptor polymorphisms influence the response to metoprolol monotherapy in

patients with essential hypertension. Clinical pharmacology and therapeutics. 2006 Jul;80(1):23–32.

153. Terra SG, Pauly DF, Lee CR, Patterson JH, Adams KF, Schofield RS, et al. beta-Adrenergic receptor polymorphisms and responses during titration of metoprolol controlled release/extended release in heart failure. Clinical pharmacology and therapeutics. 2005 Mar;77(3):127–37.

154. Beitelshees AL, Zineh I, Yarandi HN, Pauly DF, Johnson JA. Influence of phenotype and pharmacokinetics on beta-blocker drug target pharmacogenetics. The pharmacogenomics journal. 6(3):174–8.

155. Lennard MS, Silas JH, Freestone S, Trevethick J. Defective metabolism of metoprolol in poor hydroxylators of debrisoquine. British journal of clinical pharmacology. 1982 Aug;14(2):301–3.

156. Lennard MS, Silas JH, Freestone S, Ramsay LE, Tucker GT, Woods HF. Oxidation phenotype--a major determinant of metoprolol metabolism and response. The New England journal of medicine. 1982 Dec 16;307(25):1558–60.

157. Silas JH, McGourty JC, Lennard MS, Tucker GT, Woods HF. Polymorphic metabolism of metoprolol: clinical studies. European journal of clinical pharmacology. 1985 Jan;28 Suppl:85–8.

158. Freestone S, Silas JH, Lennard MS, Ramsay LE. Comparison of two long-acting preparations of metoprolol with conventional metoprolol and atenolol in healthy men during chronic dosing. British journal of clinical pharmacology. 1982 Nov;14(5):713–8.

159. Rau T, Heide R, Bergmann K, Wuttke H, Werner U, Feifel N, et al. Effect of the CYP2D6 genotype on metoprolol metabolism persists during long-term treatment. Pharmacogenetics. 2002 Aug;12(6):465–72.

160. Ismail R, Teh LK. The relevance of CYP2D6 genetic polymorphism on chronic metoprolol therapy in cardiovascular patients. Journal of clinical pharmacy and therapeutics. 2006 Feb;31(1):99–109.

161. Nozawa T, Taguchi M, Tahara K, Hashimoto Y, Igarashi N, Nonomura M, et al. Influence of CYP2D6 genotype on metoprolol plasma concentration and beta-adrenergic inhibition during long-term treatment: a comparison with bisoprolol. Journal of cardiovascular pharmacology. 2005 Nov;46(5):713–20.

162. Jin SK, Chung HJ, Chung MW, Kim J-I, Kang J-H, Woo SW, et al. Influence of CYP2D6*10 on the pharmacokinetics of metoprolol in healthy Korean volunteers. Journal of clinical pharmacy and therapeutics. 2008 Oct;33(5):567–73.

163. Bertilsson L, Dahl ML, Sjöqvist F, Aberg-Wistedt A, Humble M, Johansson I, et al. Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine. Lancet. 1993 Jan 2;341(8836):63.

164. Kirchheiner J, Heesch C, Bauer S, Meisel C, Seringer A, Goldammer M, et al. Impact of the ultrarapid metabolizer genotype of cytochrome P450 2D6 on metoprolol pharmacokinetics and pharmacodynamics. Clinical pharmacology and therapeutics. 2004 Oct;76(4):302–12.

165. Sharp CF, Gardiner SJ, Jensen BP, Roberts RL, Troughton RW, Lainchbury JG, et al. CYP2D6 genotype and its relationship with metoprolol dose, concentrations and effect in patients with systolic heart failure. The pharmacogenomics journal. 2009 Jun;9(3):175–84.

166. Baudhuin LM, Miller WL, Train L, Bryant S, Hartman KA, Phelps M, et al. Relation of ADRB1, CYP2D6, and UGT1A1 polymorphisms with dose of, and response to, carvedilol or metoprolol therapy in patients with chronic heart failure. The American journal of cardiology. 2010 Aug 1;106(3):402–8.

167. Bijl MJ, Visser LE, Van Schaik RHN, Kors JA, Witteman JCM, Hofman A, et al. Genetic variation in the CYP2D6 gene is associated with a lower heart rate and blood pressure in beta-blocker users. Clinical pharmacology and therapeutics. 2009 Jan;85(1):45–50.

168. Rau T, Wuttke H, Michels LM, Werner U, Bergmann K, Kreft M, et al. Impact of the CYP2D6 genotype on the clinical effects of metoprolol: a prospective longitudinal study. Clinical pharmacology and therapeutics. 2009 Mar;85(3):269–72.

169. Kirch W, Rämsch K, Janisch HD, Ohnhaus EE. The influence of two histamine H2-receptor antagonists, cimetidine and ranitidine, on the plasma levels and clinical effect of nifedipine and metoprolol. Archives of toxicology. Supplement. = Archiv für Toxikologie. Supplement. 1984 Jan;7:256–9.

170. Keech AC, Harper RW, Harrison PM, Pitt A, McLean AJ. Pharmacokinetic interaction between oral metoprolol and verapamil for angina pectoris. The American journal of cardiology. 1986 Sep 1;58(6):551–2.

171. Wagner F, Kalusche D, Trenk D, Jähnchen E, Roskamm H. Drug interaction between propafenone and metoprolol. British journal of clinical pharmacology. 1987 Aug;24(2):213–20.

172. Hamelin BA, Bouayad A, Méthot J, Jobin J, Desgagnés P, Poirier P, et al. Significant interaction between the nonprescription antihistamine diphenhydramine and the CYP2D6 substrate metoprolol in healthy men with high or low CYP2D6 activity. Clinical pharmacology and therapeutics. 2000 May;67(5):466–77.

173. Werner U, Werner D, Rau T, Fromm MF, Hinz B, Brune K. Celecoxib inhibits metabolism of cytochrome P450 2D6 substrate metoprolol in humans. Clinical pharmacology and therapeutics. 2003 Aug;74(2):130–7.

174. Walley T, Pirmohamed M, Proudlove C, Maxwell D. Interaction of metoprolol and fluoxetine. Lancet. 1993 Apr 10;341(8850):967–8.

175. Chittaranjan A, Chethan KB, Sandarsh S. Cardiovascular mechanisms of SSRI drugs and their benefits and risks in ischemic heart disease and heart failure. International clinical psychopharmacology. 2013 May;28(3):145–55.

176. Molden E, Garcia BH, Braathen P, Eggen AE. Co-prescription of cytochrome P450 2D6/3A4 inhibitor-substrate pairs in clinical practice. A retrospective analysis of data from Norwegian primary pharmacies. European journal of clinical pharmacology. 2005 Apr;61(2):119–25.

177. Hemeryck A, De Vriendt CA, Belpaire FM. Metoprolol-paroxetine interaction in human liver microsomes: stereoselective aspects and prediction of the in vivo interaction. Drug metabolism and disposition: the biological fate of chemicals. 2001 May;29(5):656–63.

178. Belpaire FM, Wijnant P, Temmerman A, Rasmussen BB, Brøsen K. The oxidative metabolism of metoprolol in human liver microsomes: inhibition by the selective serotonin reuptake inhibitors. European journal of clinical pharmacology. 1998 May;54(3):261–4.

179. Hemeryck A, Lefebvre RA, De Vriendt C, Belpaire FM. Paroxetine affects metoprolol pharmacokinetics and pharmacodynamics in healthy volunteers. Clinical pharmacology and therapeutics. 2000 Mar;67(3):283–91.

180. Stout SM, Nielsen J, Welage LS, Shea M, Brook R, Kerber K, et al. Influence of metoprolol dosage release formulation on the pharmacokinetic drug interaction with paroxetine. Journal of clinical pharmacology. 2011 Mar;51(3):389–96.

181. Parker RB, Soberman JE. Effects of paroxetine on the pharmacokinetics and pharmacodynamics of immediate-release and extended-release metoprolol. Pharmacotherapy. 2011 Jul;31(7):630–41.

182. Onalan O, Cumurcu BE, Bekar L. Complete atrioventricular block associated with concomitant use of metoprolol and paroxetine. Mayo Clinic proceedings. Mayo Clinic. 2008 May;83(5):595–9.

183. Yip VLM, Pirmohamed M. Expanding Role of Pharmacogenomics in the Management of Cardiovascular Disorders. American journal of cardiovascular drugs : drugs, devices, and other interventions. 2013 Apr 12;

184. WHO | Rational use of medicines. World Health Organization; [cited 2013 Apr 20]; Available from: http://www.who.int/medicines/areas/rational_use/en/

185. Shahin MHA, Johnson JA. Clopidogrel and warfarin pharmacogenetic tests: what is the evidence for use in clinical practice? Current opinion in cardiology. 2013 May;28(3):305–14.

186. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. Circulation. 2007 Nov 27;116(22):2563–70.

187. Goodman T, Ferro A, Sharma P. Pharmacogenetics of aspirin resistance: a comprehensive systematic review. British journal of clinical pharmacology. 2008 Aug;66(2):222–32.

188. Juel J, Pareek M, Jensen SE. The Clopidogrel-PPI Interaction: An Updated Mini-Review. Current vascular pharmacology. 2012 Oct 17;

189. Niemi M. Transporter pharmacogenetics and statin toxicity. Clinical pharmacology and therapeutics. 2010 Jan;87(1):130–3.

190. Lee S-J. Clinical Application of CYP2C19 Pharmacogenetics Toward More Personalized Medicine. Frontiers in genetics. 2012 Jan;3:318.

191. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta psychiatrica Scandinavica. 1983 Jun;67(6):361–70.

192. HAMILTON M. A rating scale for depression. Journal of neurology, neurosurgery, and psychiatry. 1960 Feb;23:56–62.

193. Russian cardiology society [Internet]. Available from: http://www.scardio.ru/

194. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genetic analysis : biomolecular engineering. 1999 Feb;14(5-6):143–9.

195. Garcia-Barceló M, Chow LY, Chiu HF, Wing YK, Lee DT, Lam KL, et al. Genetic analysis of the CYP2D6 locus in a Hong Kong Chinese population. Clinical chemistry. 2000 Jan;46(1):18–23.

196. Lundqvist E, Johansson I, Ingelman-Sundberg M. Genetic mechanisms for duplication and multiduplication of the human CYP2D6 gene and methods for detection of duplicated CYP2D6 genes. Gene. 1999 Jan 21;226(2):327–38.

197. Heim MH, Meyer UA. Evolution of a highly polymorphic human cytochrome P450 gene cluster: CYP2D6. Genomics. 1992 Sep;14(1):49–58.

198. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment. Oslo; 2007.

199. Herlitz J, Karlson BW, Hjalmarson A. Ten-year mortality rate after development of acute myocardial infarction in relation to clinical history and observations during hospital stay: experience from the Göteborg metoprolol trial. Coronary artery disease. 1993 Dec;4(12):1077–83.

200. Bertilsson L. Metabolism of antidepressant and neuroleptic drugs by cytochrome p450s: clinical and interethnic aspects. Clinical pharmacology and therapeutics. 2007 Nov;82(5):606–9.

201. Pratt CM, Yepsen SC, Bloom MG, Taylor AA, Young JB, Quinones MA. Evaluation of metoprolol in suppressing complex ventricular arrhythmias. The American journal of cardiology. 1983 Jul;52(1):73–8.

202. Zipes DP, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, et al. [Guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Executive summary]. Revista española de cardiología. 2006 Dec;59(12):1328.

203. Frossard M, Fuchs I, Leitner JM, Hsieh K, Vlcek M, Losert H, et al. Platelet function predicts myocardial damage in patients with acute myocardial infarction. Circulation. 2004 Sep 14;110(11):1392–7.

204. Laghrissi-Thode F, Wagner WR, Pollock BG, Johnson PC, Finkel MS. Elevated platelet factor 4 and beta-thromboglobulin plasma levels in depressed patients with ischemic heart disease. Biological psychiatry. 1997 Aug 15;42(4):290–5.

205. Sörberg M, Farra A, Ransjö U, Gårdlund B, Rylander M, Wallén L, et al. Long-term antibiotic resistance surveillance of gram-negative pathogens suggests that temporal trends can be used as a resistance warning system. Scandinavian journal of infectious diseases. 2002 Jan;34(5):372–8.

206. Goossens H. Antibiotic consumption and link to resistance. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2009 Apr;15 Suppl 3:12–5.

207. Malhotra-Kumar S, Lammens C, Coenen S, Van Herck K, Goossens H. Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. Lancet. 2007 Feb 10;369(9560):482–90.

208. McGowan JE. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. The American journal of medicine. 2006 Jun;119(6 Suppl 1):S29–36; discussion S62–70.

209. Weinstein R a. Controlling antimicrobial resistance in hospitals: infection control and use of antibiotics. Emerging infectious diseases. 2001;7(2):188–92.

210. World Health Organization. Strategy for Containment of Antimicrobial Resistance. 2001.

211. Gustafsson LL, Wettermark B, Godman B, Andersén-Karlsson E, Bergman U, Hasselström J, et al. The “wise list”- a comprehensive concept to select, communicate and achieve adherence to recommendations of essential drugs in ambulatory care in Stockholm. Basic & clinical pharmacology & toxicology. 2011 Apr;108(4):224–33.

212. Vaccheri A, Bjerrum L, Resi D, Bergman U, Nicola M. Antibiotic prescribing in general practice: striking differences between Italy (Ravenna) and Denmark (Funen). Journal of Antimicrobial Chemotherapy. 2002 Nov 1;50(6):989–97.

213. team EC for DP and C (ECDC)-HCU-E editorial. European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic use in Europe, 1998-2005. European Centre for Disease Prevention and Control (ECDC) - Health Comunication Unit; 2007.

214. Adriaenssens N, Coenen S, Versporten A, Muller A, Minalu G, Faes C, et al. European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic use in Europe (1997-2009). The Journal of antimicrobial chemotherapy. 2011 Dec;66 Suppl 6:vi3–12.

215. Gulbinovic J, Myrbäck KE, Bytautienĕ J, Wettermark B, Struwe J, Bergman U. Marked differences in antibiotic use and resistance between university hospitals in Vilnius, Lithuania, and Huddinge, Sweden. Microbial drug resistance (Larchmont, N.Y.). 2001 Jan;7(4):383–9.

216. Vander Stichele RH, Elseviers MM, Ferech M, Blot S, Goossens H. Hospital consumption of antibiotics in 15 European countries: results of the ESAC Retrospective Data Collection (1997-2002). The Journal of antimicrobial chemotherapy [Internet]. 2006 Jul [cited 2012 Aug 3];58(1):159–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16698845

217. team EC for DP and C (ECDC)-HCU-E editorial. Pathways to clean hands: highlights of successful hand hygiene implementation strategies in Europe [Internet]. European Centre for Disease Prevention and Control (ECDC) - Health Comunication Unit; 2010 [cited 2013 Apr 8]. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19560

218. Yngström D, Lindström K, Nyström K, Nilsson-Marttala K, Hillblom L, Hansson L, et al. Healthcare-associated infections must stop: a breakthrough project aimed at reducing healthcare-associated infections in an intensive-care unit. BMJ quality & safety. 2011 Jul;20(7):631–6.

219. EARS-Net database.

220. Schwaber MJ, De-Medina T, Carmeli Y. Epidemiological interpretation of antibiotic resistance studies - what are we missing? Nature reviews. Microbiology. 2004 Dec;2(12):979–83.

221. Goryachkina K, Babak S, Burbello A, Wettemark B, Bergman U. Quality use of medicines : A new method of combining antibiotic consumption and sensitivity data — application in a Russian hospital y. Pharmacoepidemiology and drug safety. 2008;17(January):636–44.

222. Sjöstedt S, Levin P, Kager L, Malmborg AS, Bergman U. Hospital and catchment area antibiotic utilization and bacterial sensitivity in primary infections following gastric surgery in Huddinge, Sweden. European journal of clinical pharmacology. 1990 Jan;39(3):211–6.

223. Laxminarayan R, Klugman KP. Communicating trends in resistance using a drug resistance index. BMJ open. 2011 Jan;1(2):e000135.

224. Sörberg M, Farra A, Ransjö U, Gårdlund B, Rylander M, Wallén L, et al. Long-term antibiotic resistance surveillance of gram-negative pathogens suggests that temporal trends can be used as a resistance warning system. Scandinavian journal of infectious diseases. 2002 Jan;34(5):372–8.

225. Mimica Matanovic S, Bergman U, Vukovic D, Wettermark B, Vlahovic-Palcevski V. Impact of restricted amoxicillin/clavulanic acid use on Escherichia coli resistance--antibiotic DU90% profiles with bacterial resistance rates: a visual presentation. International journal of antimicrobial agents. Elsevier B.V.; 2010 Oct;36(4):369–73.

226. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet. 2005;365(9459):579–87.

227. STRAMA. ESBL resistance in enteric bacteria. 2007. p. 24.

228. Bergman U, Risinggård H, Vlahović-Palcevski V, Ericsson O. Use of antibiotics at hospitals in Stockholm: a benchmarking project using internet. Pharmacoepidemiology and drug safety. 2004 Jul;13(7):465–71.

# I

Eur J Clin Pharmacol (2008) 64:275–282
DOI 10.1007/s00228-007-0404-3

PHARMACOKINETICS AND DISPOSITION

# Inhibition of metoprolol metabolism and potentiation of its effects by paroxetine in routinely treated patients with acute myocardial infarction (AMI)

**Ksenia Goryachkina · Aleksandra Burbello · Svetlana Boldueva · Svetlana Babak · Ulf Bergman · Leif Bertilsson**

Received: 18 July 2007 / Accepted: 18 October 2007 / Published online: 29 November 2007


**Abstract**
*Objective* To investigate the influence of paroxetine on metoprolol concentrations and its effect in patients treated for acute myocardial infarction (AMI) who are routinely given paroxetine as a co-treatment of depression.
*Methods* We recruited 17 depressed AMI patients who received metoprolol as a routine part of their therapy (mean dose 75±39 mg/day). Patients were genotyped for CYP2D6 *3, *4 and gene duplication. Metoprolol and α-hydroxy-metoprolol were analyzed in plasma 0, 2, 6 and 12 h post-dose. Heart rates (HR) at rest were registered after each sampling. Paroxetine 20 mg daily was then administered, and all measurements were repeated on day 8.
*Results* All patients were genotypically extensive metabolizers (EMs) (nine with *1/*1 and eight with *1/*3 or *4). Following the administration of paroxetine, mean metoprolol areas under the concentration–time curve (AUC) increased (1064±1213 to 4476±2821 n*M* × h/mg per kg, $P=0.0001$), while metabolite AUCs decreased (1492±872 to 348±279 n *M* × h/mg per kg, $P<0.0001$), with an increase of metabolic ratios (MR) (0.9±1.3 to 26±29; $P<0.0001$). Mean HRs were significantly lower after the study week at each time point. Mean area under the HR versus time curve (AUEC) decreased (835±88 to 728±84 beats × h/min; $P=0.0007$). Metoprolol AUCs correlated with patients' AUECs at the baseline (Spearman $r=-0.64$, $P<0.01$), but not on the eighth day of the study. A reduction of metoprolol dose was required in two patients due to excessive bradycardia and severe orthostatic hypotension. No other adverse effects of the drugs were identified.
*Conclusion* A pronounced inhibition of metoprolol metabolism by paroxetine was observed in AMI patients, but without serious adverse effects. We suggest, however, that the metoprolol dose is controlled upon initiation and withdrawal of paroxetine.

**Keywords** CYP2D6 · Drug interaction · Metoprolol · Myocardial infarction · Paroxetine

K. Goryachkina (✉) · A. Burbello · S. Babak
Course of Clinical Pharmacology, Department of Hospital Therapy,
St. Petersburg I.I. Mechnikov State Medical Academy,
Piskarevsky Prospect, 47,
195 067 St Petersburg, Russia
e-mail: ksenia_goryachkina@yahoo.com

S. Boldueva
Department of Faculty Therapy, Clinic of Cardiology,
St. Petersburg I.I. Mechnikov State Medical Academy,
Piskarevsky Prospect, 47,
195 067 St Petersburg, Russia

K. Goryachkina · U. Bergman · L. Bertilsson
Department of Laboratory Medicine,
Division Clinical Pharmacology, Karolinska Institutet,
Karolinska University Hospital,
141 86 Huddinge–Stockholm, Sweden

## Introduction

The estimated frequency of depression in patients after acute myocardial infarction (AMI) varies from 1.5 to 50% [1–3], and it is a known risk factor for worsened prognosis, often being associated with increased mortality [1]. Among the antidepressants currently available, SSRIs (selective serotonin re-uptake inhibitors) are considered to be safer than others for patients with ischemic heart disease (IHD) [4]. The SSRIs, however, represent a group of drugs with variable characteristics. As such, existing evidence should be carefully evaluated when choosing a member of this group for the safe and effective treatment of depression in

patients after AMI. Paroxetine is the most potent inhibitor of serotonin reuptake, with sertraline and fluoxetine being slightly less active [5]. This difference has been shown to have impact on the degree of protective effects of SSRIs on AMI occurrence, presumably due to a varying degree of platelet inactivation [6]. Paroxetine and sertraline have been best studied in IHD patients [4]. Paroxetine has shown to be absent of any cardiac adverse effects in comparison with a tricyclic antidepressant [7]; it has also been shown to have decreased platelet activity [8] and increased heart rate variability [9]. Both effects are highly beneficial for AMI patients. Sertraline has been the only SSRI studied directly in patients with depression co-morbid with AMI in a randomized placebo-controlled manner [10]. Paroxetine currently remains among the most widely prescribed drugs in patient populations [11]. The only negative feature that might lead to the need of precautions in its use in IHD patients is the ability of paroxetine to inhibit the metabolism of some essential cardiovascular drugs. Both paroxetine and sertraline are known as inhibitors of the cytochrome P450 2D6 (CYP2D6) enzyme, with paroxetine being the strongest of the two [12].

Metoprolol is a selective β1-adrenergic blocker without internal sympathomimetic activity. Randomized controlled trials have revealed that it has benefits in terms of post-myocardial infarction (MI) death prevention in a long-term follow-up and also in the reperfusion era [13–17]. The clinical effects of metoprolol have been shown to be related to the highly polymorphic CYP2D6 activity [18–22]. The clinical consequences of CYP2D6 activity with regard to metoprolol treatment have been addressed in several studies with no clear evidence of adverse effects in patients lacking CYP2D6 function [23–25]. A study performed in healthy volunteers showed that paroxetine was able to inhibit the metabolism and action of metoprolol given as a single dose [26]. Maintenance doses could add to the effects of the interaction due to active enantiomer accumulation [27].

The study reported here was performed in patients with AMI receiving both metoprolol in routinely adjusted stable doses and paroxetine routinely prescribed for depressed patients to assess the interaction between these two drugs in an actual clinical situation.

## Materials and methods

### Subjects

A total of 115 patients with confirmed AMI admitted during the study period to the clinics of cardiology in teaching hospitals of the St. Petersburg I.I. Mechnikov State Medical Academy (Russia) in whom metoprolol treatment was initiated for clinical reasons were screened for mood disorders on the seventh day of hospitalization. Of these, 26 (23%) patients presented with symptoms of mild to moderate depression, 17 of whom received paroxetine as routine antidepressant treatment. These 17 patients were naturally recruited for our study. During the study period, these patients received the following drugs without any change in dosage: aspirin (13 patients), enalapril (five), spironolactone (one), perindopril (eight), quinapril (one), mononitrate (five), trimetazidine (one), simvastatin (one), indapamide (one), clopidogrel (five), iron preparations (two), omeprazole (two), nifedipine (slow release) (three), warfarin (one), amlodipine (one), hydrochlorothiazide (one), ketorolac (one), molsidomine (one) and rosuvastatin (one). A group of non-depressed patients ($n=17$) served as the controls; these patients received a stable dose of metoprolol during a 1-week period to assess possible changes in heart rates in a natural course of the disease. All participants gave informed consent. Patients not receiving metoprolol or receiving other drugs with antiarrhythmic activity concomitantly with metoprolol were excluded from the study. Other exclusion criteria were severe thyroid dysfunction, severe diabetes mellitus, liver or kidney insufficiency and intake of other CYP2D6 substrates.

### Diagnostic criteria

The patients were considered to have AMI if at least two of the three following criteria were present: clinical symptoms, such as chest (or atypical equivalent localization) pain or an episode of severe dyspnea persistent for an extended period of longer than 30 min, specific electrocardiogram (ECG) changes in three or more neighboring leads and diagnostic elevation in myocardial damage markers (creatine-phosphokinase MB-fraction, troponine-T).

The patient was diagnosed with depression when, based on a self-test scored using the HADS (Hospital Anxiety and Depression Scale) [28], the score for depression scale exceeded 10. These results were subsequently confirmed using the HamDRS (Hamilton Depression Rating Scale) [29], which was completed by an investigator during a personal interview.

### Study design

After initial clinical adjustment of the metoprolol dose, patients received exactly the same dose during the whole study period. Blood samples for genotyping and for metoprolol plasma concentration analysis covering one dosage interval (0, 2, 6 and 12 h post-dose) were taken on days 7–10. Blood pressure (BP) and heart rate (HR) were measured by the same person (KG) in a standardized way –

with the patient in lying position 15 min after each blood sampling.

The initial treatment consisted of administering the patients paroxetine 20 mg once in the morning the day after the initial sampling had taken place. At the end of this observational period, repeated samplings for metoprolol concentrations with an additional 5-ml sample taken for determining the paroxetine concentrations were performed. The HR and BP were registered as described above.

We did not perform any genetic or kinetic analysis in the control patients.

General clinical data for all patients were retrieved from the case histories and included all laboratory testing (blood counts and chemistry, urinalysis), concomitant diseases, echocardiography, ECG and ECG-Holter monitoring. Adverse effects of metoprolol therapy (intolerable bradycardia, heart block, signs of loss of selectivity) were registered based on the reports from patients and treating physicians.

## Drugs

Patients received metoprolol tartrate or succinate (Egilok, Egilok-retard "Egis", Hungary, betaloc-zok, "AstraZeneca", Sweden). The dosage and brand of metoprolol was the same within patients during the study. Paroxetine (Rexetin) was donated to the hospital by Gedeon Richter, Hungary.

## Genotyping

A 3-ml aliquot of whole blood was collected into EDTA tubes and kept at −20°C until genotyping. DNA was isolated from the thawed whole blood using the QIAamp DNA mini kit (QIAGEN, Valencia, CA) according to the procedure described by the manufacturer. The patients were genotyped for CYP2D6 *3, *4 and gene duplication. *3 and *4 genotyping was performed with the allele-specific 5 nuclease assay using pre-developed reagents of TaqMan (Applied Biosystems, Foster City, CA,) on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, UK) [30]. Gene duplication was detected by means of a long PCR analysis, as described by Lundqvist et al. [31].

## Metoprolol and α-hydroxy-metoprolol plasma analysis

A 5-ml aliquot of blood was collected in heparinized tubes. Samples were centrifuged and the plasma was separated and stored at −20 until analysis. Metoprolol and its metabolite were separated by isocratic reverse phase high-performance liquid chromatography (HPLC). An analytic column eclipse XDB-phenyl system (column 15×4 mm ; particle diameter 5 μm; with guard column; Zorbax, Agilent technologies, USA) was used. The mobile phase consisted of 50 m*M* potassium phosphate buffer (pH 3.0): acetonitrile:tetrahydrofurane [85:13:2 (vol:vol:vol)]. The fluorescent detector was set at an excitation of 216 nm and emission of 312 nm. Retention times were 2.7, 8.4 and 10.1 min for α-hydroxy-metoprolol, metoprolol and dextrorphan (internal standard), respectively, at a flow rate of 1 ml/min. Briefly, the extraction procedure was as follows: 500 μl of plasma with 20 μl of internal standard (5 μ*M* dextrorphan water–methanol solution) was alkalinized with 200 μl of 0.1 *M* sodium hydroxide. Substances were further extracted with 3 ml dichloromethane:1-butanol [85:15 (vol:vol)]. After the extraction and subsequent evaporation of the organic phase under nitrogen flow, the samples were reconstituted in 50 μl of mobile phase. A 20-μl aliquot was injected into the chromatographic system. Calibration curves were constructed over the range from 12.5 to 400 n*M* and were linear in that range. Lower quantification limits were 6 n*M* for metoprolol and 3 n*M* for α-hydroxy metoprolol. Intra-day and inter-day variations were less than 10 and 15%, respectively.

## Paroxetine plasma analysis

A 0.5 mL aliquot of plasma together with 50 μl of internal standard (doxepin 200 μg/mL) were alkalinized with 0.5 ml 0.5 *M* sodium hydroxide and extracted into 1.5 ml 3% isoamyl alcohol-heptane. After centrifugation and freezing the organic phase was separated and substances back extracted into 75 μl of 25 m*M* acetic acid. Following centrifugation, the organic phase was discarded, and 30 μl of the solution was injected into the chromatographic system. A Waters XTerra RP 18 3.3 μm (100×3 mm) column was used. Paroxetine was detected at 293 nm using a UV detector, with gradient elution in 20–80% acetonitrile, with the addition of 20 m*M* ammonia and 6 m*M* acetic acid. Time of analysis was 18 min with a flow rate 0.6 ml/min. The range of quantification was 20–200 n*M*.

## Data analysis

Individual concentrations versus time area curves were calculated. Metabolic ratio [MR = metoprolol area under the curve (AUC)/α-hydroxy metoprolol AUC] was calculated and used for analysis.

In cases where metabolite values were below the limit of quantification, the minimal detectable value was put into the equation and the MR calculated as being above a certain value.

## Pharmacodynamics

Metoprolol effects were assessed in all of the recruited patients, including the controls. Resting HR was used as a measure of metoprolol effect. The blood pressures were

also registered, but these served only as a measure of adverse effects (excessive hypotension). Data were handled as means and areas under the effect curve (AUEC), where individual HRs were plotted against time.

Statistical analyses were performed using GRAPHPAD PRISM software. Descriptive statistics were carried out to present data as means ± SD. Areas under the curves were calculated according to the standard trapezoidal rule. The paired *t* test for log-values was used to compare the means, where a *P* value below 0.05 was considered to be statistically significant.

### Ethical issues

The study was approved by the local ethics committees of St. Petersburg I.I.Mechnikov State Medical Academy (protocol No.10; 23 September, 2004) and the Karolinska Institutet, Sweden (No. 2004-580/3).

## Results

The patient cohort consisted of 17 patients with a mean age of 66±10 years (range 47–80 years).Seven of the patients were males, eight had anterior myocardial damage and seven had a history of IHD. The left ventricular ejection fraction (LVEF) of these patients ranged from 40 to 68% (mean 55.6±8%). All patients were compliant and completed the observation study. Metoprolol doses ranged from 25 to 125 mg/day (0.3–1.8 mg/kg), with a mean dose of 75± 39 mg/day (0.8±0.4 mg/kg). Nine patients were identified as carriers of CYP2D6*1/*1, three patients as carriers of CYP2D6*1/*3 and five patients as carriers of CYP2D6*1/*4. No gene duplication was found. Metoprolol doses tended to be slightly lower in carriers of a mutated allele, but the difference was not statistically significant.

The age of the 17 control subjects ranged from 44 to 73 years (mean 58±8; years); 12 were males, eight had anterior myocardial damage and four had a previous history of IHD. The LVEF of the control subjects ranged from 47 to 71% (mean 59.9±2%). Metoprolol doses ranged from 50 to 150 mg/day (0.5–2 mg/kg), with a mean dose of 90± 35 mg/day (1.2±0.5 mg/kg).

### Pharmacokinetics

Baseline metoprolol trough concentrations ranged from below the limit of quantification (6 n*M*) to 130 n*M* (163 n*M*/mg per kilogram). The highest detected concentration during the study was 600 n*M* (545 n*M*/mg per kilogram). Baseline metoprolol/α-hydroxy metoprolol ratios (MR) ranged between 0.05 and 4.6.

Paroxetine trough plasma concentrations on day 8 ranged from 20 to 82 n*M* (80–287 n*M*/mg per kilogram).

During paroxetine co-treatment, metoprolol concentration AUC increased significantly ($P<0{,}0001$) and the metabolite levels decreased (Fig. 1; Table 1). Mean MRs increased from 0.92±1.3 to 25.9±28.5 ($P<0{,}0001$) as a reflection of CYP2D6 inhibition.

### Pharmacodynamics

Plasma metoprolol concentration AUCs significantly correlated with patients' HRs (AUEC) at baseline with a Spearman $r=-0.64$, $P<0.01$, whereas no significant correlation was observed on day 8 of the study (Fig. 2). Prior to the paroxetine treatment, the nine patients with the CYP2D6 *1/*1 genotype had a higher metoprolol AUC than the eight heterozygote patients (Fig. 2, left; $P=0.06$), but there was only a tendency for a difference during paroxetine treatment (Fig. 2, right). A significant decrease in HRs was observed when paroxetine was added to the unchanged dose of metoprolol in the study patients, but not in the controls (Fig. 3). Areas under the metoprolol effect curves decreased in the majority of study patients ($P=$ 0,0007) (Table 1).

### Treatment tolerance

Mean systolic blood pressure was 125 (range 98–147) mm Hg at the baseline and 118 (range 105–135) mm Hg after

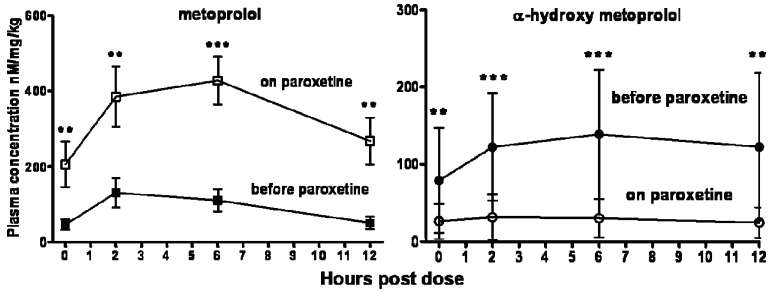



**Fig. 1** Mean ± SD plasma metoprolol (*left*) and α-hydroxymetoprolol (*right*) concentrations (n*M*/mg per kilogram) in patients before and on the eighth day of paroxetine administration in 17 patients. $^{**}P<0.01$; $^{***}P<0.001$

**Table 1** Metoprolol and metabolite pharmacokinetics (n*M* × h/mg per kilogram) and pharmacodynamics (area under heart rate versus time curve – AUEC beats × h per minute) before and after 7 days of paroxetine co-administration

| Parameter | Paroxetine treatment | | *P* value |
|---|---|---|---|
| | No | yes | |
| $AUC_{0-12}$ metoprolol[a] | 1064±1213 | 4476±2821 | < 0.0001 |
| $AUC_{0-12}$ α-hydroxy metoprolol[a] | 1492±872 | 348±272 | < 0.0001 |
| AUC ratio | 0.9±1.3 | 26±29 | < 0.0001 |
| AUEC[b] | 835±88 | 728±84 | 0.0007 |

Values are given as means ± SD; paired *t* test of log values
[a] AUC from 0 to 12 h post-dose; area under the curve
[b] AUEC, Area under the effect curve

1 week of paroxetine treatment ($P$=0.2). Two patients required the initial metoprolol dose to be reduced at the end of the study period (1 week) with paroxetine, one due to severe postural hypotension and another due to bad tolerance of bradycardia (<45 beats per min). Both patients were carriers of one non-functional CYP2D6 allele. Excessive hypotension (<100 mm Hg) was observed in four patients with initially low systolic blood pressure; this could not be exclusively assigned to metoprolol action, but rather to combined medication (β-blockers, mononitrates, ACE-inhibitors). Since this condition was well tolerated, a reduction of medication dosages was not required. No other adverse effects of either of the two drugs could be clearly distinguished.

## Discussion

A study from Norway reported that the CYP2D6 substrates codeine and metoprolol together with a CYP2D6 inhibitor paroxetine was one of the most frequently prescribed combinations according to data from a nation-wide pharmacy database [11]. The authors did not report any clinical consequences of these combination prescriptions. The magnitude at which metoprolol metabolism is inhibited by paroxetine co-administration needs to be assessed with clinically used metoprolol doses to establish whether the currently widely used combination is safe or not. In this study we investigated an interaction of routinely prescribed metoprolol and paroxetine.

We observed resting HR reduction which, together with exertion HR, is a direct clinical reflection of beta1 adrenergic receptor blockade by metoprolol [32, 33]. We avoided physical exertion tests for such tests are not part of the normal clinical routine for patients at this stage after AMI. The changes were not observed in 17 subjects who did not receive paroxetine, which therefore enabled us to consider the changes a result of the interaction.

Metoprolol has a dose-dependent effect [32], and dose adjustment is commonly performed to the highest dose tolerated in order to achieve minimal HRs in the absence of adverse effects. β-blockade reduces mortality after AMI by 23%, and the more pronounced the blockade, the more beneficial is the drug in terms of patients' survival [34]. A retrospective study found a decreased rate of metabolism to be associated with more adverse effects of metoprolol [23]. This led to the question of whether CYP2D6 inhibition would lead to adverse effects when metoprolol is given in an adjusted dose. In our study, a fourfold increase in mean metoprolol concentration AUC and a fourfold decrease in mean α-hydroxy metoprolol concentration AUC were observed, which was comparable with that shown by healthy volunteers [26]. This did not lead to any serious adverse effects. The only truly adverse effect was that of poorly tolerated bradycardia, which was not accompanied by PQ-interval prolongation on ECG, and the dose reduction was based solely on the patient's request. Another case of postural hypotension occurred in a woman with type 2 diabetes mellitus, which may well have been the main predisposing factor for this reaction.

Several interaction studies with metoprolol revealed moderately pronounced changes in metoprolol effects although a decrease in its metabolism was significant. This is explained by the preferential inhibition of the metabolism of the non-active R-enantiomer. The $C_{max}$ of S-enantiomer

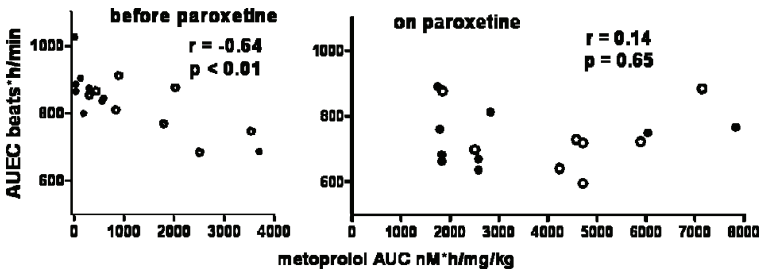



**Fig. 2** Correlation of metoprolol AUC (n*M* × h/mg per kilogram) and AUEC (beats × h/min) before (*left*) and on the eighth day of paroxetine administration (*right*). *Numbers* designate individual patients, *open circles* CYP2D6 *1/*3(*4) genotype, *closed circles* CYP2D6 *1/*1 genotype

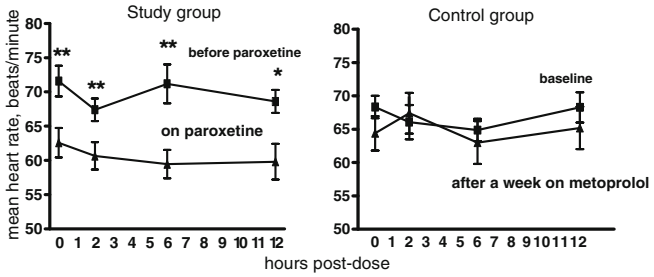



**Fig. 3** Mean heart rates (±SD) before and on the eight day of paroxetine administration in study group (*left*) and control group receiving no paroxetine (*right*). $^{**}P<0.01$; $^{*}P<0.05$

increased by only twofold compared to a threefold increase in that of R-enantiomer in healthy volunteers [26, 35]. Based on these results, one could speculate that adverse effects are more likely to occur when too high doses are prescribed, rather than in cases of decreased CYP2D6 activity. This could explain why prospective studies did not reveal any association between poor metoprolol metabolism and adverse effects [24, 25]. Much clearer was the increased duration of metoprolol action [26], which was evident in our study as well and which would be beneficial in patients taking immediate release formulation of metoprolol.

Adverse effects described in large clinical trials where AMI patients received 200 mg of metoprolol daily were hypotension (18.4 vs. 9.8% in placebo group) and fatigue (20 vs. 11% in placebo group). Atrioventricular (AV)-block I was not much more prevalent (3.3%) in the metoprolol group than in the placebo groups (2.5%), and other cardiac and non-cardiac adverse effects were even less prevalent [36]. Hypotension was present in our study patients; however, its change after paroxetine administration was not significant, and we would rather assign it to concomitant medications (nitrates).

Doses of metoprolol on days 7–10 were all below 150 mg/day in our study. This corresponds to general tendencies in AMI patients [37]. No specific change in metoprolol clearance is expected in the AMI situation that would explain the lower doses unless the drug is introduced in the acute phase of AMI when the hemodynamics is unstable and can change the rate of the drug's hepatic extraction [17]. Only two patients in our study had reduced LVEF (<50%), which could theoretically predispose to lower metoprolol clearance. Lower clearance and varying effects are more definitely associated with CYP2D6 activity in patients chronically taking metoprolol [38]. One-half of the patients in our study were carriers of one deficient CYP2D6 allele, and the other half carried two wild-type alleles. Metoprolol does not inhibit CYP2D6 in clinical doses [39]; consequently, the 100-fold variation in metoprolol concentrations observed by us might partly be assigned to the differences in CYP2D6 activity [33]. The differences in the doses prescribed in the two genotype groups were, however, insignificant. The dependence of the studied inhibition degree on initial enzyme activity has been discussed in the literature [40], and it was observed in our study as a slight tendency (not shown). Further studies with a longer observation period would be useful in determining whether the long-term co-administration of paroxetine with metoprolol has more pronounced effects.

The significant negative correlation of our patients' metoprolol concentrations and HR dynamics at the baseline ($r=-0.64$, $P<0.01$) disappeared with larger metoprolol concentrations after 1 week of paroxetine co-administration ($r=0.14$, ns; Fig. 2). If not an accidental finding, this could reflect a blockade of β1-adrenergic receptors at metoprolol concentration AUCs up to 4000 n$M$ × h/mg per kilogram with concomitant decrease of HRs. The increase in HRs at higher metoprolol concentration AUCs could either be the plateau of the concentration–effect curve or the possible recovery of the sensitivity of β-receptors under a significant β-blockade (Fig. 2 right, patient with metoprolol AUC above 6000). These findings, which may be a link between metoprolol concentrations and its specific effects in AMI patients, need further investigation on a larger cohort.

## Conclusion

We observed an inhibition of metoprolol metabolism by paroxetine co-administration in AMI patients of a similar degree compared to healthy volunteers. The assumed primary increase of inactive R-enantiomer and lower doses prescribed to most AMI patients ensured the relative safety of this inhibition. This may not be true, however, when paroxetine is initiated in ambulatory care in a patient with metoprolol dose, up-titrated to a higher degree. Hypotension could be primarily expected in the case of metabolism inhibition, although an AV-block is also possible in a susceptible population, such as older patients who commonly have more heart troubles, and women, in whom adverse effects develop more readily according to the most recent data [41]. Safety issues should be studied on a larger patient cohort receiving metoprolol and paroxetine as a routine combination.

**Acknowledgments** We thank the rector of St. Petersburg I.I.Mechnikov State Medical Academy, professor, and academician of Russian Academy of Medical Sciences Aleksandr V. Shabrov and the coordinator of the Karolinska Institute Research Training Program (KIRT) associate professor Tommy Linne. The help of biomedical analysts Lilleba Bohman in the genotyping techniques and Jolanta Widen in the HPLC techniques is gratefully acknowledged. The research was supported by the Swedish Institute (via KIRT), the Heart-Lung Foundation and the Swedish Research Council (grant No. 3902). The study was approved by the local ethics committees of both institutions.

## References

1. Sorensen C, Friis-Hasche E, Haghfelt T, Bech P (2005) Postmyocardial infarction mortality in relation to depression: a systematic critical review. Psychother Psychosom 74(2):69–80
2. Thombs BD, Bass EB, Ford DE et al (2006) Prevalence of depression in survivors of acute myocardial infarction. J Gen Intern Med 21(1):30–38
3. Chazov EI, Oganov RG, Pogosova GV et al (2007) Clinico-epidemiological program of the study of depression in cardiological practice in patients with arterial hypertension and ischemic heart disease (COORDINATA). Kardiologiia 47(3):29–37
4. Jiang W, Davidson JR (2005) Antidepressant therapy in patients with ischemic heart disease. Am Heart J 150(5):871–881
5. Tatsumi M, Groshan K, Blakely RD, Richelson E (1997) Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur J Pharmacol 340(2–3):249–258
6. Sauer WH, Berlin JA, Kimmel SE (2003) Effect of antidepressants and their relative affinity for the serotonin transporter on the risk of myocardial infarction. Circulation 108(1):32–36
7. Roose SP, Laghrissi-Thode F, Kennedy JS et al (1998) Comparison of paroxetine and nortriptyline in depressed patients with ischemic heart disease. JAMA 279(4):287–291
8. Pollock BG, Laghrissi-Thode F, Wagner WR (2000) Evaluation of platelet activation in depressed patients with ischemic heart disease after paroxetine or nortriptyline treatment. J Clin Psychopharmacol 20(2):137–140
9. Yeragani VK, Pohl R, Balon R, Ramesh C, Glitz D et al (2002) Major depression with ischemic heart disease: effects of paroxetine and nortriptyline on long-term heart rate variability measures. Biol Psychiatry 52(5):418–429
10. Glassman AH, O'Connor CM, Califf RM et al (2002) Sertraline treatment of major depression in patients with acute MI or unstable angina. JAMA 288(6):701–709
11. Molden E, Garcia BH, Braathen P, Eggen AE (2005) Co-prescription of cytochrome P450 2D6/3A4 inhibitor-substrate pairs in clinical practice. A retrospective analysis of data from Norwegian primary pharmacies. Eur J Clin Pharmacol 61(2):119–125
12. Hemeryck A, Belpaire FM (2002) Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. Curr Drug Metab 3(1):13–37
13. Hjalmarson A, Herlitz J, Holmborg S et al (1983) The Goteborg metoprolol trial. Effects on mortality and morbidity in acute myocardial infarction. Circulation 67(6 Pt 2):I26–I32
14. Chen ZM, Pan HC, Chen YP et al (2005) Early intravenous then oral metoprolol in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. Lancet 366(9497):1622–1632
15. Freemantle N, Cleland J, Young P et al (1999) Beta blockade after myocardial infarction: systematic review and meta regression analysis. Br Med J 318(7200):1730–1737
16. Olsson G, Rehnqvist N, Sjo'gren A, Erhardt L, Lundman T (1985) Long-term treatment with metoprolol after myocardial infarction: effect on 3 year mortality and morbidity. J Am Coll Cardiol 5(6):1428–1437
17. Everts B, Karlson B, Abdon N-J, Herlitz J, Hedner T et al (1997) Effects and pharmacokinetics of high dose metoprolol on chest pain in patients with suspected or definite acute myocardial infarction. Eur J Clin Pharmacol 53(1):23–31
18. McGourty JC, Silas JH, Lennard MS, Tucker GT, Woods HF et al (1985) Metoprolol metabolism and debrisoquine oxidation polymorphism-population and family studies. Br J Clin Pharmacol 20 (6):555–566
19. Ingelman-Sundberg M (2005) Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J 5(1):6–13
20. Gaikovitch EA, Cascorbi I, Mrozikiewicz PM et al (2003) Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. Eur J Clin Pharmacol 59(4):303–312
21. Rau T, Wohlleben G, Wuttke H, Thuerauf N, Lunkenheimer J et al (2002) Effect of the CYP2D6 genotype on metoprolol metabolism persists during long-term treatment. Pharmacogenetics 12(6):465–472
22. Ramenskaya GV et al (2002) Pheno- and genotyping the prescription of drugs metabolized by CYP2D6. Bull Exp Biol Med 134(2):159–160
23. Wuttke H, Rau T, Heide R, Bergmann K, Böhm M et al (2002) Increased frequency of cytochrome P450 2D6 poor metabolizers among patients with metoprolol-associated adverse effects. Clin Pharmacol Ther 72(4):429–437
24. Fux R, Morike K, Prohmer AM et al (2005) Impact of CYP2D6 genotype on adverse effects during treatment with metoprolol: a prospective clinical study. Clin Pharmacol Ther 78(4):378–387
25. Zineh I, Beitelshees AL, Gaedigk A, Walker JR et al (2004) Pharmacokinetics and CYP2D6 genotypes do not predict metoprolol adverse events or efficacy in hypertension. Clin Pharmacol Ther 76(6):536–544
26. Hemeryck A, Lefebvre RA, De Vriendt C, Belpaire FM (2000) Paroxetine affects metoprolol pharmacokinetics and pharmacodynamics in healthy volunteers. Clin Pharmacol Ther 67(3): 283–291
27. Kendall MJ, John VA, Quarterman CR, Welling PG (1980) A single and multiple dose pharmacokinetic and pharmacodynamic comparison of conventional and slow-release metroprolol. Eur J Clin Pharmacol 17(2):87–92
28. Zigmond AS, Snaith RP (1983) The hospital anxiety and depression scale. Acta Psychiatr Scand 67(6):361–370
29. Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–62
30. Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 14(5–6):143–149
31. Lundqvist E, Johansson I, Ingelman-Sundberg M (1999) Genetic mechanisms for duplication and multiduplication of the human CYP2D6 gene and methods for detection of duplicated CYP2D6 genes. Gene 226(2):327–338
32. Regardh CG, Johnsson G (1980) Clinical pharmacokinetics of metoprolol. Clin Pharmacokinet 5(6):557–569
33. Koytchev R, Alken RG, Vlahov V, Kirkov V, Kaneva R et al (1998) Influence of the cytochrome P4502D6*4 allele on the pharmacokinetics of controlled-release metoprolol. Eur J Clin Pharmacol 54(6):469–474
34. Smith SC Jr, Feldman TE, Hirshfeld JW Jr, Jacobs AK et al (2006) AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update: endorsed by the National Heart, Lung, and Blood Institute. Circulation 113(19):2363–2372
35. Toon S, Davidson EM, Garstang FM, Batra H, Bowes RJ, Rowland M (1988) The racemic metoprolol H2-antagonist interaction. Clin Pharmacol Ther 43(3):283–289

36. The MIAMI Trial Research Group (1985) Metoprolo in acute mycardial infarction. Other clinical findings and tolerability. Am J Cardiol 56(14):39G–46G
37. Herlitz J et al (2000) Long-term mortality after acute myocardial infarction in relation to prescribed dosages of a beta-blocker at hospital discharge. Cardiovasc Drugs Ther 14(6):589-595.
38. Mautz DS, Nelson WL, Shen DD (1995) Regioselective and stereoselective oxidation of metoprolol and bufuralol catalyzed by microsomes containing cDNA-expressed human P4502D6. Drug Metab Dispos 23(4):513–517
39. Brynne N, Bottiger Y, Hallen B, Bertilsson L (1999) Tolterodine does not affect the human in vivo metabolism of the probe drugs caffeine, debrisoquine and omeprazole. Br J Clin Pharmacol 47(2):145–150
40. Özdemir V, Naranjo CA, Shulman RW, Herrmann N et al (1998) Determinants of interindividual variability and extent of CYP2D6 and CYP1A2 inhibition by paroxetine and fluvoxamine in vivo. J Clin Psychopharmacol 18(3):198–207
41. Thurmann PA, Haack S, Werner U, Szymanski J et al (2006) Tolerability of beta-blockers metabolized via cytochrome P450 2D6 is sex-dependent. Clin Pharmacol Ther 80(5):551–553

# II

Eur J Clin Pharmacol
DOI 10.1007/s00228-008-0525-3

PHARMACOGENETICS

# *CYP2D6* is a major determinant of metoprolol disposition and effects in hospitalized Russian patients treated for acute myocardial infarction

**Ksenia Goryachkina · Aleksandra Burbello · Svetlana Boldueva · Svetlana Babak · Ulf Bergman · Leif Bertilsson**

Received: 22 February 2008 / Accepted: 13 June 2008


**Abstract**
*Purpose* To investigate individual metabolism-related determinants of metoprolol disposition and effects in patients receiving the drug as standard treatment for acute myocardial infarction (AMI).
*Methods* We recruited 187 AMI patients receiving metoprolol on clinical grounds and genotyped them for *CYP2D6* **3*, **4*, **10*, and gene duplication. Heart rates (HR) at admission and discharge were registered. Clinical details were derived from the case histories. Metoprolol and α-hydroxy-metoprolol were analyzed by HPLC in plasma before and after 2, 6 and 12 h post dose in the first 115 patients. HR at rest was registered after each sampling. Ventricular rhythm disturbance (VRD) association with CYP2D6 activity, found accidentally, was studied in a newly formed subgroup ($n$=23).
*Results* Metoprolol represented 85% of all beta-blocker prescriptions. *CYP2D6* genotype distribution was comparable with other Caucasian populations. Genotypically poor metabolizers (PM, $n$=2) exhibited the most pronounced bradycardia at discharge, while in the ultrarapid metabolizers (UM, $n$=7) therapeutic effect was not achieved. Metoprolol and α-hydroxy-metoprolol plasma concentration AUCs differed significantly between the genotypes corresponding to predicted metabolic activity ($P$<0.005). Correspondingly, the mean HRs were lower in PMs and increased with increasing number of active *CYP2D6* genes ($P$<0.05). Trough metoprolol concentrations were only quantifiable in patients with at least one mutated allele. Neither decreased cardiac ejection fraction nor age and gender influenced metoprolol disposition. Higher mean number of active *CYP2D6* genes was found in patients with VRDs (2.2 vs. 1.7), which could not be clearly explained by metoprolol concentrations. *CYP2D6* gene duplication was overrepresented in this group (22 vs. 2%, $P$=0.0002).
*Conclusion* Metoprolol disposition and effects are mainly controlled by *CYP2D6* genotype. Patients with gene duplication are at high risk of not benefiting from treatment due to lower metoprolol concentrations. Higher CYP2D6 activity seems to be associated with VRDs complicating AMI, being a negative prognostic factor for patients' survival.

**Keywords** *CYP2D6* · Acute myocardial infarction · Metoprolol · In-hospital treatment · Drug disposition · Therapeutic response

K. Goryachkina · A. Burbello · S. Babak
Course of Clinical Pharmacology, Department of Hospital Therapy,
St. Petersburg State Medical Academy named after I.I. Mechnikov,
Piskarevsky Prospect, 47,
195 067 St. Petersburg, Russia

S. Boldueva
Department of Faculty Therapy, Clinic of Cardiology,
St. Petersburg State Medical Academy named after I.I. Mechnikov,
Piskarevsky Prospect, 47,
195 067 St. Petersburg, Russia

K. Goryachkina (✉) · U. Bergman · L. Bertilsson
Department of Laboratory Medicine,
Division of Clinical Pharmacology, Karolinska Institutet,
Karolinska University Hospital, Huddinge,
Huddinge SE-141 86 Stockholm, Sweden
e-mail: ksenia_goryachkina@yahoo.com

## Introduction

Acute myocardial infarction (AMI) is currently the leading cause of death in the world [1]. New treatment strategies emerge continuously to improve patient survival. This laborious process requires time and great expense to introduce new treatments into practice, while older treatments with

proven efficacy and benefits that have been demonstrated in long-term patient follow-up exist that can be further improved. Beta-blockers are recommended with the highest priority by the current guidelines (class IA) at different stages of treatment for AMI patients [2]. The most evidence and long-term follow-up has been obtained with metoprolol.

Current knowledge suggests, however, that individual responses to certain medicines vary widely. With drugs like metoprolol, with definite concentration-effect relationships, different doses may lead to variable individual outcomes as was shown for metoprolol in a 10-year follow-up [3]. Also a genetic relationship with interindividual variability in metoprolol disposition has been well described experimentally [4]. It stems from individual expression of the gene encoding CYP2D6—an enzyme responsible for metoprolol metabolism. In Caucasians, 5–9% do not express the enzyme due to mutated alleles (namely **3*, **4*, **5*); these exhibit the phenotype of poor metabolizers (PM). Up to 5% express the enzyme in high quantities due to the fully active gene multiplication; these are referred to as ultrarapid metabolizers (UM). People with one or two active *CYP2D6* alleles are called extensive metabolizers (EM) [5].

Considering these rather high frequencies and that beta-blockers are consumed chronically, sometimes for life, this variability may be a rather prominent source for variable response to treatment. This assumption has not been tested in clinical practice, therefore there are no suggestions for physicians of how to make use of this knowledge, provide maximum treatment efficacy for each individual, and improve patient survival without employing high-cost technologies. In this study, we evaluate the impact of individual *CYP2D6* genotypes on metoprolol disposition and response to treatment in a noninterventional manner in patients routinely treated early after AMI in cardiology clinics of two university hospitals in St. Petersburg, Russia.

## Materials and methods

*Setting* The study was performed in cardiology clinics of two university hospitals of St. Petersburg State Medical Academy named after I.I. Mechnikov (the Peter the Great hospital and the second city hospital).

General study design

*Epidemiology of beta-blocker prescription* Retrospective analysis of summaries of patient case histories from the computer database of the cardiology clinic of the Peter the Great hospital was performed for the period of September 2004–July 2005. A total of 167 patients were diagnosed with AMI during this period of time. Beta-blocker prescriptions were evaluated.

*Consecutive patient recruitment for genotyping* All the patients with confirmed AMI for whom metoprolol was prescribed for clinical indications were considered eligible for the study. Patients were not included if they had non-compensated diabetes mellitus, thyroid dysfunction, or severe liver or kidney disease. Patients were also not included if they were taking other drugs capable of changing cardiac rhythm (cardiac glycosides or antiarrhythmics other than metoprolol) or that were substrates or inhibitors of CYP2D6. A total of 187 patients gave informed consent and were recruited for the study. All were genotyped for *CYP2D6* alleles (**3*, **4*, **10*, and gene duplication). In all 187 patients, heart rates (HR) were registered according to the data from the first and the last routine ECG record. Adverse effects of metoprolol treatment were registered from patient complaints, routine examination results, and ECG records.

*Selection of a subgroup for metoprolol pharmacokinetics evaluation* The first 115 patients in order of recruitment comprised a subgroup for analysis of metoprolol pharmacokinetics. Metoprolol concentrations were measured in plasma between the 7th and 10th days of treatment, when individual metoprolol dose had been adjusted by the treating physician and was stable for at least 3 days to assure steady-state concentrations. Blood samples (5 ml) were taken before, and 2, 6 and 12 h after metoprolol intake. At the time of sampling, HRs and blood pressure were measured in patients after at least 15 min of rest in supine position by the same person (KG) and in the same place (in the ward).

*Further selection of patients for a drug interaction substudy* The 115 patients that comprised the subgroup for metoprolol pharmacokinetics analysis were also screened for mood disorders, and 17 of those who were depressed were prescribed an antidepressant (paroxetine) and included in the previously published study of a paroxetine-metoprolol interaction [6]. These 17 patients are included here before they were treated with paroxetine.

*Substudy of association of CYP2D6 activity with ventricular rhythm disturbances (VRD)* This substudy was designed after the major data were analyzed. From all the patients recruited for this study, patients with VRDs complicating AMI were identified (12 in total). Additionally we identified patients with VRDs in the diagnosis from retrospectively analyzed case histories (September 2004–July 2005). Out of 167 retrospective case histories, we identified 31 patients in whom VRD was stated in the diagnosis as a complication of AMI and attempted to approach them. Twenty patients of these 31 were unavailable at the phone numbers we had. Eleven patients responded and agreed to participate in the study. The patients were invited to the

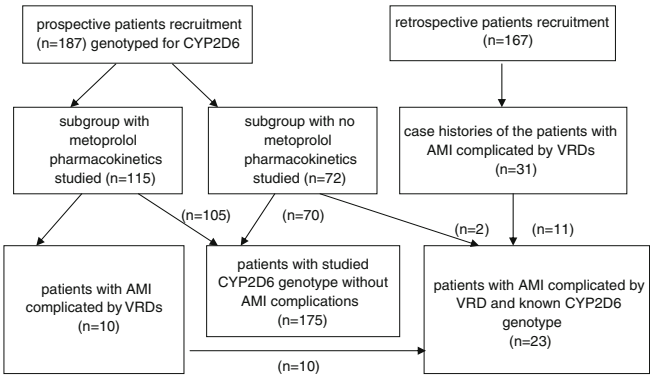



**Fig. 1** Scheme of patient selection

clinic and genotyped for *CYP2D6* mutations **3*, **4*, and gene duplication. These data were merged with the data from another 12 patients with VRDs, who were selected from the initial prospective study. The final subgroup consisted of 23 patients with VRDs (Fig. 1, Table 1, Table 2).

## Diagnostic criteria

Acute myocardial infarction was diagnosed if two of the following three criteria were present: specific pain syndrome, specific ECG changes in two or more neighboring leads, or diagnostic elevation of creatine phosphokinase-MB or troponine T.

The study was not designed to register VRDs. Therefore we only used the information available from the patients' documentation. All the registered VRDs were present either on ECG records or 24-h ECG monitors. They were classified according to Lown classification, utilized in the hospital as class I: rare monomorphic premature ventricular complexes (<30/h), class II: frequent monomorphic premature ventricular complexes (>30/h), class III: polymorphic premature ventricular complexes, class IVa: coupled premature ventricular complexes, class IVb: short-term ventricular tachycardia (3 complexes or more together), and class V: early premature ventricular complexes R/T.

## Genotyping

DNA was isolated from thawed whole blood using the QIAamp DNA mini kit (QIAGEN, USA) according to the procedure described by the manufacturer. The patients were genotyped for *CYP2D6 *3*, **4*, **10* and gene duplication. Genotyping of **3* and **4* was performed with allele-specific 5′ nuclease assay using pre-developed reagents of TaqMan (Applied Biosystems, Foster City, CA, USA) on ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, UK) [7]. **10* was analyzed with allele-specific nested polymerase chain reaction (PCR) [8]. Gene duplication was detected by long PCR as described by Lundqvist et al. [9].

## Metoprolol and α-hydroxy metoprolol plasma analysis

Separation procedure is described in the previously published paper [6]. Calibration curves were constructed over the range from 12.5 to 400 nM and were linear in that

**Table 1** Characteristics of the general study group ($n$=187), subgroup of metoprolol pharmacokinetics analysis ($n$=115), and subgroup of patients with ventricular rhythm disturbances (VRDs) ($n$=23)

| Patient group | All patients | Metoprolol pharmacokinetics study subgroup | Patients with VRDs |
|---|---|---|---|
| Number ($n$) | 187 | 115 | 23 |
| Men/women ($n$) | 114/73 | 99/16 | 18/5 |
| Age, years (mean±SD and range) | 60±11 (36–80) | 60±11 (39–80) | 58±10 (42–78) |
| Weight, kg (mean±SD and range) | 78±13 (48–130) | 79±14 (50–130) | 79±16 (50–120) |
| Anterior AMI (%) | 46 | 47 | 52 |
| Repeated AMI (%) | 28 | 27 | 30 |
| STEMI (%) | 56 | 71 | 83 |

*AMI* Acute myocardial infarction, *STEMI* ST-segment elevation myocardial infarction

**Table 2** Drugs other than β-blockers taken by patients with acute myocardial infarction ($n$=187)

| Drug group | Number of patients | Percentage |
|---|---|---|
| Antiaggregants (aspirin/clopidogrel) | 173 (139/44) | 93 |
| ACE inhibitors (perindopril, enalapril, fosinopril, lisinopril, quinapril) | 162 (78/64/2/8/10) | 87 |
| Statins (simvastatin, atorvastatin, rozuvastatin) | 78 (62/11/5) | 42 |
| Diuretics (indapamide, hydrochlorothiazide, furosemide, spironolactone) | 62 (33/26/3/12) | 34 |
| Mononitrates | 55 | 29 |
| Dihydropiridine calcium-channel blockers (nifedipine, amlodipine, felodipine, nimodipine) | 31 (16/12/2/1) | 17 |
| Anticoagulants (warfarin) | 14 | 8 |
| Metabolic drugs (trimetazidine) | 11 | 6 |
| Iron preparations | 5 | 3 |
| Hypoglycemic drugs (glibenclamide, metformin, repaglinide, gliclazide) | 6 (4/2/1/1) | 3 |
| Proton pump inhibitors (omeprazole) | 6 | 3 |
| Angiotensin receptor antagonists (losartan) | 3 | 2 |
| Molsidomine | 2 | 1 |
| Bronchodilators, mucolytics | 2 | 1 |

range. Lower quantification limits were 6 nM for metoprolol and 3 nM for α-hydroxy metoprolol. Intraday and interday variations were less than 10 and 15%, respectively.

### Data analysis

Individual areas under the concentration versus time curves (AUC) were calculated. Metabolic ratio (MR = metoprolol AUC/α-hydroxy metoprolol AUC) was calculated and used for analysis.

In cases where metoprolol or metabolite concentration values were below the limit of quantification, the minimum quantifiable value minus 1 nM was used for calculation and the MR calculated subsequently as above a certain value.

### Pharmacodynamics

Metoprolol effects were assessed in all the recruited patients. Resting HR was used as a measure. Blood pressures were also registered, but served only as a measure of adverse effects (excessive hypotension). Data were handled as means and areas under the effect curve (AUEC), where individual HRs were plotted against time.

### Statistical analyses

Statistical analyses were performed using GraphPad Prism software. Descriptive statistics were performed to present data as means ± SD. AUCs were calculated according to the standard trapezoidal rule. Paired $t$-test for log-values was used for comparisons of the means. Non-parametric methods were utilized when necessary. $P$ value below 0.05 was considered to be statistically significant. The 95% confidence intervals were calculated (CI).

### Ethical issues

The study was approved by the local ethics committee of St. Petersburg State Medical Academy named after I.I. Mechnikov, Russia (protocol no. 10; 23 September 2004) and Karolinska Institutet, Sweden (no. 2004–580/3).

## Results

### Epidemiology of beta-blocker prescriptions

Retrospective analysis of beta-blocker use in 167 patients treated for AMI in the cardiology clinics from September 2004 through July 2005 showed that beta-blockers were prescribed to 97% of patients ($n$=162). Of those, 127 received metoprolol tartrate immediate release, 14 received metoprolol tartrate or succinate extended release, 9 received carvedilol, 7 bisoprolol, 3 atenolol, and 2 nebivolol. If no beta-blocker was prescribed, patients received other drugs with indirect antianginal properties, amiodarone ($n$=3) or dilthiazem ($n$=2).

### *CYP2D6* genotype and its impact on metoprolol effects in 187 consecutive patients

In six patients no DNA could be obtained due to technical problems; in the remaining 181 prospective patients, genotypes were concordant with Hardy-Weinberg equilibrium (Table 3).

The frequency of *CYP2D6* alleles was similar to other Caucasian populations [10] and another Russian population [11] (Table 4).

**Table 3** Observed frequency of *CYP2D6* genotypes in patients with acute myocardial infarction ($n$=181)

| CYP2D6 genotype | *4/*4 | *10/*10 | *3/*10 | *4/*10 | *1/*1 | *1/*3 | *1/*4 | *1/*10 | *1/*4 × n | *1/*1 × n |
|---|---|---|---|---|---|---|---|---|---|---|
| Expected phenotype | PM | IM | IM | IM | EM | EM | EM | EM | EM/UM | UM |
| Number | 3 | 1 | 1 | 4 | 110 | 2 | 49 | 3 | 1 | 7 |
| Observed (%) | 1.7 | 0.6 | 0.6 | 2 | 61 | 1 | 27 | 1.7 | 0.6 | 4 |

*PM* Poor metabolizer, *IM* intermediate metabolizer, *EM* extensive metabolizer, *UM* ultrarapid metabolizer

The patients with different genotypes were comparable with regard to major demographic and clinical parameters, and also mean resting HRs were not different at admission with a general mean of 78±14 beats/min. We observed, however, that HRs at discharge (15–20 days after admission) differed in patients with different *CYP2D6* genotypes (Kruskal-Wallis $P<0.05$, with Dunn's multiple comparisons test). The lowest HRs were achieved by metoprolol PMs (50 and 55 beats/min), while the HR was higher in metoprolol UMs (69±8 beats/min, 95% CI: 61–78). HRs at discharge were 61±8 (95% CI: 59–64) and 62±8 (95% CI: 60–64) beats/min in carriers of one and two functional *CYP2D6* genes, respectively (Fig. 2).

## Metoprolol pharmacokinetics/pharmacodynamics in relation to *CYP2D6* genotype

The first 115 of all prospectively recruited patients made up a subgroup for metoprolol disposition analysis. Mean daily metoprolol dose was 75±38 (range 25–150) mg; 1.0±0.5 (range 0.3–2.3) mg/kg.

The patients were divided into groups corresponding to the assumed phenotype based on their genotype: PM, EM (one or two functional alleles), and UM. The patients were comparable across the groups with regard to major demographic and clinical variables, as well as the metoprolol dose and formulation prescribed (Table 5).

Plasma concentrations of metoprolol and α-hydroxy metoprolol varied widely among groups with different *CYP2D6* genotypes (Table 6). Metabolic ratio (MR) distribution was as expected from the genotypes in all patients but one, who followed the group of PM. This patient was genotyped as *CYP2D6*1*4*, but exhibited all the features of the PM phenotype; he had very high metoprolol concentration AUC and did not show any metabolite formation (Table 6). We designated him separately in the tables and figures as $PM^{*1*4}$.

Since early morning hours are considered the most dangerous with regard to the risk of the occurrence of coronary events, metoprolol concentrations before the dose intake in the morning were assessed separately. Metoprolol could be detected only in plasma of patients carrying one or two mutations in CYP2D6-encoding gene (Table 6, Fig. 3 a,b).

Correspondingly, lower HRs during the day were observed in patients with lower CYP2D6 genetically predefined activity, although the differences were not statistically significant (Table 7, Fig. 3c).

Significant, although rather moderate, correlation was observed between metoprolol concentrations (not dose-adjusted) and HRs measured simultaneously (Fig. 4).

## Metoprolol pharmacokinetics in relation to nongenetic individual variables

The experimental background allows for assumption that some nongenetic factors, namely hepatic blood flow, gender and age, may influence metoprolol disposition. We evaluated these differences in the studied group of patients.

**Table 4** *CYP2D6* allele distribution in 181 acute myocardial infarction patients ($n$ of alleles=362) compared to a Russian Voronez population [11]. Only the alleles measured in our study are given

| *CYP2D6* allele | *1 | *3 | *4 | *10 |
|---|---|---|---|---|
| Number | 289 | 3 | 60 | 10 |
| Observed frequency | 0.80 | 0.008 | 0.17 | 0.03 |
| Frequency in Voronez Russian population | 0.71 | 0.01 | 0.18 | 0.04 |

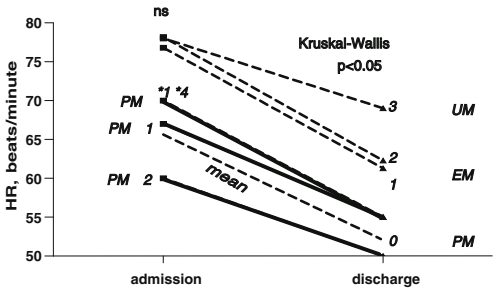



**Fig. 2** Heart rate (HR) changes at admission to the general ward (approximately 2–3 days after admission to the hospital) and after final metoprolol dose adjustment in different genotypes

**Table 5** Patient characteristics in *CYP2D6* genotype-based phenotype groups ($n$=115)

| CYP2D6 phenotype | PM | EM | | UM | p |
|---|---|---|---|---|---|
| *CYP2D6* genotype | 0 functional genes (**4/*4*) | 1 functional gene (**1/*3, *1/*4, *1/*4 × n, *10/*4*) | 2 functional genes (**1/*1, *1/*10*) | >2 functional genes (**1/*1* × n) | |
| Number | 2 | 34 | 74 | 5 | – |
| Men/women ($n$) | 1/1 | 20/14 | 46/28 | 3/2 | ns |
| Age, years (mean±SD and range) | 54, 80 | 60±10 (39–78) | 62±12 (43–80) | 53±11 (43–70) | ns |
| Anterior AMI | 0 | 18 | 35 | 3 | ns |
| Repeated AMI | 0 | 7 | 24 | 0 | ns |
| Postinfarction angina ($n$) | 2 | 23 | 36 | 3 | ns |
| LVEF, % (mean±SD and $n$[a]) | 51 (1) | 58±7 (6) | 56±9 (13) | 53±15 (1) | ns |
| Metoprolol dose, mg/kg (mean±SD) | 0.5, 0.7 | 0.9±0.5 | 0.9±0.4 | 0.9±0.4 | ns |

[a] Number of patients in whom no left ventricular ejection fractions (LVEF) were determined

First, patients were divided into two groups with different left ventricular ejection fractions (LVEF): a group with unchanged LVEF (>50%) ($n$=73) and a group with impaired LVEF (≤50%) ($n$=20) (we did not have LVEF measured in the remaining 22 patients of the group). Metoprolol concentration AUCs were not different in analysis of all the patients; a trend was observed toward higher metoprolol and α-hydroxy metoprolol concentrations in patients with lower LVEF, when only patients with both functional *CYP2D6* alleles were analyzed. Metoprolol concentration AUCs did not correlate with age in the group with no mutations in the CYP2D6-encoding gene despite a slight trend towards higher metoprolol plasma concentrations in older patients (Spearman $R$=0.23; $P$=0.06). Similarly, gender did not explain any differences in either metoprolol and α-hydroxy metoprolol concentrations or effects, although slightly higher concentrations were observed in women (data not shown).

*CYP2D6* activity in relation to complications, namely VRDs, in early period after AMI

During the data analysis we found that AMI complications differed in patients with different genotype-based *CYP2D6* activity (consult Fig. 1 for the details of the patient-group formation). Among 115 patients with studied metoprolol pharmacokinetics, 62 had complications in early AMI: VRD was seen in 10 patients and postinfarction angina (PA) in 47 patients (Table 7). Mean *CYP2D6* activity [calculated as mean number of active genes in relation to the whole gene number, minimum number of active genes ($n$=3) was given in a patient with a gene duplication] was higher in patients with VRD than in those with PA and patients with no complications. Metoprolol and α-hydroxy metoprolol concentrations were not different in these groups (Table 7).

Thirty-one patients with VRD after AMI were identified retrospectively. Eleven of them were available and recruited

**Table 6** Parameters of metoprolol pharmacokinetics and pharmacodynamics in patients with different *CYP2D6* genotypes

| Parameter | *CYP2D6 *4/*4* ($n$=2) | *CYP2D6*1/*3* (**4*), ($n$=32) | *CYP2D6*1/*1* ($n$=71) | *CYP2D6* dupl ($n$=5) | $p$[a] |
|---|---|---|---|---|---|
| Metoprolol concentrations AUC, nM·h (normalized for dose in mg/kg) | 5,167, 5,202 | 905 (705–1,809) (PM$^{*1/*4}$: 2814) | 559 (285–1063) | 336 | 0.0002[b] |
| α-Hydroxy metoprolol concentrations AUC, nM·h (normalized for dose in mg/kg) | 4, 6 | 811 (359–1,413) (PM$^{*1/*4}$: 5) | 1,215 (814–1,836) | 762 | 0.003[b] |
| MR | 1,196, 867 | 1.3 (0.6–3.5) (PM $^{*1/*4}$: 531) | 0.5 (0.3–0.7) | 0.4 | <0.0001[a] |
| Metoprolol steady-state (before dose) concentrations, nM mg$^{-1}$ kg$^{-1}$ [median (range)] | 169, 276 | 33 (1–277) (PM$^{*1/*4}$: 123) | 11 (2–295) | 18 (3–46) | 0.002[b] |
| AUEC, beats·h/min | 344, 342 | 394 (372–438) (PM$^{*1/*4}$: 360) | 403 (367–432) | 439 | 0.1[b] |
| HR (beats/min) | 54, 61 | 68 (65–70) (PM $^{*1/*4}$: 61) | 70 (66–71) | 73 (68–75) | 0.01[b] |

Unless otherwise stated, values are median (25th-75th percentile)
[a] $P$: ANOVA of log10 values
[b] $P$: Kruskal-Wallis

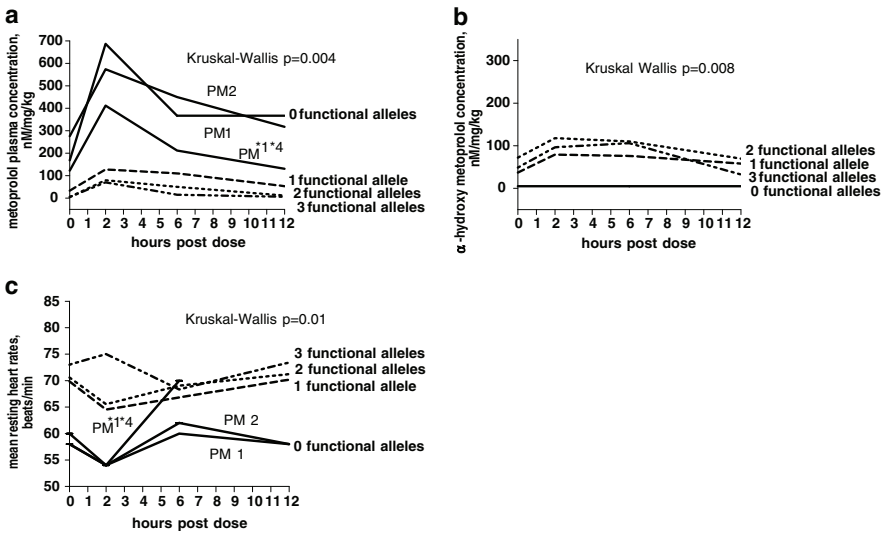



**Fig. 3** Variable median values of metoprolol (**a**) and α-hydroxy metoprolol (**b**) concentration AUCs, and mean resting heart rates (**c**) in acute myocardial infarction patients ($n$=115) with zero, one, two and more (designated as a minimum number of three) functional CYP2D6-encoding genes on the 7th to 10th day of treatment

for the study (see Fig. 1). Another twelve patients were selected from the whole group (187 patients), thus altogether 23 VRD patients were included.

Of these 23 patients, 5 carried *CYP2D6* gene duplication, 2 were genotyped as *CYP2D6*1/*4* and another 18 did not have mutations in the studied gene. The time of first VRD description in the case history varied from 2nd to 10th day of AMI. We observed that patients with VRD class II and higher had higher *CYP2D6* activity (2.1±0.5 vs. 1.6±0.6, $P$=0.0002) due to higher prevalence of *CYP2D6* duplication genotype [5 out of 18 (22%) vs. 4 out of 173 (2%), $P$=0.0002]. The only difference observed between the groups was higher proportion of ST-segment elevation myocardial infarction (STEMI) in the patients with VRD class II and higher (83 vs. 56%, $P$=0.01).

## Discussion

In this study, we evaluated the impact of *CYP2D6* genotype on disposition and effects of metoprolol in routine treatment of hospitalized patients after AMI. Metoprolol is the drug

**Table 7** CYP2D6 activity and metoprolol and α-hydroxy metoprolol concentrations in patients with complications in early period after AMI ($n$=115)

| Parameter | Ventricular rhythm disturbances (VRD) | | Early postinfarction angina | |
|---|---|---|---|---|
| Complication | Present | Absent | Present | Absent |
| Number of patients | 10 | 105 | 47 | 68 |
| CYP2D6 activity, mean±SD (median and range) | 2.2±0.8** (2*; 1–2) | 1.7±0.5** (2*; 1.5–3) | 1.6±0.6 (2; 1–2) | 1.7±0.5 (2; 1–2) |
| Metoprolol concentration AUC, nM·h (median and 25th–75th percentile)[a] | (694) 250–1,271 | (538) 342–734 | (700) 231–1,423 | (645) 270–1,110 |
| α-Hydroxy metoprolol concentration AUC, nM·h (median and 25th–75th percentile)[a] | (448) 293–1,056 | (924) 463–1,315 | (818) 444–1,727 | (901) 412–1,275 |
| MR (median and 25th–75th percentile)[ab] | (0.6) 0.4–1.9 | (0.6) 0.3–1.4 | (0.6) 0.4–1.8 | (0.5) 0.3–1.4 |

* $P$<0.05, **$P$<0.01

[a] Mann-Whitney test

[b] MR difference was calculated with parametric comparison of log values

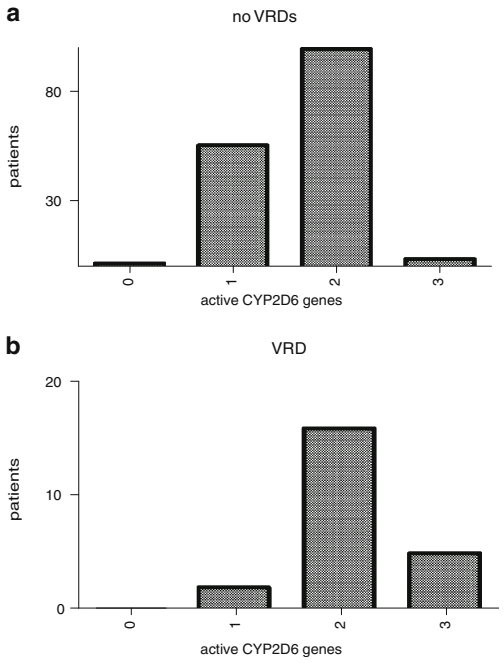



**Fig. 4** Frequency distribution of active *CYP2D6* genes in acute myocardial infarction patients without ventricular rhythm disturbances (VRDs) ($n$=177) and with VRDs ($n$=23) (for statistics, see table 7)

that is most likely to be prescribed in patients after AMI because it has been well studied in most clinical trials that demonstrated the safety and benefits of beta-blockades in AMI [2, 12]. The predominance of metoprolol prescriptions among all beta-blockers was confirmed in the studied clinic.

Metoprolol effects are predicted by experimental studies to take place in the approximate concentration range of 30–500 nM [14]. This range was not achieved on the 7th-10th day of treatment in a significant number of patients, especially at the trough levels, although it is known that cardiac events are more frequent in early morning hours [15], hence antianginal effect would be most required at this time of the day. This could be a specific problem for the hospital, where immediate-release metoprolol formulation prevailed, while extended-release formulations help maintain therapeutic concentrations without significant peaks during the whole dosing interval. However, the metoprolol plasma concentrations were low even at the time around the assumed peak concentration, indicating overall metoprolol under-dosing on the 7th–10th day of treatment. Lower doses of metoprolol than those tested in clinical trials are often prescribed in Sweden as well; this might also be relevant for other European countries and is considered to affect patient prognosis [3].

*CYP2D6* influence on metoprolol distribution at a single point after long-term intake for any reason has been shown previously [16, 17]. The *CYP2D6* allele distribution is different in Asians and Caucasians [5]. The Russian population is on the borderline as Russia has experienced a long history of Asian invasion in the past; St. Petersburg and Moscow are the cities with the most population mixture due to the highest degree of internal migration. Observed genotype distribution, however, was not clearly distinct from that in Caucasians and was within Hardy-Weinberg equilibrium, although we did note more gene duplications and fewer homozygote carriers of deficient allele **4* than was reported for the investigated Swedish populations, although the geographic location is similar for Sweden and Russia [5]. We also observed a higher frequency of the Asian **10*. Generally, the allele frequencies were comparable with those reported in a more southern Russian population of Voronez [11].

One patient was identified as exhibiting the PM phenotype with the *CYP2D6*1*4* genotype. Most probably he was carrying an unidentified nonfunctional allele other than **4* or **3*. In this patient, no α-hydroxy metoprolol could be measured, while the metoprolol concentration AUC was the highest among all non-PM subjects. Similarly to the two PM patients, he had very low HRs on the 7th day of metoprolol treatment and also at discharge (Figs. 2, 3). This shows that awareness should not be restricted to the most frequent alleles, but one should bear in mind that there are over 17 nonfunctional alleles that may come into play (http://www.cypalleles.ki.se/cyp2d6.htm) [5, 18].

We aimed to see the metoprolol concentrations during the whole dosing interval to speculate on patients' overall benefit in terms of sufficiently sustained beta-blockade. Total areas under the concentration-time curves, covering one dosing interval, were significantly different in patients with different *CYP2D6* genotypes. Trough concentrations, which we considered important as a protection against cardiovascular events in early morning hours, were also significantly controlled by CYP2D6 enzyme activity. They were sufficient enough to provide beta-blockade only in those patients who were carriers of at least one nonfunctional *CYP2D6* allele. This difference was not significantly reflected in intraday HR changes, but the higher mean HRs corresponded to the lower metoprolol concentrations ($P<$ 0.05). Mean HRs were around 70 beats/min in most patient groups, which confirmed insufficient overall dose-adjustment at about 7 days after AMI.

Resting HR efficiently reflected metoprolol concentrations in some previous studies [19], and therefore may be

used for this purpose. We tested this correspondence and observed weak correlation of HRs and metoprolol concentrations (Spearman $R$=-0.31, $P$=0.002). From the practice of the studied hospital, we know that exercise treadmill tests do not correspond to the routine at this time after AMI. None of the patients underwent early percutaneous coronary intervention (PCI).

The HR decrease from the time of admission to the general ward to the time of discharge differed significantly. Most pronounced therapeutic effect was achieved by genotypic metoprolol PMs, and almost no effect was observed in UMs. HRs at admission, however, were also lower in PMs than in the others. These data were obtained from the first routine (after permanent monitoring) ECG in the general ward, usually recorded when the metoprolol treatment is initiated in very small starting doses. It is very possible that the PMs respond more readily even to this starting dose of the drug. The strength of the evidence may be skewed by the very small size of the PM group, although the differences observed were quite prominent and corresponded to what was expected. Also more pronounced effects could be seen at a later stage after discharge, when patients commonly receive higher metoprolol doses.

We tried to also evaluate whether some nongenetic individual variables are to be considered in patients when predicting effective metoprolol dose. Metoprolol is a drug with high hepatic extraction [20]. It is possible then to assume that the changes in hepatic blood flow, e.g., in decreased cardiac pump function, would have an impact on metoprolol clearance. Increased concentrations in plasma were shown for carvedilol in patients with advanced heart failure [21], but no such data for metoprolol are available. Our data could not demonstrate more than a trend towards higher metoprolol concentrations within a group of patients with no mutations in the *CYP2D6* genes. This may be because the number of patients with significantly decreased left ventricular ejection fraction was not that high, and also because the doses prescribed were rather low.

Recent studies have discussed higher frequencies of metoprolol adverse drug reactions (ADR) in women [22]. Higher maximum metoprolol concentrations, AUCs and lower clearance were observed in a group of healthy women taking metoprolol compared to men by Luzier et al. [23]. However, clinical trials do not report gender differences in metoprolol tolerance and effects [24, 25]. Similarly our data could not demonstrate any difference but just a trend towards slightly higher metoprolol concentrations in women; it is noteworthy, however, that we had only 16 women in our pharmacokinetic group.

Good tolerance of metoprolol treatment is generally reported in older patients treated with this drug [3, 26]. Pharmacokinetic data are inconsistent, showing either no change in metoprolol disposition with age [27], or slightly decreased clearance [28]. Our study doesn't add anything but speculation, as in hospitalized patients up to 80 years, early after AMI, at doses of 75–150 mg/day, metoprolol disposition is not different in young and older patients.

An interesting finding we observed followed evaluation of the common complications in AMI. Observed frequency of complications (VRDs and PA) was not different from what is commonly known [2]. Patients were comparable with regard to common parameters, but the total number of active *CYP2D6* genes was higher in patients with VRDs. This complication is different from PA because of the relation of VRDs to adrenergic activity, while PA is demonstrative of insufficient coronary vessel diameter. Since the relation between the number of active genes and the enzyme amount is proportional, the reason for the higher complication rate could be in the differences in metoprolol concentrations, which is a known potent drug in suppression of ventricular premature complexes [29]. There was a trend towards different concentrations of metoprolol, which is what one could expect; however, it did not reach statistical significance.

Another piece of evidence against a relation between observed differences in AMI complications and metoprolol concentrations is that metoprolol concentrations that have been shown experimentally to be protective against ventricular premature complexes (72±34 ng/ml [30]) were hardly achievable at the very early time after AMI when most VRDs were reported. However, a higher number of *CYP2D6* genes was still observed in a larger group of patients with VRDs complicating AMI. This was mainly attributable to the higher (almost sixfold) prevalence of patients carrying additional copies of the CYP2D6-encoding gene. Although the observed frequencies and sample sizes ensured high statistical power to detect the difference observed, there are still many sources for bias. We could not rely on the diagnosis of VRD—the time of its registration was not specified, therefore there was a mixture of different times and types of VRD with some recorded by chance on routine ECG, and some on Holter monitor, and the chance is high that some VRDs could have been missed. Also the reason and prognostic value of VRDs at different times after AMI may have been different [31].

Together with the need to prove whether this was a chance finding, both clinical relevance and the need for explanation necessitate further investigation. There was no trend toward association of *CYP2D6* gene duplication with conventional risk factors for sudden cardiac arrhythmic death (SCD) [31]. A trend toward higher frequency of ST-elevation AMI as a debut of ischemic heart disease in these patients allows us to assume a more severe degree of initial myocardial damage in them, which may be due to increased adrenergic activity. Another hypothesis may be related to higher platelet activation, which has been shown to be an

independent predictor of myocardial damage [32] and is directly related to serotonin metabolism [33]. No specific endogenous substrates for CYP2D6 have been identified yet, although CYP2D6 is expressed in different organs and tissues, including the brain, intestines, and right cardiac ventricle [34]. Some studies suggested its involvement in catecholamine formation and have related it to different types of personality [35–37]. SCD due to arrhythmias has been repeatedly related to polymorphisms in ion channels [31]. Considering the possibility for CYP2D6 involvement in catecholamine metabolism, there could be the possibility that CYP2D6 multiplication leading to more catecholamine production may also be related to patient survival.

## Conclusion

Metoprolol disposition was shown to be controlled by the *CYP2D6* genotype, where increased activity was associated with low trough concentrations. Mean HRs were also higher in patients with the more active CYP2D6 enzyme, as carriers of the corresponding gene duplications could not achieve sufficient beta-blockade during the in-hospital metoprolol dose adjustment. This indicates that *CYP2D6* genotype plays an important role in differential response to metoprolol treatment in patients after AMI and subsequently for differential outcomes. Higher frequency of *CYP2D6* gene duplication observed in patients with VRDs after AMI may suggest another unfavorable mechanism with respect to the *CYP2D6* genotype. This is, however, just a hypothesis-generating finding requiring further evaluation.

**Acknowledgements** We thank Aleksandr V. Shabrov, the rector of St. Petersburg State Medical Academy named after I.I. Mechnikov, professor, and academician of Russian Academy of Medical Sciences; and Tommy Linne, coordinator of Karolinska Institute Research Training Program (KIRT) and associate professor. The help of biomedical analysts Lilleba Bohman in genotyping techniques and Jolanta Widen in HPLC techniques is gratefully acknowledged. The research was supported by the Swedish Institute (via KIRT), Heart-Lung Foundation and Swedish Research Council, Medicine (grant no. 3902). The study was approved by the local ethics committees of both universities.

## References

1. WHOSTAT (2005)
2. Antman EM, Anbe DT, Armstrong PW et al (2004) ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of Patients with Acute Myocardial Infarction). Circulation 110(9):e82–e292
3. Herlitz J, Karlson BW, Hjalmarson A (1993) Ten-year mortality rate after development of acute myocardial infarction in relation to clinical history and observations during hospital stay: experience from the Goteborg metoprolol trial. Coron Artery Dis 4(12):1077–1083
4. Lennard MS, Silas JH, Freestone S et al (1982) Oxidation phenotype–a major determinant of metoprolol metabolism and response. N Engl J Med 307(25):1558–1560
5. Bertilsson L (2007) Metabolism of antidepressant and neuroleptic drugs by cytochrome p450s: clinical and interethnic aspects. Clin Pharmacol Ther 82(5):606–609
6. Goryachkina K, Burbello A, Boldueva S et al (2008) Inhibition of metoprolol metabolism and potentiation of its effects by paroxetine in routinely treated patients with acute myocardial infarction (AMI). Eur J Clin Pharmacol 64(3):275–282
7. Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 14(5–6):143–149
8. Garcia-Barcelo M, Chow LY, Chiu HF et al (2000) Genetic analysis of the CYP2D6 locus in a Hong Kong Chinese population. Clin Chem 46(1):18–23
9. Lundqvist E, Johansson I, Ingelman-Sundberg M (1999) Genetic mechanisms for duplication and multiduplication of the human CYP2D6 gene and methods for detection of duplicated CYP2D6 genes. Gene 226(2):327–338
10. Bradford LD (2002) CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics 3(2):229–243
11. Gaikovitch EA, Cascorbi I, Mrozikiewicz PM et al (2003) Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. Eur J Clin Pharmacol 59(4):303–312
12. Opie L (2005) Drugs for the heart 6th edition
13. Astra Zeneca FDA report NDA 19–962/S-027. 2005 [cited January, 2008]; Available from: http://redpoll.pharmacy.ualberta.ca/drugbank/drugBank/FDA_labels/019962.pdf.
14. Elliot WJ (2001) Cyclic and circadian variations in cardiovascular events. Am J Hypertens 14(9 Pt 2):291S–295S
15. Rau T, Heide R, Bergmann K et al (2002) Effect of the CYP2D6 genotype on metoprolol metabolism persists during long-term treatment. Pharmacogenetics 12(6):465–472
16. Ismail R, Teh LK (2006) The relevance of CYP2D6 genetic polymorphism on chronic metoprolol therapy in cardiovascular patients. J Clin Pharm Ther 31(1):99–109
17. Bertilsson L, Dahl ML, Dalen P et al (2002) Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. Br J Clin Pharmacol 53(2):111–122
18. Koytchev R, Alken RG, Vlahov V et al (1998) Influence of the cytochrome P4502D6*4 allele on the pharmacokinetics of controlled-release metoprolol. Eur J Clin Pharmacol 54(6):469–474
19. Regardh CG, Johnsson G (1980) Clinical pharmacokinetics of metoprolol. Clin Pharmacokinet 5(6):557–569
20. Packer M, Lukas MA, Tenero DM et al (2006) Pharmacokinetic profile of controlled-release carvedilol in patients with left ventricular dysfunction associated with chronic heart failure or after myocardial infarction. Am J Cardiol 98(7A):39L–45L
21. Thurmann PA, Haack S, Werner U et al (2006) Tolerability of beta-blockers metabolized via cytochrome P450 2D6 is sex-dependent. Clin Pharmacol Ther 80(5):551–553
22. Luzier AB, Killian A, Wilton JH et al (1999) Gender-related effects on metoprolol pharmacokinetics and pharmacodynamics in healthy volunteers. Clin Pharmacol Ther 66(6):594–601
23. Rochon PA, Anderson GM, Tu JV et al (1999) Use of beta-blocker therapy in older patients after acute myocardial infarction in Ontario. CMAJ 161(11):1403–1408
24. Olsson G, Wikstrand J, Warnold I et al (1992) Metoprolol-induced reduction in postinfarction mortality: pooled results from five double-blind randomized trials. Eur Heart J 13(1):28–32

25. Park KC, Forman DE, Wei JY (1995) Utility of beta-blockade treatment for older postinfarction patients. J Am Geriatr Soc 43 (7):751–755
26. Herlitz J, Hjalmarson A, Holmberg S et al (1985) Tolerability to treatment with metoprolol in acute myocardial infarction in relation to age. Acta Med Scand 217(3):293–298
27. Regardh CG, Landahl S, Larsson M et al (1983) Pharmacokinetics of metoprolol and its metabolite alpha-OH-metoprolol in healthy, non-smoking, elderly individuals. Eur J Clin Pharmacol 24 (2):221–226
28. Dimenas ES, Dahlof CG, Heibel B et al (1990) Subjective symptoms and pharmacokinetics/dynamics of metoprolol CR in elderly subjects—a comparison with atenolol. Eur J Clin Pharmacol 38(6):571–578
29. Ellison KE, Gandhi G (2005) Optimising the use of beta-adrenoceptor antagonists in coronary artery disease. Drugs 65 (6):787–797
30. Pratt CM, Yepsen SC, Bloom MG et al (1983) Evaluation of metoprolol in suppressing complex ventricular arrhythmias. Am J Cardiol 52(1):73–78
31. Zipes DP, Camm AJ, Borggrefe M et al (2006) ACC/AHA/ESC 2006 Guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (writing committee to develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. Circulation 114(10): e385–e484
32. Frossard M, Fuchs I, Leitner JM et al (2004) Platelet function predicts myocardial damage in patients with acute myocardial infarction. Circulation 110(11):1392–1397
33. Laghrissi-Thode F, Wagner WR, Pollock BG et al (1997) Elevated platelet factor 4 and beta-thromboglobulin plasma levels in depressed patients with ischemic heart disease. Biol Psychiatry 42(4):290–295
34. Elbekai RH, El-Kadi AO (2006) Cytochrome P450 enzymes: central players in cardiovascular health and disease. Pharmacol Ther 112(2):564–587
35. Yu AM, Idle JR, Herraiz T et al (2003) Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine O-demethylase. Pharmacogenetics 13(6):307–319
36. Bertilsson L, Alm C, De Las Carreras C et al (1989) Debrisoquine hydroxylation polymorphism and personality. Lancet 1(8637):555
37. Llerena A, Edman G, Cobaleda J et al (1993) Relationship between personality and debrisoquine hydroxylation capacity. Suggestion of an endogenous neuroactive substrate or product of the cytochrome P4502D6. Acta Psychiatr Scand 87(1):23–28

# III

PHARMACOEPIDEMIOLOGY AND DRUG SAFETY 2008; **17**: 636–644
Published online 10 January 2008 in Wiley InterScience (www.interscience.wiley.com) **DOI**: 10.1002/pds.1543

ORIGINAL REPORT

# Quality use of medicines: A new method of combining antibiotic consumption and sensitivity data—application in a Russian hospital†

Ksenia Goryachkina MD[1,2], Svetlana Babak MD, CMSc[1‡], Aleksandra Burbello MD, DMSc[1‡], Björn Wettemark MScPharm, PhD[2] and Ulf Bergman MD, PhD[2]*

[1]*Course of Clinical Pharmacology, Department of Hospital Therapy, St Petersburg State Medical Academy named after I.I.Mechnikov, Russia*
[2]*Division of Clinical Pharmacology, Department of Laboratory Medicine, WHO Collaborating Centre for Drug Utilization Research and Clinical Pharmacological Services, Karolinska Institute, Karolinska University Hospital, Huddinge, Stockholm, Sweden*

## SUMMARY

**Purpose** Antibiotic use and resistance is subject of great concern. There is a need for internationally comparable and locally useful data collection and reporting. We developed a new method to combine and present data on antibiotic use and resistance in a figure in a Russian 1300 bed-hospital.
**Methods** We applied World Health Organization (WHO) Anatomic Therapeutic Chemical (ATC) classification/defined daily doses (DDD) analysis on *antibacterials for systemic use* (ATC: J01) delivered by the pharmacy for the years 2003–2005. Microbial resistance data were presented within the range of drugs accounting for 90% of the volume in DDD, i.e. drug utilisation 90% (DU90%).
**Results** From the DU90% profile the following was seen: in 2003, 12 of 25 drugs accounted for 90% of the volume. For six of the most commonly used antibiotics, including the two cheapest (gentamicin, ampicillin), a significant number of the strains tested were resistant. For the remaining antibiotics no resistance data were available. These data were discussed in early 2004. A general decrease of antibiotic use and resistance was seen in 2005 (by 57% from 15.5 to 8.8 DDD/100 bed days) with a concomitant decrease in expenditures (by 64%) and a shift to more potent antibiotics.
**Conclusions** The created profile highlighted potential problems in a clear and easy form. Besides being an indicator of the quality of antibiotic use it was a powerful alert and driving force for change. It can be used for external comparisons and for local monitoring of antibiotic use and resistance and can be applied with routinely available data in any hospital. 

KEY WORDS — antibiotic use; resistance; quality assessment; hospital care; Russia

*Received 3 July 2007; Revised 16 November 2007; Accepted 21 November 2007*

* Correspondence to: Professor U. Bergman, Division of Clinical Pharmacology, Department of Laboaratory Medicine, Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden.
E-mail: ulf.bergman@karolinska.se
† No conflict of interest.
‡ CMSc, Candidate of Medical Sciences—most wide spread scientific degree in Russia, often referred to as PhD; DMSc, Doctor of Medical Sciences—highest scientific degree in Russia, acquired after CMSc.

## INTRODUCTION

The 1998 European Conference 'the Microbial Threat' emphasised the importance of collecting and comparing data on antibiotic use and bacterial resistance from different countries.[1] Microorganisms

know no national boundaries, so no single country can solve the problem alone.[2] Since the conference, many cross-national studies on antibiotic use and resistance have revealed large differences in antibiotic prescribing in general practice and in hospitals.[3–10] The European Surveillance of Antibiotic Consumption (ESAC) found a three times higher antibiotic use in France compared to the Netherlands (32.2 *vs.* 10.0 defined daily doses (DDD)/1000 inhabitants per day). A strong positive correlation was later found between the extent of antibiotic use in ambulatory and hospital care.[7,9] These cross-national studies have generally used the WHO classification system ATC and the volume unit DDD (see below).[1,11,12] WHO has also recognised the need for regular local and international collection and reporting of data on antibiotic use and resistance.[13] In Russia, a federally supported Centre on Monitoring of Antimicrobial Resistance (CMAR) performs surveys and regularly provides data on antibiotic use in ATC/DDD format and microbial resistance in written publications and on the web.[14–17] Still internationally comparable data are scarce, one of the reasons for that could be the absence of routine data collection in an internationally comparable format. The objective was therefore to introduce the ATC/DDD methodology in a large Russian hospital for antibiotic utilisation assessment, and based on the phrase *a picture is worth a thousand words,* we also tested the idea of visualizing the resistance burden within a profile of antibiotic use to provide an immediate alert. Routinely available data on antibiotic use and resistance were followed for three consecutive years using the new method.

## METHODS

### *Setting*

The method was tested in a 1300-bed university hospital in St Petersburg, Russia, 2nd City Hospital with all major specialities except infectious diseases, bone marrow transplantation, haematology and tuberculosis.

The hospital has one clinical pharmacologist (SB), surveying and analysing drug utilisation (therapeutic and economical aspects) and antibiotic resistance. She routinely performs point-prevalence studies in various pharmacotherapeutic areas (antibiotics, narcotics, etc.) and provides individual consultations at the ward level. At the beginning of each year, she presents a drug utilisation report to the hospital authorities. She is also one of the members of hospital's Drug and Therapeutics Committee (DTC).

In Russia, local DTCs in hospitals are called formulary committees and usually consist of all major specialists, representative of authorities and a pharmacist, and they develop local formularies based on federal recommendations and requirements of that particular hospital.

Data on drug utilisation for inpatient care are provided by the hospital pharmacy while bacteriological laboratory provides data on microbial strains and sensitivity to antibiotics.

Data on drug use are usually analysed in terms of expenditures and divided by vital, essential and non-essential groups defined by the DTC. Microbiological data—all individual cultures from different kinds of patients' samples (blood, sputum, pus, urine, etc.) separately from patients' clinical data or antibiotic prescriptions—are presented and analysed by the hospital specialist in infectious diseases together with the clinical pharmacologist.

### *Antibiotic use data*

*ATC/DDD.* Data on consumption of antibiotics were obtained from the hospital pharmacy in numbers of packages of all antibiotics delivered to every department for the years 2003, 2004 and 2005. Drugs were classified according to the ATC classification system[12]; section J01—'antibacterials for systemic use'.[§] The amount of antibiotics in gram was transferred into the technical unit DDD, recommended by WHO as a common language for describing the use of drugs—the therapeutic intensity—in a population or in a hospital, and expressed as numbers of DDDs/100 bed days—as suggested by WHO for in-hospital use.[12] The numbers of bed days were: 212 961, 407 916, 385 911 for the years 2003, 2004 and 2005, respectively (the difference for 2003 and 2004–2005 is explained by changing treatment practices with a varying bed occupancy rate).[¶]

[§]We will apply the term *antibiotic* in this paper as it is closest to many European and Russian commonly used definitions and the one, which is being synonymous to 'antibacterials' and 'antimicrobials', implies mainly the drugs acting against bacteria.

[¶]Old recommendations were in use with long in-hospital treatment for many diseases (mostly cardiovascular), it was now administratively changed for shorter in-hospital stay with increased emphasis on ambulatory care.



DOI: 10.1002/pds

DDDs for each substance are listed in the Results section.[‖] ATC/DDD versions for the respective years were used as recommended by WHO.[12] No differences were found for any of the drugs of interest between the versions of the ATC/DDD[#] index 2003, 2004 and 2005 except amoxicillin/enzyme inhibitor (clavulanic acid) with the DDD increased from 1 to 3 g in 2005. Since this new DDD was not yet implemented 1 g was used in the analysis.[11]

*Drug utilisation 90%—DU90%.* The antibiotics were ranged in order of utilisation volume in DDDs. We focused on the drugs accounting for 90% of the volume by DDD: the drug utilisation 90% (DU90%) segment.[18–21] These DU90% profiles are commonly used to assess adherence to guidelines within a therapeutic area. We included and assessed data on the proportion of resistant strains for the various antibiotics instead.

### *Microbial resistance data*

The hospital bacteriological laboratory routinely screens microbial resistance/sensitivity for all cases requiring antibiotic therapy except for prophylactic use. Standard disc diffusion method is utilised with consequent detection of *Resistant*, *Intermediate* and *Sensitive* strains; resistance is defined according to the National Committee for Clinical Laboratory Standards (NCCLS, now Clinical and Laboratory Standards Institute, CLSI).[22] Strains isolated are tested for sensitivity to antibiotics; no diagnostic testing with primary resistance cases is routinely performed. The WHONET software developed by WHO is utilised for the data management.[23,24] The hospital specialist in infectious diseases and the clinical pharmacologist analyse the data provided by the microbiology unit and based on that create the hospital resistance profile. Duplicate samples from patients are not included in the analyses.

The number of the assays performed by the bacteriological laboratory was comparable for the years 2003 (4281), 2004 (4200) and 2005 (4150). The burden of resistance for each antibiotic was calculated as percentage of all *Resistant* + *Intermediate* among all tested isolates from all patients' samples (blood, pus, urine, sputum, etc.). For instance, for gentamicin being the mostly used sensitivity was defined for G+ (*Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus avium, Enterococcus faecalis*) and G- (*Acinetobacter, Citrobacter, Enterobacter, Escherichia coli, Haemophilus influencae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Serratia*).

### *Data management*

The antibiotic resistance data were obtained and presented within the DU90% segment in one figure. The data were visualised as a diagram combining drug utilisation in DDDs and resistance—red in each bar corresponds to the percentage of microorganisms that were *resistant or intermediate* to each antibiotic agent, respectively. The diagrams were created using simple excel based software, for the whole hospital and for the separate departments. The data were obtained at the beginning of the new calendar year (2004). All heads of the departments were given the figures both for the whole hospital and for the departments. During the year, the clinical pharmacologist held discussions with physicians on the findings while doing her routine work: screening selective case histories and performing routine consultations. The 2003 figures were also presented to the hospital authorities at the annual meeting in January 2004. The data were discussed at the meeting of the DTC in February 2004 where epidemiologists in the field of infectious diseases were present among other specialists. The changes in the formulary list of the first choice antibiotics were included in the new edition of the formulary. It was recommended that gentamicin and penicillins as well as ciprofloxacin should not be prescribed empirically, more powerful newer fluoroquinolones, and aminoglycosides, inhibitor protected penicillins were recommended instead as well as increased alertness regarding the decision to start antibiotic therapy. The data for the year 2004 and 2005 were collected and handled in the same manner.

Statistical analysis: Chi-square ($\chi^2$) test was used to compare antibiotic use in different time periods.

Ethical considerations: as this study did not collect any data on individual patients and the surveillance

[‖]Ciprofloxacin had different doses for oral and parenteral use (1 and 0.5 g). Oral formulations significantly dominated in the study hospital in all 3 years, thus we included only the oral dose for the analysis.

[#]Two of the antibacterials used in the hospital were not listed in the ATC index 2003. Request concerning suggested DDDs was sent to the WHO Collaborating Centre for Drug Statistics Methodology. Suggested DDDs were 4 g for sulperazon (combination of cefoperazon and sulbactam) and 2 g for ampioxum (combination of ampicillin and oxacillin). During the study period the request was approved at the meeting of the WHO International Working Group for Drug Statistics Methodology.



DOI: 10.1002/pds

was part of a quality assurance, approval by ethics committee was not considered necessary.

## RESULTS

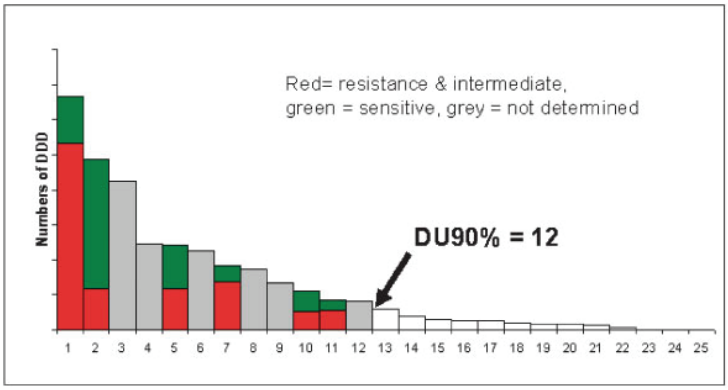
### *The atibiotic dug utilisation 90% and resistance profile*

From the antibiotic DU90% profile for 2003 the following information is highlighted at a glance Figure 1:

- Resistance rate varied from 80% (gentamicin) to 23% (cefazolin).
- No data on resistance were available for 6 of the 12 most commonly used antibiotics.
- Two of the cheapest drugs (cost/DDD) had the highest resistance rates (gentamicin, ampicillin).
- A significant amount (~40%) of the antibiotics were given by injections (gentamicin, cefazoline, benzylpenicillin and amikacin were only available as injections).

### *Antibiotic use*

In 2003, the 25 different antibiotic agents (including combinations) made up 15.5 DDD/100 bed-days and 12 of those constituted the DU90% segment (Figure 1).




| | SUBSTANCE | (DDD) | DDD | % TOT | DDD/100 BED-DAYS | COST/DDD (rubles) | % RESIST |
|---|---|---|---|---|---|---|---|
| 1 | Gentamicin | 0.24 g | 6 687 | 20% | 3,1 | 5 | 80% |
| 2 | Cefazolin | 3 g | 4 865 | 15% | 2,3 | 41 | 23% |
| 3 | Amoxicillin + clav. acid | 1 g | 4 242 | 13% | 2,0 | 105 | nd |
| 4 | Ampicillin + Oxacillin | 2UD (2g) | 2 438 | 7% | 1,1 | 13 | nd |
| 5 | Benzylpenicillin | 3.6 g | 2 435 | 7% | 1,1 | 129 | 49% |
| 6 | Pefloxacin | 0.8 g | 2 245 | 7% | 1,1 | 6 | nd |
| 7 | Ampicillin | 2 g | 1 838 | 6% | 0,9 | 10 | 74% |
| 8 | Metronidazole | 1.5 g | 1 726 | 5% | 0,8 | 50 | nd |
| 9 | Lincomycin | 1.8 g | 1 357 | 4% | 0,6 | 5 | nd |
| 10 | Amikacin | 1 g | 1 099 | 3% | 0,5 | 143 | 49% |
| 11 | Ciprofloxacin | 1 g | 845 | 3% | 0,4 | 400 | 66% |
| 12 | Pipemidic acid | 0.8 g | 828 | 2% | 0,4 | 22 | nd |
| **DU 90%** | **1 - 12** | | 30 603 | 92% | 14,4 | | |
| | 13 - 25 | | 2 477 | 8% | 1,2 | | |
| **TOTAL** | **1 - 25** | | **33 080** | **100%** | **15,5** | | |

Figure 1. Drug Utilization 90% profile for ''antibacterials for systemic use'' (J01, ATC)/DDD classification) in University hospital N2 of St Petersburg, Russia in 2003. The 12 antibiotics are ranked in order of number of DDDs corresponding to 90% of the use (data from the hospital pharmacy). Red parts correspond to percentage of resistance (resistance + intermediate) for the antibiotics, and green – percentage of sensitivity. Antibiotics not tested for bacterial sensitivity are grey (nd = not determined).



DOI: 10.1002/pds

There was a wide expected variation between different departments (from 3 DDD/100 bed-days in the 2nd neurological department, to 567 DDD/100 bed days in the 1st intensive care unit (ICU)). The most prevalent antibiotics were 'second-generation' aminoglycosides (J01GB03), penicillins (with and without beta-lactamase inhibitors) (J01C), 'first generation' fluoroquinolones (J01MA02-03) and cephalosporins (J01DB).

*Follow up in 2004 and 2005*

The total number of antibiotics used increased from 25 in 2003 to 36 in 2005 mainly due to the introduction of macrolides and third generation cephalosporins. The DU90% segment included mostly the same antibiotics, though in a different range and amounts (Figure 2).

The total amount of DDDs increased from 33 080 in 2003 (Figure 1) to 34 011 in 2005. However, with the increasing bed occupancy rate, the number of DDD/100 bed-days decreased by 57% from 15.5 to 8.8 for the whole hospital in 2005. Antibiotic resistance data were still only available for 6 of 13 antibiotics in the DU90% segment (14 of the 36 overall). Resistant microorganisms generally changed according to the change of utilisation of respective antibiotics (Figure 2). The decrease in gentamicin use from 3.1 DDD/100 bed days (20%) to 1.4 DDD/100 bed days (16%) was accompanied by the aggregated resistance decrease from 80 to 40% of resistant strains among all tested. Total expenditures for antibiotic drug purchases decreased by 64% from 2003 to 2005 (Table 1). These savings and the decrease in use of the most widely used drugs (gentamicin, penicillin, first generation cephalosporins, fluoroquinolones, etc.) allowed increased purchase of more expensive newer fluoroquinolones and cephalosporins with a wider spectrum (Table 1).

## DISCUSSION

The importance of antibiotic use for the development of antibiotic resistance has been known since many years. Many attempts have also been done to monitor and present antibiotic use and resistance data in various tables and figures.[25–29] The global aims are to provide antibiotic use surveillance internationally to timely and effectively constrain resistance.[8,9,13] We used the well-established WHO ATC/DDD methodology for drug utilisation statistics[9,12] and focused on the antibiotics accounting for 90% of the use[20] with the new dimension of adding data on microbial sensitivity (Figure 1). These data, both based on routinely available aggregate data, should be interpreted with caution and mainly serve as alerts. However, some of the points alerted may be quite obvious (no sensitivity data were available for 6 of 12 antibiotics, see also below), others may require specific surveys (to what extent are antibiotics

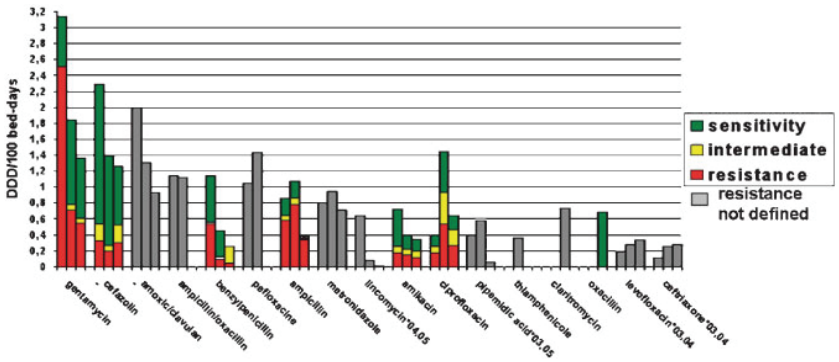



Figure 2. Antibiotics appearing in the DU90% segment in either of the years 2003, 2004 or 2005 and with resistance changes. Each antibiotic is presented in 3 bars corresponding to 3 consecutive years 2003, 2004 or 2005 in DDDs/100 bed-days. Empty spaces represent no use of the antibiotic in the respective year. Antibiotics, which were used but were not in the DU90% segment are marked with * and corresponding year. Red parts of each bar correspond to percentage of resistance for the antibiotic, yellow – percentage of intermediate sensitivity, and green – percentage of sensitivity. Antibiotics not tested for bacterial sensitivity are grey.



DOI: 10.1002/pds

Table 1. Range of all antibiotic expenditures in rubles and percentage for the years 2003 and 2005*

| Antibiotic | Costs (rubles) | Costs (percentage) |
|---|---|---|
| **Range of antibiotic expenditures 2003** | | |
| 1. Meropenem | 873 267 | 23 |
| 2. Cefepime | 517 563 | 14 |
| 3. Amoxiclav | 513 025 | 14 |
| 4. Imipenem + cilastatin | 449 806 | 12 |
| 5. Cefazolin | 201 388 | 5 |
| 6. Amikacin | 178 764 | 5 |
| 7. Vancomycin | 159 021 | 4 |
| 8. Sulperazon | 138 818 | 4 |
| 9. Ciprofloxacin | 134 751 | 4 |
| 10. Pefloxacin | 125 987 | 3 |
| 11. Ceftriaxone | 122 364 | 3 |
| 12. Levofloxacin | 83 740 | 2 |
| 13. Metronidazole | 57 364 | 1 |
| 14. Cefuroxime | 53 840 | 1 |
| 15. Ampioxum | 32 680 | 1 |
| 16. Penicillin | 29 073 | 1 |
| 17. Gentamicin | 27 458 | 1 |
| 18. Ampicillin | 18 670 | 0.5 |
| 19. Moxifloxacin | 16 877 | 0.5 |
| 20. Pipemidic acid | 16277 | 0. |
| 21. Timentin | 6226 | 0. |
| 22. Lincomycin | 5523 | 0. |
| 23. Nitroxoline | 1359 | 0. |
| 24. Unasyn | 846 | 0. |
| 25. Ofloxacin | 167 | 0. |
| TOTAL | 3 765 266 | 100 |
| **Range of antibiotic expenditures 2005** | | |
| 1. Ceftazidim | 480 752 | 35 |
| 2. Levofloxacin | 153 998 | 11 |
| 3. Cefazolin | 134 606 | 10 |
| 4. Cefepim | 94 835 | 7 |
| 5. Amoxiclav | 84 538 | 6 |
| 6. Meropenem | 72 656 | 5 |
| 7. Imipenem + cilastatin | 65 031 | 5 |
| 8. Metronidazole | 49 705 | 4 |
| 9. Moxifloxacin | 48 965 | 4 |
| 10. Vancomycin | 29 660 | 2 |
| 11. Pefloxacin | 28 642 | 2 |
| 12. Linezolid | 21 441 | 2 |
| 13. Claritromycin | 17 892 | 1 |
| 14. Ciprofloxacin | 16 402 | 1 |
| 15. Ampicillin | 15 262 | 1 |
| 16. Amikacin | 12 599 | 1 |
| 17. Amoxicillin | 5620 | 0. |
| 18. Erhytromycin | 5489 | 0. |
| 19. Ceftriaxone | 5027 | 0. |
| 20. Ofloxacin | 4840 | 0. |
| 21. Azitromycin | 3960 | 0. |
| 22. Ampioxum | 3930 | 0. |
| 23. Pipemidic acid | 3536 | 0. |
| 24. Gentamicin | 2829 | 0. |
| 25. Oxacillin | 1079 | 0. |
| 26. Lincomycin | 956 | 0. |
| 27. Norfloxacin | 604 | 0. |
| 28. Canamycin | 411 | 0. |
| 29. Doxycycline | 272 | 0. |
| 30. Benzylpenicillin | 338 | 0. |
| 31. Tetracycline | 169 | 0. |
| TOTAL | 1 366 044 | 100 |

*For the sake of space the 2004 data were omitted. (Ruble = 35 Euro and 30 USD in 2003, Ruble = 36 Euro and 28 USD in 2005.)

appropriately given by injections, what specific resistance patterns are involved in the overall growth of resistance to an excessively used antibiotic). There are simple protocols available for point-prevalence studies that may illustrate the clinical significance of these problems.[8,10]

Although the DU90% profile may have some limitations with regard to the relevance of the DDDs in the hospital setting (DDD is defined for the major indication in adults) the DDDs used were given in the profile (Figure 1) and the relevance can thus easily be assessed. General limitations of DDDs are described elsewhere and are common for all aggregate data.[11,12,18,30,31]

Also the aggregate microbiology data is subject to limitations (no spectrum of strains analysed considered, selection of analysis with increasing bed-days occupancy could have taken place). With all the limitations the figures should not be taken literarily but rather as an illustration of the principle when applying this methodology.

Here is an example of the data interpretation from the studied setting.

We showed a high rate of resistance among the most commonly used antibiotics (gentamicin was the most commonly used antibiotic with the highest rate of resistance (80%)). This suggests that its use was not based on empirical data and was therefore probably not very effective. The pressure of selected ineffective antibiotics decreased in 2005 (gentamicin constituted 20% of all antibiotics use in 2003 and 16% in 2005; difference significant with $p < 0,0001$ $\chi^2$ test). Concomitantly we showed the decreased resistance level for gentamicin (80 to 40%—same strains were tested). This provides an overall impression of coincidence of resistance and antibiotic overuse. The initial finding serves as a quick alert, leading to the decrease of gentamicin use, and also it stimulates further investigations to identify the details of the problem. Here it was found, for instance, that resistance of *P. aeruginosa* to gentamicin, was changed from 64% of resistant strains in 2003, to 69% in 2004 and 41% in 2005; whereas *E. coli* remained moderately resistant. This means that for *P. aeruginosa* infections the change led to improved sensitivity, though it's clear that gentamicin still cannot be used for this kind of infection; for *E. coli* infection the benefit was the decrease of the use of this poorly effective antibiotic.

From the DU90% profile it is also obvious that sensitivity test was only available for 6 of the 12 antibiotics. According to the routine protocols, a sensitivity test is normally taken in all patients receiving antibiotics



DOI: 10.1002/pds

except for cases of prophylactic use. However, it is also obvious that there was an 'empirical' prescribing that was not based on empirical data for several of the most commonly used antibiotics. This lack of integration between microbiology and clinical routine was earlier discussed in Russia.[16] Now it was brought to the attention of the hospital authorities and the practicing physicians.

Drug cost analyses, routinely performed in Russian (as well as in most) hospitals, cannot identify the actual problem with antibiotic use.[32] We found that two of the cheapest drugs (cost/DDD) had the highest resistance rates (gentamicin, ampicillin) reflecting a cost-ineffective use.

A high use of antibiotics given by injections was revealed with 4 out of 12 antibiotics in the DU90% segment being available for parenteral use only (gentamicin, cefazolin, benzylpenicillin and amikacin). It has been shown that rather than being due to severity of disease this may often reflect a psychological assumption that intravenous antibiotics are more effective, despite the lack of evidence for this general approach.[5,8]

Here we showed in a real clinical setting some possibilities of a new method for antibiotic utilisation studies. Firstly, it demonstrated the usefulness of the DU90% method in focusing on the bulk of antibiotic utilisation, and secondly, the possibility to include resistance in this general assessment. The main goal is to make powerful improvement areas obvious for responsible people. Although this clinical setting data were just to exemplify the new methodology, and this was not an experimental study with a control group, the hospital administration has reacted to these data with the recommendations to reduce gentamicin and unprotected penicillins empirical prescribing and the available 2003 profile seems to have had a marked impact on the antibiotic use in the following 2 years. There were major organisational changes with regard to bed-occupancy in this hospital, which could have influenced the patterns of drug use. However, although the total number of DDDs of antibiotics used in the hospital increased slightly from 2003 to 2005 (3%) the relative use (per 100 bed-days) decreased considerably (57%). The number of beds was unchanged so was the composition of diseases treated. With the pattern of antibiotics used, the resistance pattern was reduced as well as the costs (64%) suggesting that there might have been a more effective antibiotic use in 2005.

We believe that our method includes various forms of potentially effective intervention strategies: educational (data distribution and discussion), restrictive (formulary changes) and structural (establishment of a new quality monitoring mechanism) as stated in a recent Cochrane review.[33] Many of them has in other studies shown to be effective in the decrease of unnecessary antibiotic use. Restrictive methods are most widely spread and they are easier to perform. Guidelines changes are based on local utilisation and microbial patterns. Certain restrictions are usually considered to have the greatest immediate impact on prescribing pattern. The persuasive studies are more difficult to evaluate, but they are considered important and sometimes more cost-effective. Our method has the strength of influencing both—recommendations for the new guidelines and persuasion. It is important to evaluate this methodology's interventional power in a well-designed randomised controlled study. However, even in this early attempt it was capable of identifying obvious and severe problems in antibiotic utilisation in the hospital and changes in the prescribing.

Similarly, our simple methodology has identified factors for a cost effective use of antibiotics in a hospital, the clinical relevance of which might be further elucidated by use of a simple protocol for point prevalence quality of care studies.[8,10]

## CONCLUSION

The method of an integrated graphic presentation of antibiotic use and resistance (expressed in DDD/100 bed-days and with focus on the volume of use) in a Russian hospital proved to be easy to do. It provided a clear message concerning inappropriate antibiotic use both for practicing physicians and hospital authorities that probably resulted in rather quick changes. The method serves just as a general alert. A detailed investigation based on the signals revealed may be needed. The simplicity of the methodology seems to

KEY POINTS

- Combined presentation of antibiotic utilisation and resistance provides clear information on both problems in relation to each other.
- Use of combined aggregate data on antibiotic utilisation and resistance may be useful to identify general trends and stimulate deeper investigations.
- Enrichment of DU90% profile of antibiotics with resistance is easily understood by health professionals and may be a tool in changing prescribing patterns.



DOI: 10.1002/pds

be beneficial for local regulation of antibiotic use and resistance and can in principle be applied in any hospital where consumption and resistance data exists.

## ACKNOWLEDGEMENTS

KG is a KIRT fellow (Karolinska Institute Research Training Programme supported by the Swedish Institute) at the Division of Clinical Pharmacology, Karolinska University Hospital, Huddinge. We thank the administration of the hospital of St Petersburg and St Petersburg State Medical Academy named after Mechnikov for the possibility to collect data.

## REFERENCES

1. Pedersen KB, Rosdahl VT. *The Copenhagen recommendations report from the Invitational EU Conference on The Microbial Threat*, Copenhagen, Denmark 9-10 September 1998, 52 blade (Ministry of Health Ministry of Food, [Kbh.], 1998).
2. Smith RD, Coast J. Antimicrobial resistance: a global response. *Bull World Health Organ* 2002; **80**: 126–133.
3. Cars O, Mölstad S, Melander A. Variation in antibiotic use in the European Union. *Lancet* 2001; **357**: 1851–1853. DOI: 10.1016/S0140-6736(00)04972-2
4. Gulbinovic J, Myrbäck KE, Bytautienė J, Wettermark B, Struwe J, Bergman U. Marked differences in antibiotic use and resistance between university hospitals in Vilnius, Lithuania, and Huddinge, Sweden. *Microb Drug Resist* 2001; **7**: 383–389. DOI: 10.1089/10766290152773392
5. Vaccheri A, Bjerrum L, Resi D, Bergman U, Montanaro N. Antibiotic prescribing in general practice: striking differences between Italy (Ravenna) and Denmark (Funen). *J Antimicrob Chemother* **50**: 989–997. DOI: 10.1093/jac/dkf239
6. Bronzwaer S, Buchholz U, Courvalin P, *et al.* Comparability of antimicrobial susceptibility test results from 22 European countries and Israel: an external quality assurance exercise of the European Antimicrobial Resistance Surveillance System (EARSS) in collaboration with the United Kingdom National External Quality Assurance Scheme (UK NEQAS). *J Antimicrob Chemother* 2002; **50**: 953–964. DOI: 10.1093/jac/dkf231
7. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 2005; **365**: 579–587. DOI: 10.1016/S0140-6736(05)17907-0
8. Vlahović-Palčevski V, Dumpis U, Mitt P, Gulbinovič J, Struwe J, Palčevski G, Štimac D, Lagergren Å, Bergman U. Benchmarking antimicrobial drug use at university hospitals in five European countries. *Clin Microbiol Infect* 2007; **13**: 277–283. DOI: 10.1111/j.1469-0691. 2006.01613.x
9. Vander Stichele RH, Elseviers MM, Ferech M, Blot S, Goossens H. Hospital consumption of antibiotics in 15 European countries: results of the ESAC Retrospective Data Collection (1997–2002). *J Antimicrob Chemother* 2006; **58**: 159–167. DOI: 10.1093/jac/dkl147
10. Struwe J, Dumpis U, Gulbinovic J, Lagergren A, Bergman U. Healthcare associated infections in university hospitals in Latvia, Lithuania and Sweden: a simple protocol for quality assessment. *Euro Surveill* 2006; **11**: 167–171.
11. Ronning M, Blix HS, Harbo BT, Strom H. Different versions of the anatomical therapeutic chemical classification system and the defined daily dose—are drug utilisation data comparable? *Eur J Clin Pharmacol* 2000; **56**: 723–727, DOI: 10.1007/s002280000200
12. WHO. ATC index with DDDs, (World Health Organization: World Health Organisation Collaborating Centre for Drug Statistics Methodology, 2003–2005). Available at www.whocc.no/atcddd [accessed 30, June 2007].
13. WHO global strategy for containment of antimicrobial resistance http://www.who.int/csr/drugresist/guidance/en/ [accessed 30, June 2007].
14. Antibiotics and antimicrobial therapy http://www.antibiotic.ru/index.php?newlang=eng [accessed 30, June 2007].
15. Stratchounski L, Bedenkov A, Hryniewicz W, *et al*. The usage of antibiotics in Russia and some countries in Eastern Europe. *Int J Antimicrob Agents* 2001; **18**: 283–286. DOI: 10.1016/S0924-8579(01)00381-8
16. Stratchounski LS, Dekhnitch AV, Kozlov RS. Infection control system in Russia. *J Hosp Infect* 2001; **49**: 163–166. DOI: 10.1053/jhin.2001.1042
17. Palcevski G, Ahel V, Vlahović-Palcevski V, *et al.* Antibiotic use profile at paediatric clinics in two transitional countries. *Pharmacoepidemiol Drug Saf* 2004; **13**: 181–185. DOI: 10.1002/pds.880
18. Birkett D, Smet Pd, Ofori-Adjei D, Trolin I, Bergman U, Strøm H. *Introduction to Drug Utilization Research*, (WHO Library Cataloguing-in-Publication data, Oslo, 2003).
19. Sjöqvist F, Bergman U, Dahl M-L, Gustafsson L, Hensjö L-O. Drug and Therapeutics Committees: a Swedish experience. *WHO Drug Inf* 2002; **16**: 207–213.
20. Bergman U, Popa C, Tomson Y, Wettermark B, Einarson TR, Åberg H, *et al.* Drug utilization 90%—a simple method for assessing the quality of drug prescribing. *Eur J Clin Pharmacol* 1998; **54**: 113–118, DOI: 10.1007/s002280050431
21. Bergman U, Risinggård H, Vlahovic-Palcevski V, Ericsson O. Use of antibiotics at hospitals in Stockholm: a benchmarking project using internet. *Pharmacoepidemiol Drug Saf* 2004; **13**: 465–471. DOI: 10.1002/pds.898
22. NCCLS—National Committee for Clinical Laboratory Standards www.nccls.org [accessed 30. June 2007].
23. O'Brien TF, Stelling JM. WHONET: an information system for monitoring antimicrobial resistance. *Emerg Infect Dis* 1995; **1**: 66.
24. WHO. WHONET5 Microbiology Laboratory Database Software. in WHO/CDS/CSR/DRS (1999).
25. Sjostedt S, Levin P, Kager L, Malmborg AS, Bergman U. Hospital and catchment area antibiotic utilization and bacterial sensitivity in primary infections following gastric surgery in Huddinge, Sweden. *Eur J Clin Pharmacol* 1990; **39**: 211–216. DOI: 10.1007/BF00315098
26. Pestotnik SL, Classen DC, Evans RS, Burke JP. Implementing antibiotic practice guidelines through computer-assisted decision support: clinical and financial outcomes. *Ann Intern Med* 1996; **124**: 884–890.
27. Mölstad S, Cars O. Major change in the use of antibiotics following a national programme: Swedish strategic programme for the rational use of antimicrobial agents and surveillance of resistance (STRAMA). *Scand J Infect Dis* 1999; **31**: 191–195.
28. Vlahovic-Palcevski V, Morovic M, Palcevski G, Betica-Radic L. Antimicrobial utilization and bacterial resistance at three different hospitals. *Eur J Epidemiol* 2001; **17**: 375–383. DOI: 10.1023/A:1012742314070
29. Kuperman GJ, Gibson RF. Computer physician order entry: benefits, costs, and issues. *Ann Intern Med* 2003; **139**: 31–39.

30. Coenen S, Ferech M, Goossens H. Antibiotic prescribing quality indicators. in ESF EMRC exploratory workshop. (7–9 September, 2005).
31. Rønning M, Blix HS, Strøm H, *et al.* Problems in collecting comparable national drug use data in Europe: the example of antibacterials. *Eur J Clin Pharmacol* 2003; **58**: 843–849. DOI: 10.1007/s00228-003-0572-8
32. Gendel I, Azzam ZS, Braun E, Levy Y, Krivoy N. Antibiotic utilization prevalence: prospective comparison between two medical departments in a tertiary care university hospital. *Pharmacoepidemiol Drug Saf* 2004; **13**: 735–739. DOI: 10.1002/pds.976
33. Davey P, Brown E, Fenelon L, *et al.* Interventions to improve antibiotic prescribing practices for hospital inpatients. *Cochrane Database Syst Rev CD003543* 2005. DOI: 10.1002/14651858.CD003543.pub2

# IV

**Combined presentation of the Drug Utilization 90% segment of antibiotic use and cumulative resistance – a tool for quality assessment and intervention**

Ksenia Zagorodnikova (Goryachkina) MD CMSci[1,2], Ulf Bergman MD PhD[2], Annika Hahlin MSciPharm[3], Aleksandra Burbello MD DMSci[1], Anastasiya Fedorenko MD CMSci[1], Natalia Zaharova MD DMSci[1], Christian G. Giske MD PhD[4]

[1]*Department of Clinical Pharmacology and Therapeutics, North-western Medical University named after I.I.Mechnikov*

[2]*Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska Institutet, Sweden*

[3]*Strama-group, Stockholm County Council*

[4]*Department of Clinical Microbiology, MTC - Karolinska Institutet, Karolinska University Hospital, Sweden*

Corresponding author: Ksenia Zagorodnikova. 105067 Piskarevsky prospect, 47 St Petersburg, Russia. Tel/fax: +78125430522. ksenia.zagorodnikova@gmail.com

Keywords: antibiotic utilization, microbial resistance, quality indicators, Russia, Sweden, hospital setting, Drug Utilization 90%

Abstract

We evaluated the methodology of combined presentation of the 90% segment of antimicrobial Drug Utilization (DU90%) and cumulative microbial resistance in hospital settings. Drug utilization data were obtained from pharmacies in two hospitals in Russia and one in Sweden. The WHO ATC classification/ Defined Daily Dose (DDD) methodology was used to identify antimicrobials for systemic use and calculate utilization per year. Key microorganisms were identified according to the indications for which the antimicrobials were used in each setting and used to calculate cumulative resistance for each antibiotic within the DU90% segment based on routine microbiology data. In one of the Russian hospitals the figures were presented to the prescribers during 2007-2011, while they were created retrospectively for 2009-2011 for another Russian and a Swedish hospital. The DU90% segment comprised a lower number of antimicrobials in the Russian hospitals (n=8-14) with prevalence of fluoroquinolones, extended-spectrum cephalosporins and aminopenicillins. In the Swedish hospital a broader range was used (n=19) including penicillins, fluroquinolones, cephalosporins and carbapenems. The DU90% segment- resistance figure showed that resistance rates were higher in the two Russian hospitals. Significant changes in antimicrobial use, but not resistance were observed in the hospital where the figure was presented to prescribers in contrast to

the other two hospitals. We conclude that the DU90%-cumulative resistance figure gives information of the rationality of antimicrobial use in terms of its potential effectiveness against key microorganisms. It showed to be a promising tool to change utilization profile, though susceptibility restoration could not be achieved.

**Introduction**

During the 60 years with antibiotics resistance of microbes has increased dramatically. The society now has to face a threat of decreased potency of existing antibiotics and lack of new ones [1]. This problem was formulated in the World Health Organization global strategy 2001 for containment of antimicrobial resistance [2]. A number of factors promoting microbial resistance were identified, with two of them being the most important - poor infection control and extensive antibiotic exposure [3]. It is generally recognized that in order to improve quality of antibiotic use it is important to be able to measure it properly [4,5]. Clear relation of antibiotic use and resistance became obvious after the introduction of methods to measure antimicrobial use and relate it to the microbial resistance rates[6]. The World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification/Defined Daily Dose (DDD)[7,8] methodology was developed to make drug utilization

data comparable on different health care levels and is therefore extensively used for antibiotic consumption surveillance[9]. In order to make utilization data a possible instrument for changes in local prescribing patterns and make resistance an indicator of quality of antibiotic use it is important to perform combined surveillance of antibiotic use and resistance and find a way of comprehensive and feasible presentation of these data in combination. We have previously proposed such a method of graphic presentation of antibiotic utilization profile and cumulative resistance and tested it in a Russian hospital[10]. The graph helped to easily visualize existing problems of inexpensive antibiotic prevalence and high resistance of microbes to the most utilized agents. We could also observe decrease of overall antibiotic utilization and change in utilization profile after presenting these data.

In this study we were aiming to apply this method prospectively and test its interventional power in a more controlled manner in a Russian hospital similar to the one described in the previous paper. We were also aiming to describe patterns of antibiotic utilization and resistance in another hospital in Russia and in Sweden.

**Methods**

**Study hospitals**

The study university hospital (SPH1) in St Petersburg, Russia is a 1,300 bed tertiary care hospital with all the general departments

excluding pediatrics, neurosurgery, transplantation, haematology, infectious

diseases[1] and psychiatry. Antibiotics are purchased based on the departments' needs and are regulated by the restrictive list – hospital formulary – which, however, contains most antibiotic groups present on the international market and does not restrict prescriber's choice within this class of medications. The hospital has a system of partly restricted antibiotic distribution, according to which carbapenems, monobactams, glycopeptides, linezolid, levofloxacin, moxifloxacin and antipseudomonal penicillins and cephalosporins (ceftazidime, cefoperazone, cefepime) can be given via personal order from the department only after approval of a clinical pharmacologist.

A Russian comparison hospital (SPH2) was a tertiary care hospital in St Petersburg, where we collected the microbiology data for the period 2009 – 2011, which was the only period available to us from the hospital administration. This hospital has 1,050 beds and the same structure and patient categories as the study hospital. It has a similar way of antimicrobial use control.

The procedure of drug purchase by the hospitals in Russia is similar. The list of drugs for purchase is created when needed and is based on the requirements from the departments and analyses of

[1] In Russian hospitals infectious diseases are treated in different departments without patients' transfer to a specific infectious unit

approximate rate of utilization and medications presence in the hospital formulary list. The drugs are purchased according to this list from distributing companies via the open governmentally controlled procedure. In some cases original drugs may be replaced with generics if no specific objections are stated by the ordering hospital. Antibiotic utilization and resistance patterns in these Russian hospitals were also compared to those in a Swedish hospital (SWH) – Karolinska University Hospital, Solna for the period of 2009 – 2011. This hospital has 800 beds and is a referral hospital. Pediatric departments were excluded from the analysis. Other departments were the same with the exception of neurosurgery, infectious disease[1], hematology and transplantation that are absent in the study hospital in Russia.

**Antibiotic utilization data**

Data on antibiotic use were obtained from the hospital pharmacies and analyzed using the Anatomical Therapeutic Chemical (ATC) classification / Defined Daily Dose (DDD) methodology[8] for corresponding years. Since our first publication[10] this type of analysis was introduced in many hospitals in St Petersburg and is currently a hospital routine incorporated in a hospital internal analytic network and analyzed by clinical pharmacologists. Antibiotics in the hospital are not purchased by patients themselves, but are generally provided by the hospital. All agents within the ATC code J01

(antimicrobials for systemic use) were included in the analysis.

**Drug Utilization measurements**

The antibiotics were ranged in order of utilization volume in Defined Daily Doses (DDDs). DDD is the average maintenance dose of the drug when used on its major indication in adults [11]. It is the WHO recommended methodology for drug utilization studies. We focused on the drugs accounting for 90% of the volume by DDD: the Drug Utilization 90% (DU90%) segment[12][13]. These DU90% profiles are commonly used to assess adherence to guidelines[14][15], also within a therapeutic area[16]. Instead we assessed data on the proportion of resistant strains for the various antibiotics.

**Microbiology data**

Data on microbial susceptibility test results were obtained from the hospital bacteriological laboratories. The disk diffusion method on the Mueller-Hinton agar is used for susceptibility testing in all of the laboratories. The breakpoints were used in accordance with the Clinical and Laboratory Standards Institute (CLSI) standards[17] and national guidelines[18] in Russia; the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards[19,20] after 2011 and standards developed by the Swedish Reference Group for antibiotics[21] before 2009 in Sweden (using also another agar type, Iso-Sensitest Agar). The WHONET software[22] was used for the microbiology data management in

Russia. All sources of isolates were analyzed together. Percentage of resistance was calculated as a number of resistant + intermediate isolates/total number of isolates tested. Only the first isolate from a single patient was considered in the analysis. A list of "key" microorganisms was created for the analysis. We took into account the most relevant species for the particular indication each antimicrobial is used for in the respective countries (Table 1). Only susceptibility of naturally susceptible key microbes was analyzed for each antimicrobial agent. *Staphylococcus aureus* susceptibility to oxacillin was tested as the only way of MRSA detection in Russian hospitals while cefoxitin was used and meticillin resistance was confirmed by polymerase chain reaction (PCR) in the respective Swedish laboratory. For cumulative resistance only the key microorganisms with natural susceptibility were included.

**Instrument of combined presentation of the utilization and resistance data.**

The method first presented in our paper in 2008[10] was tested as a way of alerting prescribers against excessive use of antimicrobials. Antibiotics utilization data were structured in descending order and presented in a diagram, the focus was put on the antibiotics within the DU90% segment as the segment that is most influential[12]. Each bar in the diagram was then divided into two differently colored segments. The whole bar represents 100% of microbial

susceptibility to this particular antimicrobial agent. The part of the bar corresponding to the percentage of strains of the key microbes naturally susceptible to this agent that were found to be resistant during the observation year was colored red, while the rest part corresponding to the percentage of sensitive strains was colored green (Figure 1).

The figure was presented to the heads of the departments in the hospital and to the hospital authorities on an annual conference, where people responsible for treatment policies are present. Similar data were collected each year and discussed on the annual meetings with the hospital authorities since early 2008. Similarly utilization and resistance data were presented each year for the administration. The observation period was five years (2007-2011). For comparison we used another Russian hospital (SPH2), but these data were not shown to the hospital authorities. We also created similar figures for a Swedish hospital (SWH) to test the method in different circumstances and compare patterns of antibiotic use and resistance internationally. In these two hospitals the observation period was 2009-2011.

**Statistical analysis**

Chi-square ($\chi 2$) test was used to compare antibiotic use in different time periods, Spearman rank correlation test was used for defining relations of antibiotics use and microbial resistance. Results from

long-term observations were presented as the median [min, max].

**Ethical considerations**

No data on individual patients were collected in this study and the surveillance was part of a quality assurance. Ethics committees were approached, and ethical approval was not considered necessary.

**Results**

*Antibiotic utilization profiles in the three study hospitals*

Total antimicrobial use in the Russian hospital was 22.8; 31.2; 25.1; 24.5 and 28.7 DDD/100 bed-days in 2007; 08; 09; 10 and 2011 respectively (Figure 2). The number of beds was not changed during the study period. The number of antibiotics within the DU90% segment varied from 11 to 14, and the total number of antibiotics used varied from 31 to 43 during the observation period. Quinolone antibacterials (J01M), penicillins with extended spectrum (J01CA), and extended-spectrum cephalosporins (J01DD) prevailed in the beginning of observation in 2007, with only minor use of potent antibiotics active against multi-resistant microorganisms.

Prevalence of antimicrobials was comparable although slightly higher in the comparison Russian hospital: 38; 43 and 43 DDDs/100 bed-days in 2009; 10 and 2011 respectively (Figure 2a). The list of antibiotics used comprised 36; 32 and 33 different agents, while the DU90% segment consisted of eight antibiotics: ciprofloxacin, ceftriaxone, cefazolin, metronidazole, ampicillin, ampicillin with

enzyme inhibitor, clarithromycin and an aminoglycoside (gentamicin/amikacin).

In the Swedish hospital (SWH) antimicrobial exposure was 58 DDD/100 bed-days in 2009; 57 in 2010 and 58 in 2011 (Figure 2a). The total number of antibiotics used was 49; 47 and 49 in 2009; 2010 and 2011 respectively with 19 antibiotics present in the DU90% segment each year. The segment contained beta-lactamase sensitive penicillins (J01CE) beta-lactamase resistant penicillins (J01CF), 2nd and 3rd generation cephalosporins (J01DC; J01DD), carbapenems (J01DH) and vancomycin.

Distribution of major antibiotic classes for each hospital is presented in Figure 3.

*Microbial resistance in the three study hospitals*

High cumulative resistance levels were observed in both Russian hospitals during the whole observation period (Figure 2), which was confirmed by the detailed resistance pattern (Table 2 a,b) comprising cumulative resistance. Presence of the *mecA* gene was not tested, nor was the cefoxitin disc method used for MRSA detection,as recommended in the EUCAST methodology..

In the Swedish hospital cumulative resistance levels were low (Table 2c).

*Cumulative utilization/resistance figure*

The figure showing antibiotic use and cumulative resistance of the

key microorganisms (Figure 1) was presented to the study hospital prescribers and authorities in early 2008. It revealed a general problem of high levels of resistance in the whole segment of agents used. Prospective annual surveillance of antibiotic use and resistance in combination was established in the hospital after the first intervention (Table 1). Next year figure showed that the use of ciprofloxacin decreased from 5.2 DDD/100 bed-days to 3.5 DDD/100 bed-days ($p<0.0001$); ampicillin use increased from 3.1 to 6.3 DDD/100 bed-days ($p<0.0001$). There was also an increase in cephalosporins use: ceftriaxone+cefotaxime use increased from 2.4 DDD/100 bed-days to 4.5 DDD/100 bed-days ($p<0.0001$).

The next year, 2009 showed return of fluoroquinolones to the top position, and the whole segment of DU90% was changing each year. General trends were in increase of beta-lactams with enzyme inhibitors use (amoxicillin/clavulanate + amlicillin/sulbactam use was 0.4 DDD/100 bed-days (1.6%) in 2007, 1.3 DDD/100 bed-days (4%) in 2008, 2.2 DDD/100 bed-days (8.6%) in 2009, 3.2 DDD/100 bed-days (13%) in 2010 and 4.8 DDD/100 bed-days (16.8%) in 2011). Despite utilization changes we did not observe any significant changes in the susceptibility levels of microorganisms (Table 2a).

In the comparator Russian hospital (SPH2) the DU90% segment was stable during all three years of observation (Figure 2).

Cumulative resistance rates were also generally stable with slight increase of resistance to fluoroquinolones (53-64%). Oxacillin resistant *Staphylococcus aureus* rates were 48%-55%-44% in 2009, 2010 and 2011 respectively. Tendency to increased resistance of *Pseudomonas aeruginosa* to imipenem was observed (46%, 54% and 66% in 2009, 2010 and 2011 respectively).

Antibiotics utilization profiles in the Swedish hospital was stable without significant changes in 2009-2011. The only tendency to gradually decreased cefuroxime use was not statistically significant. Cumulative resistance rates were also generally unchanged.

*Statistical relations of utilization and resistance*

We found correlation of aminopenicillins use and corresponding *E.coli* resistance (Spearman r 1, p=0.02), but no correlation of *E.coli* resistance and use of extended-spectrum cephalosporins, fluoroquinolones or aminoglycosides. There was also no correlation between *K. pneumoniae* and *P.aeruginosa* resistance and use of any group of antibacterials. We did not study correlations for the other two hospitals due to a shorter observation period.

**Discussion.**

*Antimicrobial utilization patterns*

Antimicrobial use have been related to bacterial resistance in many studies[23], therefore its surveillance is considered essential. Large international programs have been established in order to monitor

and compare antibiotic utilization internationally[5]. The first international comparison of antibiotic consumption in hospital setting performed by the European network for Surveillance of Antimicrobial Consumption (ESAC-net) group was retrospective[24]. There are also a number of point-prevalence studies directly comparing antibiotic use in different hospitals [13,25,26]. In this study we were testing the methodology that would help to evaluate data on antibiotic consumption and resistance in hospital settings at a glance. Total number of antibiotics used in a Russian study hospital (SPH1) varied during the observation period between 23 and 31 DDDs/100 bed-days with general slight tendency to increase. In the Swedish university hospital this number was almost twice higher. This corresponds to previous comparisons of antibiotic utilization in a Baltic region hospital in Vilnius and Huddinge, where antibiotic utilization was three times higher in the Swedish University hospital, than in Vilnius (43 vs 15 DDD/100 bed-days)[25]. This difference could only be partly explained by differences in the hospital structures. One difference was the absence of oncology and hematology departments in the Russian hospitals, but still these departments together were responsible only for 5% of the consumption in the Swedish hospital in 2011 (oncology 3% and hematology 2%). Slight differences could be brought by variation in prescribed doses that

may not equal the assigned DDDs, though the differences observed are too high to be explained by this fact only. It was shown in the literature that antibiotic utilization is lower in Scandinavian countries both in the outpatient [6,27] and in the hospital setting[24], and lower rates of resistance were attributed to this. However, Russian data were not published in the international context, and our data cannot be related to other publications. The spectra were also different. Prevalence of aminopenicillins, extended-spectrum cephalosporins and fluoroquinolones in the Russian hospitals correspond to the structure of antibiotic use in Europe according to the ESAC-Net data [24]. These spectra were different from the one described in our previous study from another similar hospital [10], where aminoglycosides (gentamicin) represented 20% of the whole antimicrobial utilization pattern. A similar picture could be seen in the mentioned above Lithuanian study [25]. The Swedish hospital was different in that higher prevalence of the most potent antibiotics – carbapenems and vancomycin – together with beta-lactamase resistant penicillins was observed (Figure 3), which may be explained by different patient characteristics, but also by different strategies of antibiotic use, because prevalence of consumption of beta-lactamase resistant penicillins and carbapenems was characteristic for Sweden in the above mentioned ESAC-Net study[24]. As we mentioned in the methods section all classes of

antibiotics are generally available on both the Russian and the Swedish market. The amount of purchase, however, is confined by hospital budgets. According to the world health statistics data total expenditures on health as a percentage of gross domestic product are almost twice higher in Sweden than in Russia[28] which may lead to higher prevalence of cheaper drugs and generics. Also lower prevalence of carbapenems may be explained by restrictive pre-authorized approach to prescription of this group in Russian hospitals.

*Microbial resistance*

Despite lower antibiotic use we observed considerably higher levels of microbial resistance in both Russian hospitals. We did not differentiate between the origins of the species tested following the idea that overall antibiotic pressure would guide overall resistance of primarily hospital microbes. In many cases resistance levels exceeded 50%. In the study hospital the data could be uncertain due to low number of species tested, but we observed similarly high resistance levels in the second Russian hospital (SPH2), where the number of species was in most cases sufficient for such calculations (Table 2b). In contrast to recent European studies[29] we did not observe correlation of fluoroquinolones use and resistance of Enterobacteriaceae. A possible explanation could be circulation or resistant strains without sufficient control of this transfer. There have

been good studies demonstrating that infection control may decrease infections and microbial resistance in health-care settings[30,31]. These strategies are not fully implemented in the study hospitals (no infection control team or controlled strategy) which may explain high resistance and lack of its direct association with utilization of antimicrobials.

*Methodology of combined presentation of utilization and resistance*

We tested the methodology of combined presentation of utilization and resistance. In our previous study [10] the figure was created and revealed clear problems in the rationality of antimicrobials use – prevalence of a limited number of antibiotics, and high levels of microbial resistance. In the current study we improved the methodology to make it more informative and setting-specific. We defined the key-microorganisms for each setting, which would help to evaluate figures in terms of practical use of antibiotics. There have been numerous attempts to combine antibiotic utilization and resistance in one indicator[32,33], since these relationships are obviously important in long-term perspective[6,34,35]. A «drug resistance index», which is based on an advanced calculation and composite evaluation of antibacterial use and a certain microbial resistance was recently proposed [33]. This index shows trends of resistance related to use for each drug-bacteria combination, which

makes it unfeasible for prescribers to grasp general situation. Another indicator used is correlation of percentage of patients receiving antibiotic and rate of infections caused by resistant flora[29]. This approach requires specific selection of a pair antibiotic-resistant microbe and does not allow seeing the whole picture of rational antimicrobials use. The figure combining key microorganisms susceptibility data and prevalence of corresponding antibiotic utilization has a benefit of highlighting the problem in a way easily interpreted by both prescribing physicians and hospital management. We used a concept of DU90%, based on the fact that this segment is the most influential and could be used for quality evaluation[12,14]. In these studies we are developing the concept further using the assumption that resistance might be looked at as inappropriate quality of antibiotic use. The differences observed between the figures from the Russian hospitals and the Swedish hospital indicate differences in potential effectiveness of the most widely prescribed antibiotics against microbes, i.e. differences in rationality of use.

In our previous paper [10] we observed changes following the presentation of the figure of combined utilization-resistance profile to the hospital authorities. In the current study we could also observe significant changes in the utilization profile – decrease of fluoroqhinolones consumption increase of aminopenicillins, and

general increase of the number of various antibiotics the next year after the intervention. The changes, however, were unstable and resulted in return of fluoroquinolones and extended-spectrum cephalosporins, and increase of aminopenicillins combined with enzyme inhibitor use. The assumption that these changes could be mostly due to the figure presentation is supported by the fact that there were no changes in our control hospital (SPH2) with a similar structure of drug utilization and infection control despite similar problems in microbial resistance observed. There were also no changes in the Swedish hospital. In local perspective it could be seen from the figure that resistance is present for extended-spectrum cephalosporins and trimetoprim-sulfamethoxazole, which is most obvious from separate microbial resistance data (Table 2a).

An unexpected finding was that lower overall antibiotic use in Russian hospitals was accompanied by much higher resistance levels. These high levels were not changed after the changes in utilization profile. At the same time in the Swedish hospital resistance levels were generally low despite high exposure. This may indicate that infection control strategies besides antimicrobial use are important to combat microbial resistance. Since many years Sweden has had an aggressive strategy of combating spread of antimicrobial resistance in health care institutions. Active screening, high focus on hand hygiene and contact precautions, as well as using cohort care

or patient isolation, have all been part of the strategy to limit nosocomial spread[36].

*Limitations.*

Our study was based on a natural observation with some elements of intervention. Since the periods of time for the data collection was different during 2007-2008 the study cannot be considered as controlled. The DDD concept has certain methodological limitations that have been widely discussed and that can also be referred to the current study. These are the problems of differences in DDDs and PDD (Prescribed Daily Doses) that may vary in time and lead to certain variability in the final data. Number of admitted patients may also vary and influence the results which has been repeatedly addressed in the literature[37]. With regard to microbiology data one limitation was the very low number of strains analyzed that were used to produce percentage of resistance in the Russian hospitals. We relied upon internal quality control in the bacteriology laboratory and did not perform external quality check of the results, There was also a possible bias in the selection of species for susceptibility testing. Some of the resistance rates reported for Sweden in this paper are significantly higher than those reported in the EARS-Net surveillance system[38]. The main bias is that some bacteria are only tested against some compounds in specific situation, e.g. when the isolate is derived from specific important departments or when the

isolate is resistant to a lot of first-line agents. This is a likely explanation for the high rates of resistance to extended-spectrum cephalosporins in *E. coli* and methicillin-resistance in *S. aureus*. One way to come around this problem is to focus the susceptibility data on blood culture isolates only, as they are normally tested against all antimicrobial agents. However, trends of resistance development within one institution will not be affected by this bias, so it will mainly be a problem when trying to extract information on resistance levels of specific bacteria-antimicrobial combinations. For better quality of cumulative resistance data a stable number of isolates and definite sources are both important, and the data observed in the current naturalistic study should not be interpreted in a more global perspective, but rather should be a screening tool for local or regional use [39].

## Conclusion

The figure of combined presentation of antimicrobial utilization 90% profile and key microbes resistance is a useful tool to reveal irrational antimicrobial use. It might be of value in international comparisons where different key microbes could be identified making the figure more setting specific and therefore valuable, but should not be overestimated due to biases related to all cumulative data. It may be successfully used as a component of local quality control and demonstration of such data may influence prescribers'

behavior. Further development of this method as a quality metrics could be inclusion of patient outcome data – expressed in numbers of treatment failure. The approach to local infection control should be multifaceted and include thorough microbiology and drug utilization screening together with measures to improve local hygiene, and the methodology presented could be a part of antibiotic stewardship programs but is not powerful enough to improve quality of treatment if used alone.

Reference list


1. Clarke T. Drug companies snub antibiotics as pipeline threatens to run dry Europe offers grants to young stars. Nature. 2003;425(September):2003.
2. World Health Organization. Strategy for Containment of Antimicrobial Resistance. 2001.
3. Weinstein R a. Controlling antimicrobial resistance in hospitals: infection control and use of antibiotics. Emerging infectious diseases [Internet]. 2001;7(2):188–92. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2631704&tool=pmcentrez&rendertype=abstract
4. Rosdahl VT, Pedersen KB. The Copenhagen Recommendations The Microbial Threat. Copenhagen; 1998.
5. Adriaenssens N, Coenen S, Versporten A, Muller A, Vankerckhoven V, Goossens H. European Surveillance of Antimicrobial Consumption (ESAC): quality appraisal of antibiotic use in Europe. The Journal of antimicrobial chemotherapy [Internet]. 2011 Dec [cited 2012 Aug 3];66 Suppl 6:vi71–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22096068
6. Goossens H, Ferech M, Vander Stichele R, Elseviers M.

Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet [Internet]. 2005;365(9459):579–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15708101

7. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment. Oslo; 2007.

8. WHOCC - Home [Internet]. Available from: http://www.whocc.no/

9. Ibrahim OM, Polk RE. Benchmarking antimicrobial drug use in hospitals. Expert review of anti-infective therapy [Internet]. 2012 Apr;10(4):445–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22512754

10. Goryachkina K, Babak S, Burbello A, Wettemark B, Bergman U. Quality use of medicines : A new method of combining antibiotic consumption and sensitivity data — application in a Russian hospital y. Pharmacoepidemiology and drug safety. 2008;17(January):636–44.

11. WHO | Drug Utilization: ATC/DDD. World Health Organization; [cited 2013 Apr 3]; Available from: http://www.who.int/medicines/areas/quality_safety/safety_efficacy/utilization/en/

12. Bergman U, Popa C, Tomson Y, Wettermark B, Einarson TR, Aberg H, et al. Drug utilization 90%--a simple method for assessing the quality of drug prescribing. European journal of clinical pharmacology [Internet]. 1998 Apr [cited 2012 Aug 15];54(2):113–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9626914

13. Vlahović-Palcevski V, Dumpis U, Mitt P, Gulbinovic J, Struwe J, Palcevski G, et al. Benchmarking antimicrobial drug use at university hospitals in five European countries. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases [Internet]. 2007 Mar;13(3):277–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17391382

14. Gustafsson LL, Wettermark B, Godman B, Andersén-Karlsson E, Bergman U, Hasselström J, et al. The “wise list”- a comprehensive concept to select, communicate and achieve adherence to recommendations of essential drugs in ambulatory care in Stockholm. Basic & clinical pharmacology & toxicology [Internet]. 2011 Apr [cited 2013 Feb 19];108(4):224–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21414143

15. Wettermark B, Vlahovic-Palcevski V, Laing R, Bergman U. Adherence to WHO's Model List of Essential Medicines in two European countries. WHO drug information. 2006;20(2):78–85.

16. Vlahovic-Palcevski V, Wettermark B, Bergman U. Quality of non-steroidal anti-inflammatory drug prescribing in Croatia (Rijeka) and Sweden (Stockholm). European journal of clinical pharmacology [Internet]. 2002 Jun [cited 2013 Feb 19];58(3):209–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12107607

17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. M100-S23. 2013;33(1).

18. Guidelines for susceptibility testing of microorganisms to antibacterial agents. Klinicheskaya microbiologiya i antimicrobnaya chimioterapiya. 2004;6(4):306–59.

19. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters [Internet]. 2013 [cited 2013 Feb 22]. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf

20. Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, Macgowan a P, et al. EUCAST expert rules in antimicrobial susceptibility testing. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases [Internet]. 2013 Feb;19(2):141–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22117544

21. Swedish Reference Group for Antibiotics [Internet]. [cited 2013 Feb 22]. Available from: www.srga.org

22. World Health Organization. WHONET software [Internet]. [cited 2013 Feb 22]. Available from: http://www.who.int/drugresistance/whonetsoftware/en/

23. Jensen US, Skjøt-Rasmussen L, Olsen SS, Frimodt-Møller N, Hammerum AM. Consequences of increased antibacterial consumption and change in pattern of antibacterial use in Danish hospitals. The Journal of antimicrobial chemotherapy [Internet]. 2009 Apr [cited 2012 Aug 15];63(4):812–5. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/19240071

24. Vander Stichele RH, Elseviers MM, Ferech M, Blot S, Goossens H. Hospital consumption of antibiotics in 15 European countries: results of the ESAC Retrospective Data Collection (1997-2002). The Journal of antimicrobial chemotherapy [Internet]. 2006 Jul [cited 2012 Aug 3];58(1):159–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16698845

25. Gulbinovic J, Myrbäck KE, Bytautienĕ J, Wettermark B, Struwe J, Bergman U. Marked differences in antibiotic use and resistance between university hospitals in Vilnius, Lithuania, and Huddinge, Sweden. Microbial drug resistance (Larchmont, N.Y.) [Internet]. 2001 Jan;7(4):383–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11822778

26. Dumpis U, Gulbinovic J, Struwe J, Lagergren a, Griskevicius L, Bergman U. Differences in antibiotic prescribing in three university hospitals in the Baltic region revealed by a simple protocol for quality assessment of therapeutic indications. International journal of clinical pharmacology and therapeutics [Internet]. 2007 Oct;45(10):568–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17966843

27. Cars O, Mölstad S, Melander A. Variation in antibiotic use in the European Union For personal use . Only reproduce with permission from The Lancet Publishing Group . Lancet. 2001;357(62):1851–3.

28. Health financing: Health expenditure ratios by country [Internet]. [cited 2013 Apr 8]. Available from: http://apps.who.int/gho/data/view.main.1901

29. team EC for DP and C (ECDC)-HCU-E editorial. Trends in yearly prevalence of third-generation cephalosporin and fluoroquinolone resistant Enterobacteriaceae infections and antimicrobial use in Spanish hospitals, Spain, 1999 to 2010 [Internet]. European Centre for Disease Prevention and Control (ECDC) - Health Comunication Unit; 2011 [cited 2013 Apr 2]. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19983

30. team EC for DP and C (ECDC)-HCU-E editorial. Pathways to clean hands: highlights of successful hand hygiene implementation strategies in Europe [Internet]. European Centre for Disease Prevention and Control (ECDC) - Health Comunication Unit; 2010 [cited 2013 Apr 8]. Available from:

http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19560

31. Yngström D, Lindström K, Nyström K, Nilsson-Marttala K, Hillblom L, Hansson L, et al. Healthcare-associated infections must stop: a breakthrough project aimed at reducing healthcare-associated infections in an intensive-care unit. BMJ quality & safety [Internet]. 2011 Jul [cited 2013 Apr 8];20(7):631–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21642444

32. Sjöstedt S, Levin P, Kager L, Malmborg AS, Bergman U. Hospital and catchment area antibiotic utilization and bacterial sensitivity in primary infections following gastric surgery in Huddinge, Sweden. European journal of clinical pharmacology [Internet]. 1990 Jan [cited 2013 Feb 21];39(3):211–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2257854

33. Laxminarayan R, Klugman KP. Communicating trends in resistance using a drug resistance index. BMJ open [Internet]. 2011 Jan [cited 2013 Feb 21];1(2):e000135. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3221297&tool=pmcentrez&rendertype=abstract

34. Sörberg M, Farra A, Ransjö U, Gårdlund B, Rylander M, Wallén L, et al. Long-term antibiotic resistance surveillance of gram-negative pathogens suggests that temporal trends can be used as a resistance warning system. Scandinavian journal of infectious diseases [Internet]. 2002 Jan;34(5):372–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12069023

35. Mimica Matanovic S, Bergman U, Vukovic D, Wettermark B, Vlahovic-Palcevski V. Impact of restricted amoxicillin/clavulanic acid use on Escherichia coli resistance--antibiotic DU90% profiles with bacterial resistance rates: a visual presentation. International journal of antimicrobial agents [Internet]. Elsevier B.V.; 2010 Oct [cited 2012 Aug 16];36(4):369–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20688486

36. STRAMA. ESBL resistance in enteric bacteria. 2007. p. 24.

37. Bergman U, Risinggård H, Vlahović-Palcevski V, Ericsson O. Use of antibiotics at hospitals in Stockholm: a benchmarking project using internet. Pharmacoepidemiology and drug safety [Internet]. 2004 Jul [cited 2013 Feb 23];13(7):465–71. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/15269930

38. EARS-Net database.

39. Schwaber MJ, De-Medina T, Carmeli Y. Epidemiological interpretation of antibiotic resistance studies - what are we missing? Nature reviews. Microbiology [Internet]. 2004 Dec [cited 2013 Apr 2];2(12):979–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15550943

**Figure 2. Drug utilization 90% - cumulative resistance profiles in the Russian study hospital (SPH1) in 2007-2011**

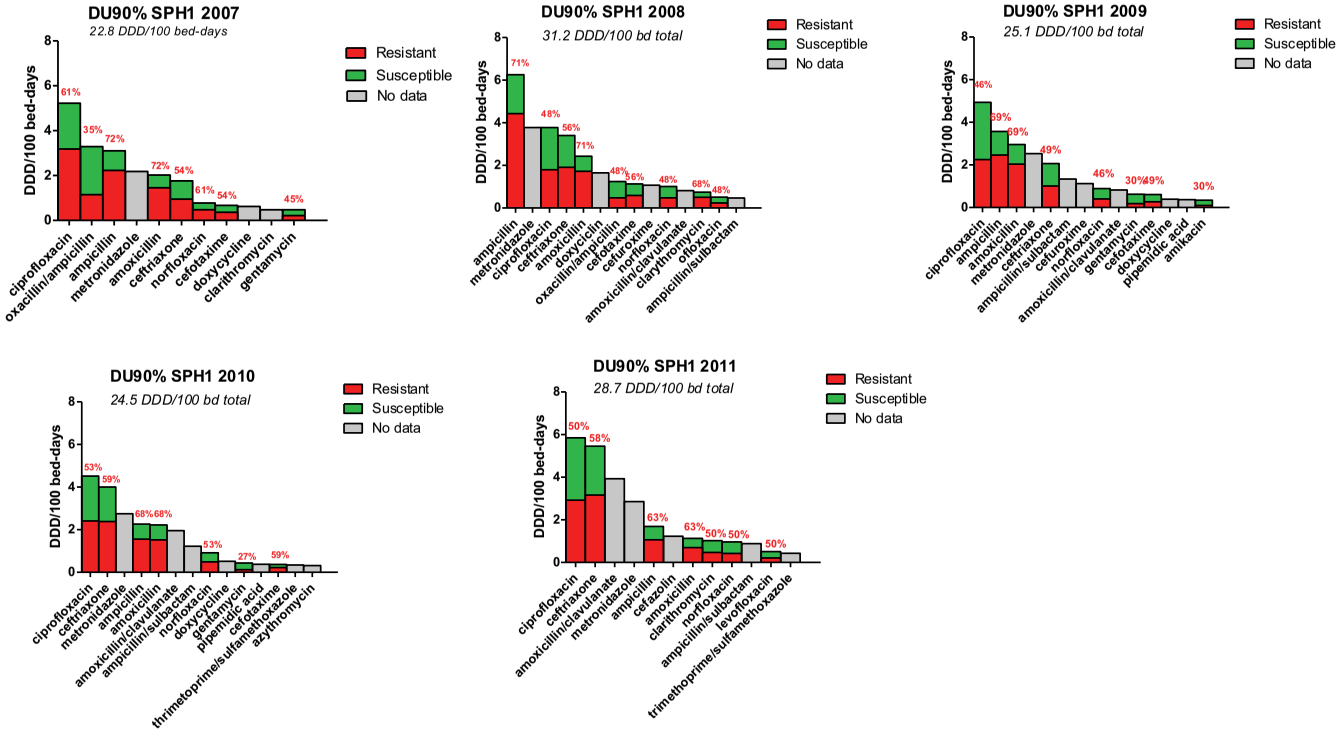


Figure 2a. Drug utilization 90% - cumulative resistance profiles in the Russian control hospital (SPH2) – upper row, and the Swedish hospital (SWH) – lower row in 2009-2011

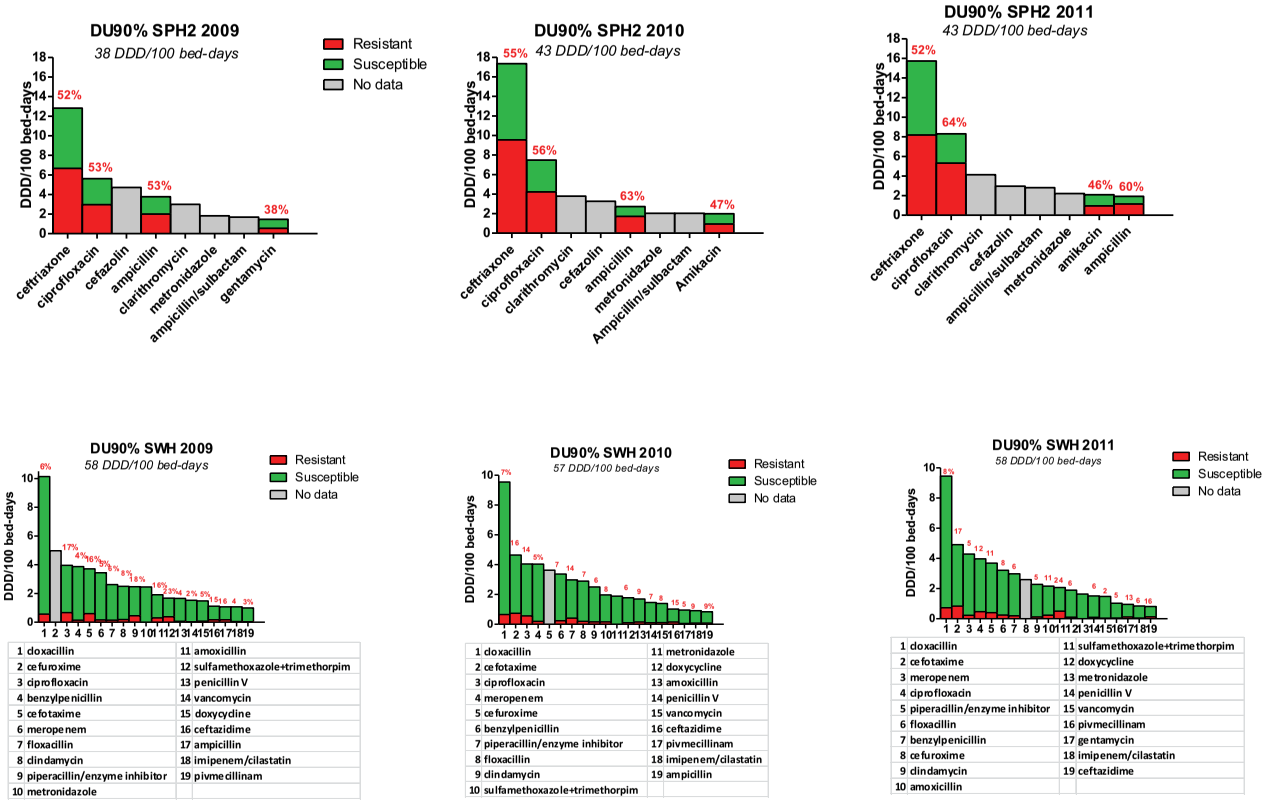


Figure 3. Antibiotic use and distribution of main classes in three study hospitals during the observation years (2007-2011 or 2009-2011)

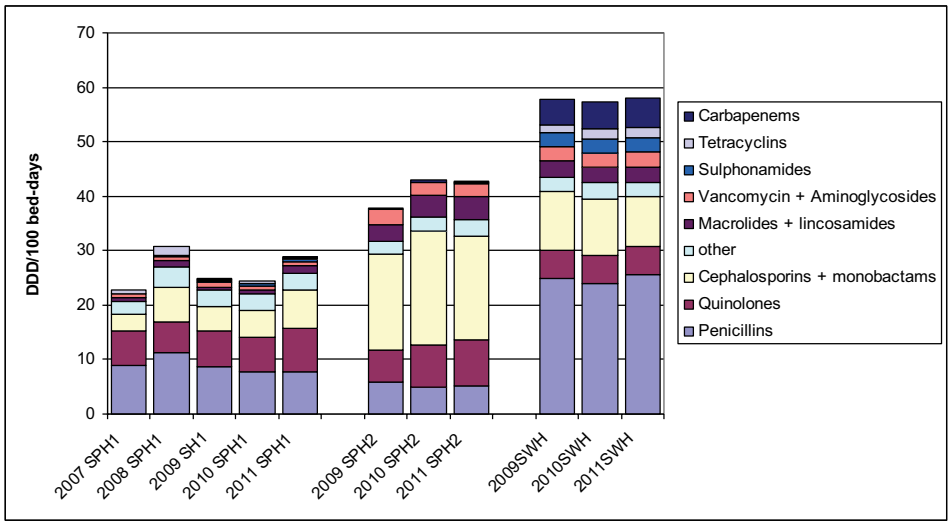


Table 1.

# Key microorganisms in Russian and Swedish hospitals

| Antibiotic | Key microorganisms in Swedish hospitals | Key microorganisms in Russian hospitals | Notes |
|---|---|---|---|
| **Penicillins** | | | |
| benzylpenicillin | S. pneumoniae, S.pyogenes | S. pneumoniae, group A streptococcus | |
| amoxicillin | H.influenzae | E.coli, Enterococcus faecalis, | H.influenzae was rarely detected in Russian study hospitals |
| ampicillin | H.influenzae | E.coli, Enterococcus faecalis, streptococci | H.influenzae was rarely detected in Russian study hospitals |
| cloxacillin | S. aureus | No | Not in use in Russia |
| flucloxacillin | S. aureus | No | Not in use in Russia |
| fenoximethylpenicillin | S. pneumoniae, group A streptococcus | No | Not in use in Russia |
| oxacillin/ampicillin | No | staphylococci | An outdated combination never used in Sweden |
| amoxicillin/clavulanate | | Enterobacteriaceae, streptococci | |
| ampicillin/sulbactam | | Enterobacteriaceae, streptococci | |
| piperacillin/tazobactam | Enterobacteriaceae, P. Aeruginosa, Acinetobacter, B. fragilis group | No | The drug was not in use during the test period in Russia |
| **Cephalosporins** | | | |
| cefazolin | No | No | |
| cefuroxime | No | staphylococci | No testing is routinely performed in Sweden, |
| ceftriaxone | Enterobacteriaceae | Enterobacteriaceae | |
| cefotaxime | Enterobacteriaceae | Enterobacteriaceae | |
| cefoperazone | | Enterobacteriaceae, P. aeruginosa | these agents are used in Russia mostly where pseudomonas is suspected |
| ceftazidime | Enterobacteriaceae | Enterobacteriaceae, P. aeruginosa | these agents are used in Russia only where pseudomonas is suspected |
| cefoperazone/sulbactam | | Enterobacteriaceae, P. aeruginosa | these agents are used in Russia mostly where pseudomonas is suspected |
| **Carbapenems** | | | |
| meropenem | Enterobacteriaceae, P. Aeruginosa, Acinetobacter, B. fragilis group | Enterobacteriaceae, P. aeruginosa, Acinetobacter | No anaerobe testing is routinely performed in Russia |
| doripenem | | Enterobacteriaceae, P. aeruginosa, Acinetobacter | Was not in use during the study period in Sweden |
| imipenem/cilastatin | Enterobacteriaceae, P. Aeruginosa, Acinetobacter, B. fragilis group, E. Faecalis | Enterobacteriaceae, P. aeruginosa, Acinetobacter, E. faecalis | No anaerobe testing is routinely performed in Russia |
| **Lincosamides** | | | |
| clindamycin | S. aureus, group A S.pyogenes | S. aureus, group A streptococcus | |
| lincomycin | S. aureus, group A streptococcus | S. aureus, group A streptococcus | |
| **Fluoroquinolones** | | | |
| ciprofloxacin | Enterbacteriaceae, P. aeruginosa, Acinetobacter | Enterbacteriaceae, P. aeruginosa, Acinetobacter | |
| norfloxacin/ofloxacin /pefloxacin | | Similar to ciprofloxacin | |
| levofloxacin | | Enterobacteriaceae, S.pneumoniae | |
| moxifloxacin | | Enterobacteriaceae, S.pneumoniae | |

| | | | |
|---|---|---|---|
| **Quinolones** | | | |
| pipemidic acid | | no | |
| **Tetracyclines** | | | |
| doxycycline | H.influenzae, S. pneumoniae | H.influenzae, S. pneumoniae | |
| **Aminoglycosides** | | | |
| gentamycin | E. coli, K. Pneumoniae, S. aureus | E. coli, K. pneumoniae, S. aureus | |
| amikacin | E. coli, K. Pneumoniae, S. aureus | E. coli, K. pneumoniae, S. aureus | |
| **Macrolides** | | | |
| clarithromycin | | no | Hospital use is mostly for H.pylori in Russian hospitals |
| azythromycin | | no | |
| **Various** | | | |
| metronidazole | B.fragilis group | no | No anaerobes were cultured in Russian hospitals |
| trimethoprim-sulfamethoxazole | Enterobacteriaceae, H. influenzae, S. Pneumoniae | | |
| vancomycin | S. aureus, E. Faecium | S. aureus, E. faecium | |

Table 2a. Rates of resistance of selected bacteria to antimicrobial agents during 5 consecutive years SPH1l

| Antibiotic | Resistant isolates, % (n of strains) | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | | | *K.pneumoniae* | | | | | *P.aeruginosa* | | | | | *S.aureus* | | | | | *Enterococcus spp.* | | | | |
| | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 | 2007 | 2008 | 2009 | 2010 | 2011 |
| Ampicillin | 73 (96) | 67 (40) | 63 (90) | 46 (26) | 66 (345) | 100 (48) | 100 (7) | 93 (15) | 100 (2) | 90 (95) | | | | | | | | | | | 42 (76) | 54 (46) | 50 (66) | 60 (5) | 33 (255) |
| Oxacillin | | | | | | | | | | | | | | | | 40 (158) | 36 (63) | 30 (76) | 17 (97) | 32 (452) | | | | | |
| Ciprofloxacin | 62 (97) | 27 (15) | 45 (87) | 46 (93) | 48 (352) | 77 (48) | 71 (7) | 73 (15) | 59 (17) | 61 (113) | 58 (48) | 60 (10) | 57 (14) | 42 (19) | 62 (55) | 43 (160) | 42 (36) | 38 (86) | 20 (97) | 30 (356) | | | | | |
| Ceftriaxone | 55 (95) | 41 (17) | 42 (86) | 45 (49) | 45 (497) | 72 (47) | 83 (6) | 78 (14) | 58 (12) | 71 (138) | | | | | | | | | | | | | | | |
| Gentamicin | 58 (96) | 36 (39) | 34 (90) | 24 (58) | 27 (422) | 77 (48) | 71 (7) | 60 (15) | 63 (8) | 46 (117) | 61 (49) | 50 (14) | 33 (15) | No data | 50 (104) | | | | | | | | | | |
| Vancomycin | | | | | | | | | | | | | | | | | | | | | 7 (80) | 9 (46) | 3 (67) | 0 (26) | 0.3 (358) |
| Ceftazidime | | | | | | | | | | | 60.4 (48) | 71 (14) | 40 (15) | 21 (14) | 35 (84) | | | | | | | | | | |
| *N of isolates* | *97* | *40* | *91* | *115* | *610* | *48* | *7* | *15* | *18* | *147* | *49* | *15* | *15* | *23* | *129* | *160* | *66* | *90* | *98* | *524* | *81* | *49* | *67* | *39* | *495* |

# Table 2b. Rates of resistance of selected bacteria to antimicrobial agents during 3 consecutive years SPH2

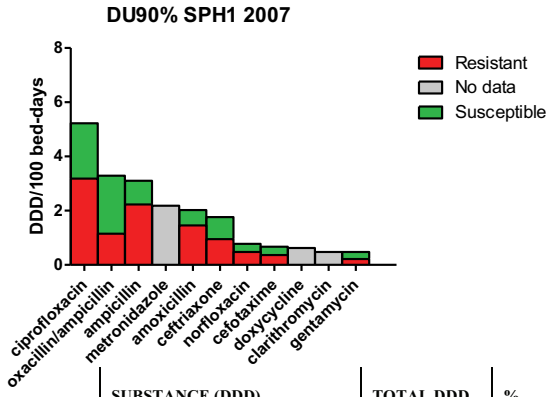
| Antibiotic | Resistant isolates, % (n of strains) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | *K.pneumoniae* | | | *P.aeruginosa* | | | *S.aureus* | | | *Enterococcus spp.* | | |
| | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 |
| Ampicillin/amoxicillin | 58 (426) | 66 (341) | 81 (304) | 99 (195) | 100 (182) | 99 (136) | | | | | | | 26 (288) | 38 (291) | 46 (197) |
| Oxacillin | | | | | | | | | | 45 (282) | 52 (272) | 42 (234) | | | |
| Ciprofloxacin | 36 (425) | 44 (149) | 17 (12) | 82 (195) | 75 (88) | | 52 (98) | 54 (67) | 65 (99) | 52 (273) | 58 (256) | 52 (229) | | | |
| Ceftriaxone | 30 (431) | 41 (420) | 42 (595) | 82 (197) | | 77 (290) | | | | | | | | | |
| Amikacin | | | | | | | 6 (48) | 47 (61) | 15 (101) | | | | | | |
| Gentamicin | 22 (424) | 31 (421) | 35 (562) | 70 (191) | | | 26 (60) | 24 (42) | | | | | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vancomycin | | | | | | | | | | 0 (231) | 0 (272) | 1 (227) | 2.6 (267) | 1 (347) | 6 (387) | |
| Imipenem | 2 (427) | 4 (422) | 3 (562) | 17 (196) | | 8 (269) | 45 (97) | 43 (82) | 57 (114) | | | | | | | |
| Ceftazidime | | | | | | | 46 (54) | 37 (60) | 29 (100) | | | | | | | |

Table 2c. Rates of resistance of selected bacteria to antimicrobial agents during 3 consecutive years (SWH)

| Antibiotic | Resistant isolates, % (n of strains) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *E.coli* | | | *K.pneumoniae* | | | *P.aeruginosa* | | | *S.aureus* | | | *Enterococcus spp.* | | |
| | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 |
| Ampicillin | | | | | | | | | | | | | 0.3 (1541) | 0.2 (3739) | 0.1 (2282) |
| Pivmecillinam | 2 (14436) | 5 (244347) | 4 (29739) | 16 (1579) | 10 (1852) | 9 (2514) | | | | | | | | | |
| Cloxacillin | | | | | | | | | | 5.6 (12962) | 6.8 (21214) | 7.7 (26749) | | | |
| Ciprofloxacin | 17 (16234) | 14 (27738) | 12 (34166) | 17 (2065) | 9.6 (2395) | 7.5 (4044) | 18 (1681) | 19 (3059) | 17 (3814) | | | | | | |
| Cefotaxime | 17 (3581) | 17 (8559) | 19.5 (11191) | 6 (994) | 9.5 (2389) | 7.5 (2705) | | | | | | | | | |
| Trimethoprim/sulfamethoxazole | 24 (14675) | 26 (27502) | 26 (33819) | 23 (992) | 20 (3292) | 18 (3919) | | | | | | | | | |
| Clindamycin | | | | | | | | | | 7.7 (4623) | 6.6 (11237) | 5.5 (14576) | | | |
| Vancomycin | | | | | | | | | | 0 (262) | 0 (54) | no data | 2.6 (660) | 8.6 (938) | 2.3 (1148) |
| Meropenem | 0.08 (3504) | 0.01 (6264) | 0.09 (7960) | 0.3 (961) | 1.3 (1577) | 1.3 (1715) | 20 (1679) | 19 (3058) | 19 (3783) | | | | | | |
| Piperacillin/tazobactam | | | | | | | 12 (1684) | 13 (3064) | 13.5 (3774) | | | | | | |
| Ceftazidime | | | | 6 (989) | 10 (2386) | 8 (2690) | 13 (1683) | 14 (3059) | 13 (3777) | | | | | | |

Figure 1. Method of combined presentation of DU90% volume of antibiotic use and cumulative resistance (intervention tool)




| | SUBSTANCE (DDD) | TOTAL DDD | % | DDD/100 BED-DAYS | % RESISTANCE |
|---|---|---|---|---|---|
| 1 | ciprofloxacin (0.5 g P*; 1 g O*) | 15827 | 22,91 | 5,22 | 61 |
| 2 | oxacillin/ampicillin (2 g) | 9980 | 14,45 | 3,29 | 35 |
| 3 | ampicillin (2 g) | 9399 | 13,61 | 3,10 | 72 |
| 4 | metronidazole (1.5 g P) | 6610 | 9,57 | 2,18 | no data |
| 5 | amoxicillin (1g) | 6132 | 8,88 | 2,02 | 72 |
| 6 | ceftriaxone (2g) | 5335 | 7,72 | 1,76 | 54 |
| 7 | norfloxacin (0.8g) | 2371 | 3,43 | 0,78 | 61 |
| 8 | cefotaxime (4g) | 2038 | 2,95 | 0,67 | 54 |
| 9 | doxycycline (0.1g) | 1871 | 2,71 | 0,62 | no data |
| 10 | clarythromycin (1g P; 0.5g O) | 1460 | 2,11 | 0,48 | no data |
| 11 | gentamicin (0.24g) | 1454 | 2,10 | 0,48 | 45 |
| **DU90%** | **1-11** | **62476** | **91** | **20.6** | |
| 12 | amoxicillin/clavulanate (3g P; 1g O) | 1120 | 1,62 | 0,37 | |
| 13 | pipemidic acid (0.8g) | 820 | 1,19 | 0,27 | |
| 14 | cefuroxime (3g P; 0.5g O) | 788 | 1,14 | 0,26 | |
| 15 | lincomycin (1.8g) | 665 | 0,96 | 0,22 | |
| 16 | cefoperazone (4g) | 649 | 0,94 | 0,21 | |
| 17 | benzylpenicillin (3.6g) | 635 | 0,92 | 0,21 | |
| 18 | amikacin (1g) | 426 | 0,62 | 0,14 | |
| 19 | vancomycin (2g) | 282 | 0,41 | 0,09 | |
| 20 | pefloxacin (0.8g) | 220 | 0,32 | 0,07 | |
| 21 | ceftazidime (4g) | 208 | 0,30 | 0,07 | |
| 22 | azithromycin (0.5g P; 0.3g O) | 198 | 0,29 | 0,07 | |
| 23 | nitroxoline (1g) | 125 | 0,18 | 0,04 | |
| 24 | trimethoprim/sulfamethoxazole (2g**) | 116 | 0,17 | 0,04 | |
| 25 | cefazolin (3g) | 108 | 0,16 | 0,04 | |
| 26 | levofloxacin (0.5g) | 85 | 0,12 | 0,03 | |
| 27 | fosfomycin (8g P; 3g O) | 53 | 0,08 | 0,02 | |
| 28 | meropenem (2g) | 40 | 0,06 | 0,01 | |
| 29 | cefoperazone/sulbactam (4g) | 30 | 0,04 | 0,01 | |

| | | | | | |
|---|---|---|---|---|---|
| 30 | cefepime (2g) | 25 | 0,04 | 0,01 | |
| 31 | imipenem/cilastatin (2g) | 13 | 0,02 | 0,00 | |
| | **12-31** | **6606** | **9** | **2.2** | |
| **TOTAL** | **1-31** | **69082** | **100** | **22.8** | |

* P- DDD for parenteral use, O – DDD for oral use

* for sulfamethoxazole

Karolinska Institutet

**Department of Laboratory Medicine, Division of Clinical Pharmacology**

# Clinical studies on drug treatment of hospitalised patients: general infectious diseases and acute myocardial infarction

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Karolinska Institutet, Alfred Nobels Alle 8, Sal 7B, våning 7, 14152 Huddinge

**Måndagen den 27 maj, 2013, kl 13.00** av

**Ksenia Zagorodnikova (Goryachkina)**
MD, CMSci

*Huvudhandledare:*
professor Ulf Bergman
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Pharmacology

*Bihandledare:*
professor Leif Bertilsson
Karolinska Intitutet
Department of Laboratory Medicine
Division of Clinical Pharmacology

professor Aleksandra Burbello
North-Western State Medical University
n.a.I.I.Mechnikov, St Petersburg, Russia
Department of Therapeutics and Clinical Pharmacology

professor Svetlana Boldueva
North-Western State Medical University
n.a.I.I.Mechnikov, St Petersburg, Russia
Department of Hospital and Faculty Therapy

*Fakultetsopponent:*
professor Staffan Hägg
Linköping Unversity
Division of Drug Research,
Department of Medical and Health Sciences

*Betygsnämnd:*
ass. professor Henrik Green
Linköping University,
Dearpment of Medicine and Health Science,
Division of Drug Research and Clinical Pharmacology

professor Otto Cars
Uppsala Unversity
Department of Medical Sciences, Infectious Diseases

ass. professor Bror Jonzon
Medical Products Agency, Uppsala

**Stockholm 2013**

## ABSTRACT

Treatment of hospitalised patients is generally governed by the pre-developed algorithms and common guidelines. These approaches are helpful in most, but not all cases. Treatment of hospitalised patients is limited to the time of hospital stay and is therefore directed to immediate help. There are diseases for which immediate help is as important as its long-term consequences. General infections and ischemic heart disease are among the most prominent examples. Cardiovascular diseases (CVD) remain the leading cause of death in developed countries. While immediate treatment of acute myocardial infarction (AMI) is currently dependent on rapid surgery and management of thrombosis, adequate long-term treatment with other agents including beta-blockers may prolong time to further cardiovascular events and therefore prolong patients' life. It is important to achieve adequate effects as early as possible and avoid adverse drug reactions (ADR) to fulfill primary goals of the treatment. Factors that affect individual treatment response may be inherited (genetic polymorphisms) or exogenous (drug interactions). General infectious diseases represent another problem where hospital lethality is traditionally high and is dependent on a number of factors, mainly timeliness of diagnosing and susceptibility of pathogenic microorganisms to available antibiotics. This susceptibility is a changing parameter and may be dependent on the pattern of traditional antibiotic use in a given hospital, which is related to selection of resistant pathogens potentially worsening patients' survival. This also has a more global consequence of cultivation of multiple resistant pathogens, which may then be spread over the hospital, region and even country borders.

General aim of the current thesis was to increase knowledge of specific factors that may affect quality of hospital care in the treatment of general infections and acute myocardial infarction and suggest methods to minimize their negative influence in hospitalised patients.

We found that CYP2D6 is a major factor of metoprolol disposition and effects in AMI patients and also a major determinant of individual variability of response to the treatment. Common exogenous medications prescribed for treatment of depression complicating AMI namely selective serotonin reuptake inhibitor (SSRI) paroxetin significantly increase metoprolol concentrations in patients and put them at risk of excessive bradycardia. Based on our findings we suggested that CYP2D6 genetically defined activity may be related to ventricular rhythm disorders (VRD) complicating early period after acute myocardial infarction, though not in patients undergoing percutaneous coronary intervention.

In our studies on surveillance of antibiotic use and resistance we applied a method of Drug Utilization 90% (DU90%), and modified it with cumulative microbial resistance data. From this combination it was clear that most widely utilized antibiotics are not suitable for treatment of registered infections due to high resistance of the microbes. We showed that this method of combined presentation of antibiotic 90% use and microbial resistance reflects existing situation in a comprehensive and easy way both – for prescribers and authorities. When this method was tested in a Russian hospital we observed antibiotic use and resistance during five consecutive years, we could not see any change in resistance despite obvious changes in utilization profile. We considered these changes attributable to our intervention because they were not observed in a control Russian hospital. We also observed antibiotic utilization and key microorganisms resistance in a Swedish hospital. Overall antibiotic use was much higher in that hospital, antibiotics active against multiple resistant microorganisms were present within DU90% segment, despite that resistance of key microorganisms in this segment was low during the whole observation period. We concluded that the instrument of combined presentation of antibiotic use and cumulative resistance is an effective tool to show in an easy and comprehensive way rationality of antibiotic use and change utilization profile. It was also clear that in hospitals with high resistance of microorganisms to the most agents used other methods of infection control are required.

Our studies demonstrate two principally different approaches to improvement of drug treatment of hospitalised patients. In cardiovascular diseases we showed clinical importance of pharmacogenetics and drug interactions, which may further be continued by studies defining place for pharmacogenetic tests in clinics. For patients suffering from general infections we proposed a more general approach of antibiotic use and resistance surveillance that will help to define existing problems. It is a crucial step to improved treatment of patients in a particular hospital but also may have global contribution to containment of world dissemination of resistant microbes.