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

ECOLOGICAL IMPACT OF ANTIBIOTIC TREATMENT ON HUMAN NORMAL MICROFLORA

Mamun-Ur Rashid

Stockholm 2013

To my mother and father

ABSTRACT

The skin and the mucosal surfaces of humans are colonized with microorganisms which are often referred as the normal microflora. There is a biological balance between the human host and the normal microflora in health. The extensive use of antibiotics in both humans and animals has caused the development of many resistant bacteria. Administration of antibacterial agents can cause disturbances in the ecological balance between the host and microorganisms.

Ceftobiprole is a new broad-spectrum cephalosporin active against methicillin-resistant *Staphylococcus aureus*. Twelve healthy volunteers received ceftobiprole. Plasma and fecal samples were collected according to the study design for analysis. Plasma concentrations of ceftobiprole were 14.7- 24.5 mg/l. No measurable concentrations of ceftobiprole were found in feces. There were minor to moderate changes in the numbers of enteric bacteria, enterococci, *Candida albicans*, bifidobacteria, lactobacilli, clostridia and *Bacteroides* spp. No *Clostridium difficile* strains and no new colonizing bacteria were found.

Ciprofloxacin is a well-known fluoroquinolone active against Gram-negative and Gram-positive bacteria. Thirty-six healthy female volunteers according to the study design received either the extended release formulation of ciprofloxacin or the immediate release formulation. Mean fecal concentrations were 453 mg/kg and 392 mg/kg, respectively. The numbers of *Escherichia coli* were significantly suppressed while the enterococci decreased moderately in both treatment groups. No toxigenic *C. difficile* strains were found.

Telavancin is a new glycopeptide for the treatment of Gram-positive infections. Thirteen healthy volunteers received telavancin. Fecal and urine samples were collected according to the study design. There were no measurable concentrations of telavancin in feces. No significant effects on the number of Enterobacteriaceae, enterococci, *C. albicans*, bifidobacteria, lactobacilli, clostridia and *Bacteroides* spp. were observed in the study. No *C. difficile* strains and no new colonizing Gram positive bacteria were found.

Thirty-four healthy volunteers were included and received either doxycycline or placebo for 16 weeks. Plasma, saliva and fecal samples were collected according to the study design. The plasma concentrations of doxycycline in the doxycycline group were 0.20-1.49 mg/l. The fecal concentrations of doxycycline in the doxycycline group were 0-4.10 mg/kg. Minor effects on the oropharyngeal microflora were observed in both groups. There were minor changes in the number of enterococci and *E. coli* in both groups. No *C. difficile* strains were isolated.

This thesis shows that intravenous administration of antibiotics (ceftobiprole and telavancin) had less impact on the intestinal microflora. Both antibiotics caused minor disturbance on the normal microflora indicting a low risk to develop *C. difficile* infection. Ciprofloxacin had impact on the microflora regardless of the formulation of the drug. Doxycycline sub-antimicrobial dose had minor effect on the normal microflora and development of resistance.

Keywords: Ceftobiprole, Ciprofloxacin, Telavancin, Doxycycline, Oropharyngeal microflora, Intestinal microflora, Ecological impact, Normal flora, Health, Subantimicrobial dose, Antibiotic resistance.

LIST OF PUBLICATIONS

I. Bäckström T, Panagiotidis G, Beck O, Asker-Hagelberg C, **Rashid MU**, Weintraub A, Nord CE. Effect of ceftobiprole on the normal human intestinal microflora. Int J Antimicrob Agents 2010; 36:537-41.

II. **Rashid M**, Weintraub A, Nord CE. Comparative effects of the immediate and the extended release formulations of ciprofloxacin on normal human intestinal microflora. J Chemother 2011; 23:145-9.

III. **Rashid MU**, Weintraub A, Nord CE. Effect of telavancin on human intestinal microflora. Int J Antimicrob Agents 2011; 38:474-9.

IV. **Rashid MU**, Panagiotidis G, Bäckström T, Weintraub A, Nord CE. Ecological impact of doxycycline at low dose on normal oropharyngeal and intestinal microflora. Int J Antimicrob Agents 2013; 41:352-7.

OTHER PUBLICATIONS NOT INCLUDED IN THE THESIS

I.   **Rashid MU**, Lozano HM, Weintraub A, Nord CE. In vitro activity of cadazolid against *Clostridium difficile* strains isolated from primary and recurrent infections in Stockholm, Sweden. Anaerobe 2013; 20:32-5.

II.   **Rashid MU**, Weintraub A, Nord CE. Effect of new antimicrobial agents on the ecological balance of human microflora. Anaerobe 2012; 18:249-53.

III.   Amaya E, Reyes D, Paniagua M, Calderon S, **Rashid MU**, Colque P, Kühn I, Möllby R, Weintraub A, Nord CE. Antibiotic resistance patterns of *Escherichia coli* isolates from different aquatic environmental sources in León, Nicaragua. Clin Microbiol Infect 2012; 18:347-54.

CONTENTS

LIST OF ABBREVIATIONS

AEs	Adverse events
ATCC	American Type Culture Collection
AUCtau	Area under the concentration–time curve over a dosing interval
CDI	*Clostridium difficile* infection
CFU	Colony-forming units
CHAPS	3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate
CL	Total body clearance
CLR	Renal clearance
CLSI	Clinical and Laboratory Standards Institute
Cmax	Maximum drug concentration in plasma
Cmin	Minimum drug concentration
CNS	Central nervous system
CRF	Case report form
cSSSI	Complicated skin and skin-structure infections
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ERT	Extended release formulation ciprofloxacin treatment
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
i.v	Intravenous
IRT	Immediate release formulation ciprofloxacin treatment
LC-MS/MS	Liquid chromatography tandem mass spectrometry
Li	Lithium
M	Molar
MedRA	Medical Dictionary for Regulatory Activities
MIC	Minimum inhibitory concentration
MRM	Multiple reaction monitoring
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NaOH	Sodium hydroxide
ND	Not Detected
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
PT	Preferred term
RNA	Ribonucleic acid
SOC	System organ class
SPSS	Statistical Package for the Social Sciences
$t_{1/2}$	Half-life
TEAEs	Treatment-emergent adverse events
TFS	Trial Form Support
UPLC-MS/MS	Ultra-performance liquid chromatography–tandem mass spectrometry

UTI	Urinary tract infection
UV	Ultraviolet
Vd_{ss}	Volume of distribution in steady state
VRE	Vancomycin-resistant Enterococci
VRSA	Vancomycin-resistant *Staphylococcus aureus*
V_z	Volume of distribution based on terminal phase

1 INTRODUCTION

The skin and the mucosal surfaces of human are colonized with microorganisms which are often referred to as the normal microflora [1]. There is a biological balance between the human host and the normal microflora in health [1]. The normal microflora varies between individuals depending on different diet and lifestyle [1]. In an adult individual's intestine there are around 300-500 different species of bacteria, with 30-40 species comprising up to 99% of the total colonization [1, 2]. Bacteriological studies of the fecal microflora show that strict anaerobic bacteria outnumber aerobes by a factor of 100 to 1000 [1-4]. The composition of the colonizing microflora influences individual variations in immunity against different diseases [5].

The most frequent and important cause of instability in the normal microflora is the administration of antimicrobial agents [6-12]. To what extent changes of normal microflora and instability occur depends on the spectrum, the dose, the route of administration, the pharmacokinetic and pharmacodynamic properties of the agent and the in vivo inactivation of the antimicrobial agent [6, 7, 9, 11-18]. Antimicrobial agents that change and affect the normal microflora also promote the emergence of antimicrobial-resistant strains and the risk of super-infection [1, 9-12, 19-21]. Antibiotic-resistant organisms have steadily increased for the last 15-20 years, which renders threat to present disease management [22, 23]. The resistant bacteria can be transmitted to other sites within the host and from individual to individual in the hospital environment [24-29]. Inhibition of intestinal flora by antimicrobial drugs creates a microbiologic vacuum and these sites may be colonized by antibiotic-resistant microorganisms normally excluded [30-35]. Some bacteria of the normal microbiota not affected by the antimicrobial agent may also cause overgrowth [6, 8, 11, 18, 33, 35-37]. If the individual is compromised by surgery, advanced age or immunosuppressive therapy, opportunistic bacteria can cause severe infections [10, 11, 18, 38]. *Clostridium difficile* infection (CDI) is one of such infection caused by an opportunistic bacterium named *C. difficile* [11, 38-40]. The exact mechanism by which *C. difficile* overgrowth occurs is still unclear, but antibiotics are supposed to be the main important risk factor for *C. difficile* infection by reducing the colonization resistance of the intestine followed by colonization with *C. difficile* [11, 38-40]. Antibiotic resistance mechanisms exist in both pathogenic bacteria and commensal bacteria surviving the antimicrobial attack [37]. Resistance can be inherent, in the genetic composition of that bacterial species and can be acquired also, by which bacteria acquires deoxyribonucleic acid (DNA) encoding for resistance or the DNA mutates to become resistant [37].The bacteria that are pathogenic and newly established in the gastrointestinal tract are often resistant to one or more antimicrobial drugs [11, 37, 38, 41]. Careful investigation of the effect of antibiotic treatment on the normal microflora is of importance since alteration of the normal flora balance, qualitatively and/or quantitatively, may facilitate colonization by new potentially pathogenic strains or enable microorganisms already present in the normal flora to develop resistance [3, 6, 7, 9, 10, 20, 38, 42, 43].

1.1 NORMAL FLORA OF THE OROPHARYNX

The normal flora of the oropharynx includes a large number of aerobic and anaerobic bacterial species [18, 32, 34, 44-47]. Approximately $1x10^9$ bacteria per ml presents in

saliva which are mostly anaerobic bacteria. The number of anaerobic bacterium is 10 to 100 for every aerobic bacterium. Cultureable predominant microorganisms of saliva are streptococci, pneumococci, staphylococci, diphtheroids, *Haemophilus* spp, neisseria, micrococci, *Peptostreptococcus* spp, anaerobic cocci, lactobacilli, *Branhamella* spp, actinomyces, *Fusobacterium* spp, leptotrichia, *Bacteriodes* spp, *Veillonella* spp, *Prevotella* spp, *Porphyromonas* spp, *Candida albicans*, various other Gram-negative rods, spirochaetes and filamentous forms [35, 44, 46-48]. The normal flora of saliva remains relatively constant and is rarely responsible for disease, unless exogenous factors such as antibiotic treatment disrupt the balanced flora [18, 34, 35, 45-48].

1.2   NORMAL FLORA OF THE INTESTINE

The small intestine is colonized with many different aerobic and anaerobic bacteria such as streptococci, enterococci, bifidobacteria, lactobacilli, clostridia, pepto-streptococci, porphyromonas, prevotella, fusobacteria and bacteroides etc [3, 10, 11, 34, 46, 47]. The motility of small intestine, p^H and the presence of bile are inhibiting bacterial multiplication and therefore bacterial concentrations are usually between 1x 10^2 to 1x 10^5 colony forming units per ml small intestinal content [3, 46, 47]. A small number of *Salmonella* and *Campylobacter* spp can be present asymptomatically in the small intestine [3, 46, 47].

The normal microflora of large intestine or colon has at least 10^{12} colony-forming unit (CFU) per gram feces. More than 500 bacterial species have been identified and 95-99% of them belong to anaerobic bacteria such as *Bacteroides, Bifidobacterium, Eubacterium, Peptostreptococcus* and *Clostridium* [3, 46, 47, 49]. In this highly anaerobic region of the intestine, these bacteria proliferate and colonize most available niches [3, 46, 49]. The strict anaerobic conditions, colonization resistance and bacterial waste products are factors that inhibit the growth of other bacteria in the large intestine or colon [3, 46, 47, 49]. Enterobacteriaceae, enterococci and *C. albicans* are predominant among aerobic and facultative anaerobic microorganisms [3, 6, 46]. Mostly *Escherichia coli* is dominant from the Enterobacteriaceae group [3, 46, 47, 49].

1.3   SIGNIFICANCE OF THE NORMAL FLORA

Normal microflora varies and is controlled at different body sites by p^H, temperature, redox potential, oxygen, water, nutrient, peristalsis, lysozyme and immunoglobulins [3, 5, 32, 46, 47]. Normal microflora influences human anatomy, physiology, lifespan and ultimately cause of death [3, 5, 32, 46, 47]. Normally the opportunistic organisms are not causing disease but may do so when the host defenses are impaired, such as when the normal flora is altered by an antibiotic [3, 32, 46, 47]. Suppressed immune system is also a cause of opportunistic bacterial infection [5, 46]. *C. difficile,* which is an opportunistic bacterium, remains viable in a patient undergoing antimicrobial therapy and causes CDI [46, 50]. So the infection caused by the normal intestinal flora is secondary to another problem [5, 32, 35, 46].

Normal microflora in the intestine produces vitamins such as vitamins B_{12}, K, folate, riboflavin and helps to break down food that are normally indigestible by the host [5, 32, 46]. Administration of certain antimicrobial agents causes vitamin K deficiency by disrupting normal microflora [51, 52]. Normal microflora and diet play role in the development of cancer and obesity [5, 53]. The normal microflora colonizes the favorable ecological niches and inhibits colonization of pathogenic bacteria [5, 35, 46,

2

47]. Normal microflora inhibits pathogen organisms multiplication by competing with nutrients and production of antibacterial chemicals as a side product of their metabolism, thus generating a local antibiotic effect which inhibits the colonization of pathogenic microorganisms [3, 5, 32, 46]. Normal microflora helps in the maturation of our immune system and keeps it in tune [5, 19, 32, 46].

1.4   DISTURBANCE OF THE NORMAL MICROFLORA

Disturbances of the normal microflora in the oropharynx and intestine may be caused by antibiotics, malnutrition, contaminated food, contaminated water, surgical procedures, emotional stress, environment, food habit, hygiene, age, obesity, immune response etc [5, 19, 32, 46]. The most significant and common cause of disturbances in the normal oropharyngeal and gastrointestinal microflora is the administration of antimicrobial agents [8, 32, 46]. Incomplete absorption of perorally administered agents is one of the factors for the disturbances of the microflora [8, 18, 32]. Poorly absorbed drugs and antimicrobial agents that are secreted by the salivary glands, in bile and by the intestinal mucosa are disrupting the normal microflora [8, 18, 32]. As a consequence, this promotes the emergence of resistant microorganisms in oropharyngeal and intestinal microflora, as well as dissemination of resistant microorganisms [8, 18, 32]. Antimicrobial treatment may lead to a dramatic shift in bacterial colonization. [8, 11, 32]. As a consequence, several unwanted effects may result, such as overgrowth of already present microorganisms, development of resistance, superinfection, colitis etc [8, 11, 32]. Approximately 5% of healthy adults asymptomatically carry low numbers of *C. difficile* in the colon and the growth of these bacteria has been shown in vitro to be held in balance by the intestinal normal microflora [11, 54, 55]. *C. difficile* is implicated in 20 to 30% of patients with antibiotic-associated diarrhea, in 35 to 50% of those with antibiotic-associated colitis and in more than 90% of those with antibiotic-associated pseudomembranous colitis [54, 55]. The incidence of CDI ranges from 1 in 100 to 1 in 1,000 hospital discharges depending on the antibiotic prescribing habits of the hospital [56]. The incidence may change over time at the same hospital as it did in one study from approximately 1 in 300 to 1 in 100 hospital discharges [56]. Use of antibiotics may lead to the emergence of a new variant of *C. difficile*, which is competent of secreting elevated amounts of toxin A and B and is more resistant to the recommended antibiotic treatment [41, 57]. This hypervirulent variant, PCR ribotype 027 of *C. difficile,* has been reported in Canada, USA, and Europe [41, 57]. Among all the patients with *C. difficile* infections, recurrence occurs in 15-35% of patients [56, 58].

1.5   HISTORY OF ANTIBIOTICS

For a long time the leading cause of death in humans are infections [59, 60]. The main causes of death during the 19th century were pneumonia, tuberculosis, diarrhea and diphtheria in children and adults [60]. The beginning of industrial revolution and upcoming urbanization led to a shift of population to the cities that consequently increased the incidence of diseases such as tuberculosis and syphilis [60]. It was possible to correlate the existence of microscopic pathogens with the development of various diseases in the late 19th century [60]. The antiseptic procedures were introduced by Semmelweis and Lister [60, 61]. As a consequence, the mortality due to postsurgical infections began to be reduced [60, 61]. A significant role was also played

by sanitation and hygiene in the reduction of the mortality due to several infectious diseases [60]. In 1911 the first compound with antimicrobial activity was introduced by Ehrlich [60, 62-64]. His theory was that the immune system of humans could have been aided by the use of chemical compounds [62, 63]. His research activity was focused on the discovery of a "magic bullet" to treat syphilis [62-64]. Arsphenamine was the first sulfa drug or magic bullet [62-64]. The first compound with antimicrobial activity was very successful for controlling many diseases [60, 62-65]. Despite antiseptics and magic bullet in hospital and post-surgical, infections induced by Gram-positive bacteria remained a common cause of death [60, 62, 65]. The antimicrobial treatment concept was revolutionized by Alexander Fleming [66-70]. His curiosity in microbiology and antiseptics brought him to the discovery of penicillin, one of the most important drugs of the last century [66-70]. Discoveries of more and more new antimicrobials gave clinicians more therapeutic options for previously life-threatening diseases [60, 71]. By changing the morbidity and mortality, antibiotics have had an effect not only on the treatment of infections but also on the society [60, 71]. However, the wide use of antimicrobial drugs in humans, animals and agriculture has introduced a new era in which clinicians have to face the emergence of drug resistant pathogens [72-78]. The condition is provoked by a significant weakening in research and development into antibacterial agents [22, 79, 80].

1.6 ANTIMICROBIAL RESISTANCE

The leading causes to the emergence and spread of antibiotic resistance include absence of regulation in the proper use of antibiotics, transmission of antibiotic resistance genes in the community through normal microflora, improper disposal of antibiotics used in animals and agriculture [81-90]. Globalization also has an impact on the transmission of antibiotic resistance genes in bacteria through immigration and export/import of foods [25, 83, 91-93]. Antibiotic resistance is a major problem for the treatment of infections and the origin of many antibiotic resistance mechanisms can be traced back to environmental organisms [81, 94, 95]. In nature there exists a gene pool for resistance to antibiotics for self-defense, homeostasis, detoxification, cell signaling etc [94, 95]. There, antibiotics act as weapon, signal and manipulator [81, 94, 95]. The spread and maintenance of antibiotic resistant genes are influenced by anthropogenic activities [81, 94-96]. Antibiotic resistance genes find their way into the pathogenic microorganisms in that way rendering them resilient to most of the antibiotics [81, 94, 95].

Bacteria have the ability to transfer genes from one bacterium to another by lateral gene transfer and three steps are required: delivery of the donor DNA into the recipient cell, incorporation of the alien genes into the genome of the recipient cell and expression of the acquired genes in a manner that benefits the recipient microorganism [91, 94, 97]. Delivery of the donor DNA and incorporation of the alien genes into the genome can take place by transformation, transduction or conjugation [91, 98]. The resistance mechanisms can also be transferred by plasmids [99]. Antimicrobial resistance includes three most important mechanisms, i. e. drug target alteration, production of antibiotic-inactivating enzymes and the cellular membrane barrier preventing drug accessibility (a result of decreased influx and increased efflux) [100, 101]. These mechanisms frequently interplay synergistically to increase antibiotic resistance levels significantly [100, 101]. Antibiotic resistance mechanisms exist in both pathogenic and commensal

bacteria surviving the antimicrobial attack [6, 8, 33, 37]. Resistance can be inherent (in the genetic composition of that bacterial species), or acquired (bacteria acquires DNA encoding for resistance or the DNA mutates to become resistant) [37].

The penalties of antimicrobial resistance are longer duration of treatment, higher mortality, expensive drugs treatment, costly health system, complex surgeries, development of patient as a reservoir of resistant microorganisms for the community and health-care personnel and massive impact on the economy [81, 91, 94, 95, 97, 102].

1.7 CEFTOBIPROLE

Ceftobiprole is a novel, broad-spectrum and β-lactamase-stable cephalosporin group antibiotic [103, 104]. Ceftobiprole is administered as ceftobiprole medocaril [105]. Ceftobiprole medocaril is a water-soluble prodrug for i.v. administration which is rapidly converted to ceftobiprole [105]. Ceftobiprole is primarily eliminated by the kidneys as unchanged drug [105]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged in hospitals and in the community [106, 107]. Vancomycin is an effective antibiotic against MRSA and wide use of vancomycin has led to the development of MRSA isolates with reduced susceptibility [108]. For the treatment of MRSA, daptomycin, linezolid, quinupristin– dalfopristin and tigecycline are available on the market [108, 109]. MRSA are resistant to most existing β-lactam antibiotics due to their production of penicillinase, a low-affinity to penicillin-binding protein (PBP) and PBP2a [110, 111]. Ceftobiprole binds strongly to PBP2a and makes it active against MRSA [103, 110, 111]. Ceftobiprole also strongly binds to PBP2x that is liable for β-lactam resistance in streptococci [103, 111-113]. Moreover, ceftobiprole strongly binds to PBP2 and PBP3 in *E. coli* [103, 111, 112]. It binds to PBP1a-b, PBP2, PBP3, and PBP4 in *Pseudomonas aeruginosa* [111, 112, 114]. It also binds to PBPs in *Enterococcus faecalis* [112, 114]. Ceftobiprole is hydrolyzed by class A cephalosporinase, extended-spectrum β-lactamases and carbapenemases [104, 115-117].
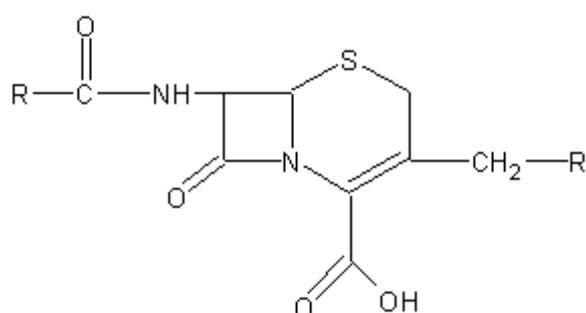
Basic structure of cephalosporin
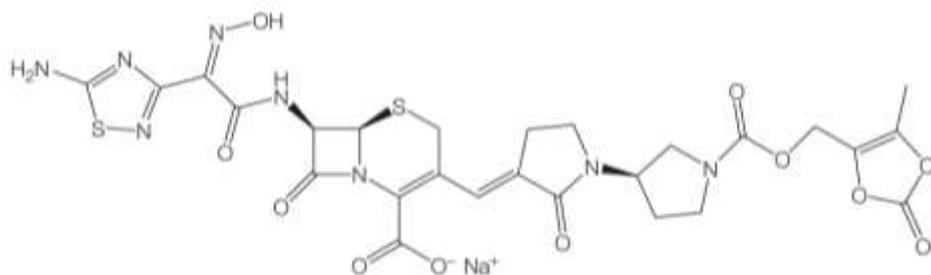
Ceftobiprole medocaril (BAL5788) pro-drug
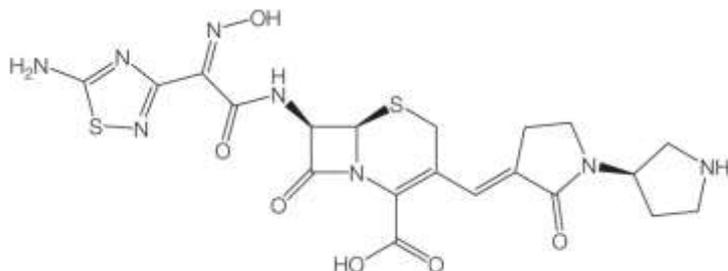
Ceftobiprole (BAL9141) active drug

Ceftobiprole is active against most aerobic Gram-positive bacteria including MRSA and methicillin-susceptible *S. aureus* (MSSA), vancomycin-resistant *E. faecalis* and Gram-negative bacteria such as Enterobacteriaceae and *Pseudomonas* spp [110, 117]. It is the first cephalosporin to demonstrate clinical efficacy in patients with infections due to MRSA [110]. Anaerobic Gram-positive bacteria such as bifidobacteria, propionibacteria and peptostreptococci are susceptible while clostridia are variable in susceptibility to ceftobiprole. The minimum inhibitory concentration value for *C. difficile* strains is 8.0 mg/l [118]. *Bacteroides fragilis* and *Prevotella* species are resistant to ceftobiprole [118]. Ceftobiprole has revealed a low potential to select for resistance [111]. Ceftobiprole is a promising antimicrobial for monotherapy of complicated skin and skin-structure infections (cSSSIs) and pneumonias that have required combination therapy in the past [110, 117]. The impact of ceftobiprole on the human microflora has not been studied before.

1.8 CIPROFLOXACIN

Ciprofloxacin is a commonly used fluoroquinolone [119]. It has high bactericidal activity against uropathogens [120]. An extended-release formulation of ciprofloxacin delivers systemic drug exposure comparable with that achieved with twice-daily administration of immediate-release ciprofloxacin [121, 122]. Extended-release formulation of ciprofloxacin achieved higher maximum plasma concentrations with less inter-patient variability and maintained throughout the 24-hour dosage interval [121, 122]. Extended-release formulation of ciprofloxacin is as safe and effective as the conventional or immediate-release formulation of ciprofloxacin [121, 122]. It may decrease the risk of infection recurrence and occurrence of antimicrobial resistance [121]. Since its introduction in the 1980s, the rates of ciprofloxacin resistance have remained low [123, 124]. Urinary tract infections (UTIs) are more common in females

[119, 125]. Almost 80% of uncomplicated UTIs are caused by *E. coli [119, 125]*. Other microorganisms responsible for UTIs are enterococci, *Staphylococcus saprophyticus*, *Klebsiella* spp. and *Proteus mirabilis* [119, 125]. For UTIs in females, the recommended first-line treatment is cotrimoxazole and its clinical utility is increasingly compromised by the emergence of resistance [119, 126]. Increase of resistance to cotrimoxazole has prompted physicians to use ciprofloxacin for UTIs in females [119].
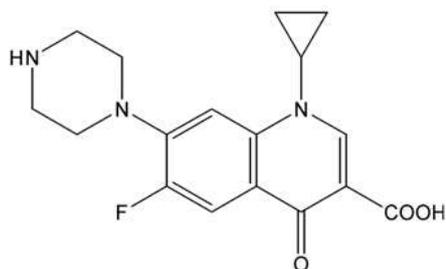
Ciprofloxacin

For favorable pharmacological profile and high antibacterial activity against clinically important Gram-negative and Gram-positive pathogens, ciprofloxacin has become widely accepted for the treatment of a wide range of infections including UTIs, sexually transmitted infections, skin and bone infections and gastrointestinal infections [123, 127]. The impact of ciprofloxacin on the human intestinal microflora has been studied before; measurable concentration of ciprofloxacin in feces had been detected [8, 16, 31]. The aerobic and anaerobic bacteria in the fecal flora were suppressed markedly during the prophylactic period as well as during the treatment period [6, 16, 31]. The intestinal microflora was almost normal within 2 weeks after treatment [6, 16, 31]. The concentrations of ciprofloxacin in the intestinal mucosa and feces were in excess of the MICs for most aerobic and anaerobic bacteria [6, 16, 31].

1.9   TELAVANCIN

Telavancin is a semisynthetic lipoglycopeptide [128].   Telavancin is invented by alkylation of vancomycin to add an extended lipophilic tail [128]. It improved the antimicrobial activity and addition of a hydrophilic moiety improved pharmacokinetics [128]. Telavancin inhibits bacterial cell-wall synthesis by binding with lipid II and inhibiting transglycosylation ten times more than vancomycin [128]. Disruption of the functional integrity of the bacterial membrane is another action of telavancin [128, 129]. Vancomycin does not have this disruption property [128, 129]. Telavancin also binds to bacterial membranes, inducing dissipation of membrane potential and disruption of bacterial membrane permeability, activities that lead to inhibition of lipid, protein, DNA and ribonucleic acid (RNA) synthesis, which results in bacterial cell death [128, 129]. Telavancin is primarily eliminated by kidneys without metabolism [130].

Vancomycin   Telavancin
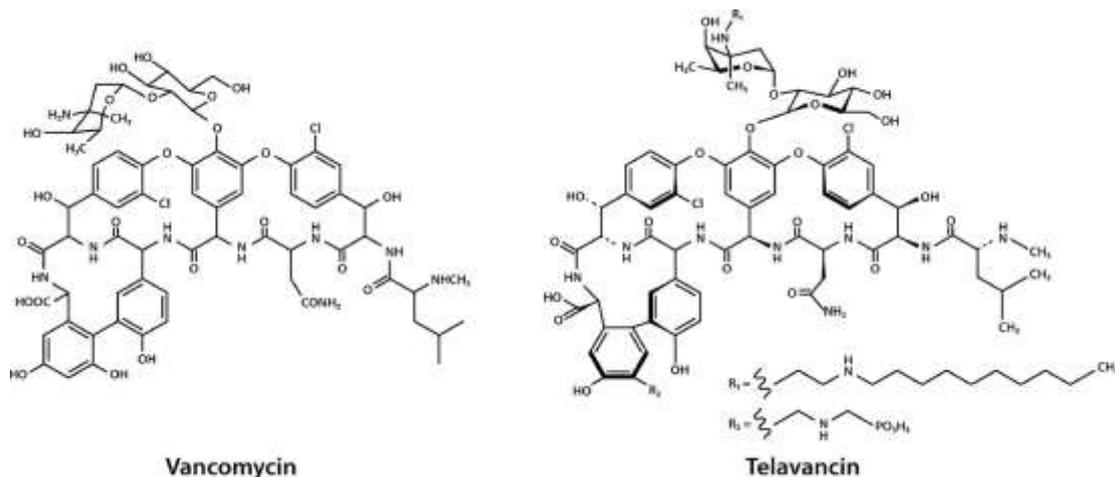
Nosocomial pneumonia is a common infection related with significant mortality [131-133]. It is the second most common hospital acquired infection [133]. Nosocomial pneumonia caused by MRSA is increasing and treatment options for this pathogen are limited [133]. The coverage for MRSA is important in the empiric treatment of nosocomial pneumonia [134, 135]. Recommending vancomycin or linezolid for coverage of MRSA as empiric treatment is not appropriate in all settings, for instance in cases of multidrug-resistant strains [134, 135]. As a consequence, there is an urgent need for new antimicrobials with activity against MRSA [134-136]. Telavancin should be used in known or suspected cases where other alternatives are not suitable [137]. Telavancin is approved in the USA and Canada for treatment of cSSSIs and in Europe for the treatment of adults with nosocomial pneumonia [137]. Telavancin is active against a range of Gram-positive isolates, including MRSA, MSSA, vancomycin-resistant *S. aureus* (VRSA), streptococci and vancomycin-susceptible enterococci, but it is less active against vanA isolates of vancomycin-resistant enterococci [137-140]. Telavancin once-daily dosing makes it a more convenient dosing schedule compared with β-lactam antibiotics or vancomycin [141]. The impact of telavancin on the human microflora has not been studied before.

1.10 DOXYCYCLINE

Tetracyclines are an amazing class of antibacterial agents with a lot of therapeutic potential [142]. Today the most widely used tetracyclines are minocycline and doxycycline [142]. Chlortetracycline discovered in 1945 and tetracycline in 1953 were naturally occurring molecules formed by *Streptomyces aureofaciens* [143] . The tetracycline compounds were chemically adjusted to the semi-synthetic doxycycline in 1967 [143-145]. Tetracyclines are active both against Gram-positive and Gram-negative bacteria, thus becoming the first class of broad-spectrum antibiotics [142, 145]. Tetracyclines were found to be highly effective against various pathogens and infections including rickettsiae, anthrax, chlamydial infections, community-acquired pneumonia, Lyme disease, cholera, syphilis, acute Q fever, *Yersinia pestis*, dermatological diseases, behavior and mental disorders, immune system disorders, cardiovascular diseases, nervous system diseases rheumatoid arthritis, corneal inflammation, periodontal infections, allergen-induced inflammation and cancer [142,

8

146-148]. Doxycycline may be used in infections with penicillin resistant streptococci [146].
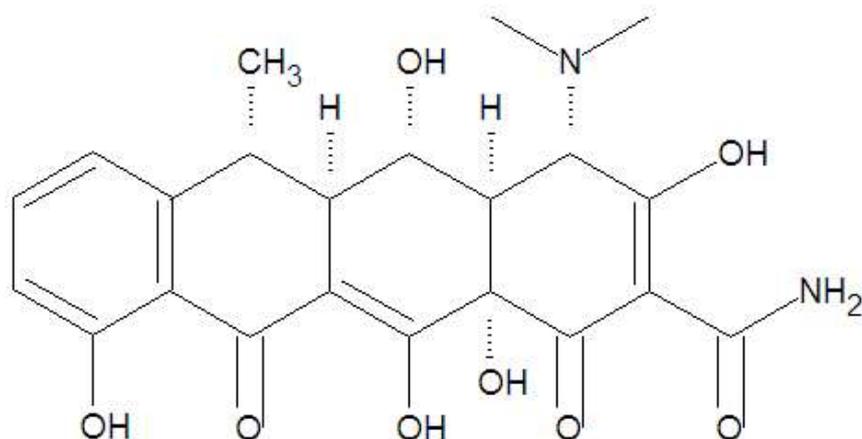
Doxycycline, a tetracycline antibiotic

The extensive use of tetracyclines in both humans and animals has caused the development of many resistant bacteria and subsequently limited their use in therapy [149-151]. Resistance is undoubtedly not limited to the tetracyclines and has been reported amongst most classes of antibacterials [150, 151]. Three mechanisms are responsible for tetracycline resistance – efflux pump, ribosomal protection and chemical modification. Efflux pump and ribosomal protection are the most clinically important mechanisms [152-155]. Through the acquisition of tetracycline resistance genes, resistance occurs [153-155]. The tet genes are encoded on plasmids, conjugative transposons and integrons [150, 151, 153].

Tetracyclines have many other interesting properties not related to their antibiotic activity [142, 156]. These other interesting properties have led to widely divergent experimental and clinical use of tetracycline [156, 157]. Doxycycline has anti-protease activities [142, 156, 157]. Doxycycline can inhibit matrix metalloproteinases which contribute to tissue destruction activities in diseases such as periodontitis [142, 156, 157]. Tetracyclines have independent anti-inflammatory effects at sub-antimicrobial doses [158-163]. It has immune-modulating and neuroprotective effects [158-163]. Studies have provided evidence for the anti-inflammatory properties of tetracyclines, as well as in the management of acne and rosacea [158-168]. Traditional tetracycline dose has effect on antibiotic susceptibility and resistance of the host microflora [9, 169-172]. Subantimicrobial doxycycline dose has raised questions about potential changes in antibiotic susceptibility of the host microflora [88]. Many studies have shown that long-term subantimicrobial doxycycline dose does not contribute to changes in antibiotic susceptibility and resistance of the host microflora [173-177]. But studies also reported that subantimicrobial dose exposure to microorganisms may select bacteria having enhanced multidrug efflux pump activity, which deliver both resistance to microorganisms and cross-resistance to multiple antibiotics [88]. It also showed that continuous long-term exposure to low level of antibiotics lead to antibiotic resistance in pathogenic microorganisms [88].

2 AIMS OF THE THESIS

2.1 PRIMARY OBJECTIVES

To assess the effect of antibiotic treatment on the intestinal microflora before, during and after administration of ceftobiprole (Paper I) or telavancin (Paper III) given to healthy volunteers;

To evaluate the ecological impact of the extended release formulation ciprofloxacin in comparison with immediate release formulation ciprofloxacin on the intestinal microflora in healthy volunteers (Paper III);

To investigate whether a subantimicrobial dose of doxycycline (40 mg) for 16 weeks had any ecological impact on the oropharyngeal and intestinal microflora of healthy human volunteers (Paper IV).

2.2 SECONDARY OBJECTIVES

To explore the potential for development of resistance by measuring the MICs of new colonizing isolated bacterial strains during and after antibiotic administration (Paper I, II, III and IV);

To correlate the intestinal and oropharyngeal microflora patterns with drug concentrations measured in feces (Paper I, II, III and IV), saliva (Paper IV) and plasma (Paper I);

To determine the pharmacokinetics of telavancin in plasma and urine (Paper III);

To assess the safety of the drug (Paper I, II, III and IV).

3 MATERIALS AND METHODS

3.1 SUBJECTS

3.1.1 Paper I

This was an open-label, non-comparative, multiple-dose, single-center study. Twelve healthy volunteers (6 males and 6 females) aged between 20 and 31 years were included in the study. They were recruited through information and advertisement about the study on the Clinical Pharmacology Trial Unit website (http://www.karolinska.se/KarolinskaUniversitetslaboratoriet/Kliniker/Klinisk-farmakologi/Humanlaboratoriet/) of the Karolinska University Hospital, Stockholm, Sweden.

3.1.2 Paper II

This was a randomized, two-armed, parallel study. Thirty-six healthy female volunteers aged between 18 and 45 years were included in the study. Half of the volunteers were 18-35 years and another half of the volunteers were 36-45 years. Trial Form Support (TFS), Lund, Sweden, recruited all the volunteers through advertisement.

3.1.3 Paper III

This was an open-label, single-dose, single-center study. Thirteen healthy volunteers (6 males and 7 females) aged between 18 and 40 years were included in the study. All the volunteers were admitted to the clinical research unit (PRA International, Zuidlaren, The Netherlands) by advertisement.

3.1.4 Paper IV

This was a double blind, randomized, placebo-controlled, parallel group study. Thirty-four healthy volunteers (16 males and 18 females) aged between 19 and 37 years were included in the study. The volunteers were recruited through information and advertisement about the study on the Clinical Pharmacology Trial Unit website of the Karolinska University Hospital, Stockholm, Sweden.

3.2 INCLUSION CRITERIA

Necessary physical examinations were carried out on each volunteer at the screening visit, including measurements of blood pressure, heart rate, electrocardiogram (ECG) and clinical laboratory safety tests as well as an interview on medical and surgical history. Female volunteers were tested for pregnancy. Included volunteers had to adhere to the visit schedule and concomitant therapy prohibitions and be compliant with the treatment. Volunteers aged between 18 and 45 years with regular defecation (five or more per week) and normal findings in the medical history and physical examination were included in the studies. Body weights were 60.0–100.0 kg for male subjects and 50.0–90.0 kg for female volunteers, with a body mass index between 18.0 kg/m2 and 26.0 kg/m2 both for male and female volunteers. Female volunteers of childbearing potential were required to use a highly effective and approved contraceptive method during the entire study period and 3 months after completion of

the studies. During this period, other antibiotic treatment was prohibited. In paper II the healthy volunteers were females and in the papers I, III and IV both males and females were included.

3.3 EXCLUSION CRITERIA

Volunteers were not eligible if any of the following criteria was met: Regular use of medication (except contraceptive tablets); treatment with antimicrobial agents or participation in a trial with another investigational drug within the 3 months preceding inclusion in the study; presence of any gastrointestinal disease 1 month preceding the study; use of probiotic products; presence of any surgical or medical condition that might interfere with the absorption, distribution, metabolism or excretion of drugs; known case of CDI, central nervous system (CNS) disorder, abnormal blood pressure (above 140 mmHg systolic and/or above 90 mmHg diastolic; below 100 mmHg systolic and/or below 60 mmHg diastolic), abnormal heart rate (above 110 beats/min and/or below 50 beats/min), decreased creatinine clearance (<80 mL/min), positive screen for hepatitis B or C or human immunodeficiency virus (HIV) and alcohol or substance abuse disorder; pregnant, breast-feeding or having the intention of becoming pregnant or not using acceptable contraceptive measures; donation of blood or blood products within 1 month prior the study; medical or physical findings considered to be clinically significant; volunteers suffering from constipation; history of hypersensitivity to β-lactam antibiotics (paper I); history of hypersensitivity to quinolones or history of tendon disorders related to quinolones administration (paper II); known or suspected hypersensitivity to telavancin (paper III); known or suspected hypersensitivity to tetracycline (paper IV) or to any components of the formulation used; hypersensitivity to the excipients and concomitant direct exposure to either extensive sunlight or ultraviolet (UV) irradiation; recent travel history to tropical countries (within last 3 months); deviating renal function; decreased amount of thrombocytes; any clinically significant abnormality following the investigator's review of the pre-study physical examination, ECG and clinical laboratory tests; or any other clinical conditions that in the opinion of the responsible physician would not allow safe completion of the study.

3.4 INFORMED CONSENT

According to the inclusion/exclusion criteria, the volunteers were informed about the study both verbally and by written information. The volunteers had enough time to consider participation and opportunity to ask the physician. When a volunteer participated, she/he signed a consent form, after which study activities had been performed. The volunteer was also given a copy of the signed consent form.

3.5 STUDY DESIGN

3.5.1 Paper I

The volunteers were admitted to the study center the day before the first drug administration and discharged from the study center on Day 8. Each volunteer included in the study participated at follow-up visits on Days 10, 14 and 21.
From each volunteer, 13 plasma samples were collected as followed: one at pre-dose (Day −1), 3 samples each on Days 1, 4 and 7 and 1 sample each on Days 10, 14 and 21.

From each volunteer, 7 fecal samples were collected at pre-dose (Day −1) and on Days 2, 4, 7, 10, 14 and 21.

3.5.2 Paper II

Each volunteer passed inclusion criteria was allocated to one of the following treatments groups, the extended release formulation ciprofloxacin treatment (ERT) or the immediate release formulation ciprofloxacin treatment (IRT), according to a computer-generated randomization code list prepared by the TFS, Lund, Sweden. The treatment randomization was stratified by age. The study treatment was not blinded for the volunteers and the clinical staff. Intestinal microflora assessments were blinded. First fecal samples were collected for the study on the screening day. The study drug for the whole treatment period was dispensed to the volunteers. The volunteers were informed about how to take the antibiotics and how to proceed if one dose was forgotten. Feces collection tubes were handed out together with information on how and when to carry out samplings. Included volunteers visited the site 4 times during the study: Visit 1 screening/including randomization/start of treatment; Visit 2, end of treatment; Visit 3, 7 days after the end of treatment; Visit 4, 2 weeks after the end of treatment.

3.5.3 Paper III

Volunteers were admitted to the clinical research unit the day before the first dose of antibiotic administration and discharged from the clinical research unit on Day 9. Volunteers visited the clinical trial center on Days 10, 14 and 21 for follow up.
For microbiological analysis and for bioassay of telavancin, seven feces samples were collected: at pre-dose (Day −1) and on Days 2, 5, 7, 9, 14 and 21. Plasma samples were collected to evaluate the pharmacokinetics of telavancin on Day -1 (pre-dose), on Days 5, 6 and 7. For pharmacokinetics analysis additional plasma samples were taken on Day 7 at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h after start of infusion. Urine was collected over 24 h after the last dose to determine the excretion of telavancin.

3.5.4 Paper IV

Volunteers visited the clinical trial center six times as followed screening day, Day -1 (pre-administration) and at Weeks 4, 8, 16 and 20.
For pharmacokinetics analysis from each volunteer, five plasma samples were collected at baseline visit (2 h after the oral dosing) and at Weeks 4, 8, 16 and 20. Saliva and feces samples were collected on Day -1 and at 4, 8, 16 and 20 weeks post dosing for pharmacokinetic and microbiological analyses.

3.6 ETHICS COMMITTEE'S APPROVAL

The study protocols were submitted to the Ethics Committee of Karolinska Institutet, Stockholm, Sweden (Paper I and IV), the Medical Products Agency, Uppsala, Sweden (Paper I, II and IV), the Ethics Committee of the Lund University, Lund, Sweden (Paper II) and were approved before the trials were started. The study protocol for paper III was submitted to the local ethics committee by the clinical research unit of PRA International, Zuidlaren, The Netherlands and approved before the clinical trial was started.

3.7 DRUG ADMINISTRATION

3.7.1 Ceftobiprole

By intravenous infusion, 500 mg of ceftobiprole was given to each volunteer over 120 minutes every 8 h (q8h) for 7 days.

3.7.2 Ciprofloxacin

Extended release formulation ciprofloxacin (Utiminx® 500 mg, Rottapharm Madaus SpA, Monza, Italy) was taken once daily together with a meal for 3 days. The comparator immediate release formulation ciprofloxacin (Ciproxin® 250 mg, Bayer HealthCare AG, Leverkusen, Germany) was taken twice daily for 3 days. The tablets were swallowed whole with fluid, not cut, crushed or chewed. The first dose was administered after the first feces sampling.

3.7.3 Telavancin

By intravenous infusion of 10 mg/kg body weight, telavancin was given over a 60-min period once every 24 h for 7 days.

3.7.4 Doxycycline

Orally, 17 volunteers were given Doxycycline 40 mg capsules (Efracea®; Galderma, Sophia Antipolis, France) and 17 volunteers received placebo 40 mg capsules (Galderma) for 16 weeks, once daily.

3.8 TREATMENT COMPLIANCE

The medications were supervised to ensure treatment compliance by the responsible persons or staffs of clinical research or trial unit. Staffs performed drug accountability and recorded the relevant information in the case report form (CRF).

3.9 SAMPLING PROCEDURE

3.9.1 Feces

Samples from feces were collected according to the study design in conjunction with each visit, either at the volunteer's home or at the clinical trial unit during the study period in a sterile container and were recorded with the study number, volunteer number and date and time of collection. The collection containers were filled up to the top. If the feces sample was collected at home, it was kept at $+4°C$ or at $-20°C$ until it was brought to the site of the clinical trial unit. In the CRF the time of collections was also recorded. The first specimen collected was analyzed if more than one feces specimen were collected on a given day for pharmacokinetic and microbiological analyses. If none was passed on a given day, the first specimen passed after that day was collected for analyses.

3.9.2 Blood

Samples from blood for evaluation of pharmacokinetics (Paper I, III and IV) and for bioassay (Paper I) were collected into sterile blood collection tubes, containing sodium–heparin as anticoagulant according to the respective study design and were labeled appropriately with the study number, volunteer number, date and time of collection. Collected blood samples were immediately put on ice and were centrifuged within 30 minutes at 1500 × g for 10 min at 4°C to obtain plasma.

3.9.3 Saliva

Saliva (Paper IV) was collected in a sterile container and labeled with the study number, volunteer number, date and time of collection. Samples were collected according to the study design for pharmacokinetic, microbiological analyses and bioassay.

3.9.4 Urine

Samples from urine (Paper I, II, III and IV) were collected at the site of clinical trial unit and pregnancy tests were completed by the clinical staff. For bioanalysis of telavancin (Paper III), urine was collected and labeled appropriately. In all containers 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) was added to prevent adsorption of telavancin by the container wall. The sample was inverted gently several times to thoroughly mix the contents and divided into three aliquots at the end of each interval. Aliquots were labeled with the study number, volunteer number, date and time of collection.

3.9.5 Storage and transportation

At site of clinical trial unit all samples (Paper II and III) were frozen immediately in a -70°C freezer and time was recorded in the CRF. According to the study design, relevant samples were shipped with adequate dry ice to the Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska University Hospital, Stockholm, Sweden, for microbiological analysis and bioassay. Feces and plasma samples (Paper I and IV) were transported to the Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska University Hospital, within 30 min of collection time and were stored at -70°C until processed.

3.10 DETERMINATION OF ANTIBIOTIC CONCENTRATION IN FECES, PLASMA, SALIVA AND URINE

3.10.1 Antibiotic concentration in feces by bioassay (Paper I, II, III and IV)

Fecal concentrations of ceftobiprole (Paper I), ciprofloxacin (Paper II), telavancin (Paper III) and doxycycline (Paper IV) were assayed by the agar well (4 mm in diameter) diffusion method. The agar plates were made by antibiotic medium No. 1 (Paper I, II and III) (Difco, Sparks, MD, USA) or nutrient broth (Paper IV) (BBL, Cockeysville, MD, USA) and agarose (Sigma, St Louis, MO, USA) with p^{H} 8, on Nunc bioassay plates 24 cm × 24 cm (Thermo Fisher Scientific, Waltham, MA, USA). *Micrococcus luteus* ATCC 9341 (Paper I and III), *E. coli* ATCC 25922 (Paper II) and

Bacillus cereus ATCC 11778 (Paper IV) were used as the indicator strains. The respective indicator strain was suspended in 0.9% NaCl with a density of 10^7 CFU/ml and the agar surface was inoculated by the suspension [178, 179]. Samples were always analyzed in duplicate and put in randomized order. Fecal samples were first diluted 1:4 (w/v) in 0.1 M NaOH (p^H 8). Samples were homogenized thoroughly by vortex and then centrifuged at 5000 rpm for 12 min. The supernatants were diluted in 0.15 M phosphate buffer (p^H 8) according to the need. The standards of ceftobiprole, ciprofloxacin, telavancin and doxycycline according to the companies' provided instructions. Inhibition zones were measured after incubation for 18 h at 37°C and standard curves were used to calculate the concentration. The standard curves were based on a logarithmic regression model and the correlation coefficients of the standard curves were 0.99 for all plates. For the final calculation of antibiotic measurement mean values from the duplicates were taken. The lower limit of sensitivity was 0.25 to 1 mg/kg feces,

3.10.2 Antibiotic concentration in plasma (Paper I) and saliva (Paper IV) by bioassay

In the plasma or saliva, concentrations were determined on antibiotic medium no. 1 (Difco) with *Micrococcus luteus* ATCC 9341 (Paper I) or *Bacillus cereus* ATCC 11778 (Paper IV) as indicator strain [178, 180]. The standards of ceftobiprole were prepared in human serum in the range 0.25−64 mg/l and plasma samples were also diluted in healthy human serum according to the need. The normal human serum used here was collected from the Transfusion Medicine Department of Karolinska University Hospital that was collected from healthy humans with no history of antibiotic exposure in 3 months. The determination of the drug concentration in plasma followed the same protocol as for the feces described above.

3.10.3 Ceftobiprole (Paper I) plasma and fecal concentrations by high-performance liquid chromatography (HPLC)

Ceftobiprole plasma and feces concentrations were determined by the following developed and validated methods. An ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) system (Waters Corp., Milford, MA, USA) was used for determining the ceftobiprole in lithium (Li) heparin human plasma (p^H 8). Samples were prepared by protein precipitation with 1% formic acid in acetonitrile containing BAL0009141-d4 as an internal standard. Into the UPLC–MS/MS system the final extract of plasma samples was injected.

In the same way as plasma, concentrations of ceftobiprole in fecal extract (supernatant from human fecal acidic homogenized with phosphate-buffered saline solution) were determined.

An ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm) (Waters Corp.) using a gradient run with mobile phase A of 0.1% formic acid in water and mobile phase B of acetonitrile separation of analytes was used. By using a Micromass Quattro Premier XE mass spectrometer (Waters Corp.) operating in positive electrospray ionization mode utilizing multiple reaction monitoring for the transitions 535 → 203 m/z for ceftobiprole and 539 → 207 m/z for IS (International System of Units) analytes were

detected. The calibration curve in plasma was linear over the range 0.05–30 mg/l plasma and in fecal extract over the range 1.00–143 mg/kg feces.

3.10.4 Doxycycline plasma concentrations by HPLC (Paper IV)

Samples from plasma were assayed for doxycycline using a validated liquid chromatography tandem mass spectrometry (LC–MS/MS) method. The quantification limit was 15 ng/ml plasma.

3.10.5 Antibiotic concentration in saliva by bioassay (Paper IV)

Doxycycline concentrations in saliva samples were determined in the same way as the feces concentration was measured and the indicator strain was *B. cereus* ATCC 11778 [178-181].

3.10.6 Telavancin concentrations in plasma and urine by HPLC (Paper III)

Plasma or urine samples (50 μl) spiked with the internal standard (deuterated telavancin) were acidified and loaded into Strata-X-C (3 mg) 96-well plates (Phenomenex Inc., Torrance, CA, USA) and were washed with 0.1 M HCl and then with methanol. Methanol elution was performed with ammonium hydroxide. Before being injected in a LC–MS/MS system (Sciex, Foster City, CA, USA), elute was evaporated to dryness and was reconstituted again.

From the matrix telavancin was separated by liquid chromatography and was detected by mass spectrometry. Using a gradient mobile phase, chromatographic separation was carried out in an Agilent 1200 SL G1312B LC system equipped with a Hypersil Gold column (2.1 mm I.D. × 150 mm length, 5 μm) (Agilent Technologies Inc., Santa Clara, CA, USA). Mobile phase A consisted of 1% formic acid/5% methanol in water. Mobile phase B was 1% formic acid/20% water in acetonitrile. With an increasing concentration of phase B, the separation was performed. The flow rate was 400 μl/min. API 4000 triple quadrupole mass spectrometer (Sciex) equipped with an electrospray interface operated in multiple reaction monitoring (MRM) positive ion mode was used for the quantification of telavancind. The temperature in the source was 550°C, ion spray voltage 5.2 kV and the dwell time 75 ms. The following transitions were selected for MRM: 586.2 > 112.2 for telavancin and 593.2 > 112.2 for the internal standard. Analyst® software (Sciex) was used for the data acquisition and analysis.

For plasma, the limit of quantification of the method was 0.10 μg/ml and 0.25 μg/ml for urine. For plasma, the response of telavancin was linear in the range 0.10–25.0 μg/ml and for urine 0.25–80.0 μg/ml. With accuracy and precision better than 15%, the coefficient of variation was always >0.99

3.11 ESTIMATION OF TELAVANCIN PHARMACOKINETIC PARAMETERS

Telavancin pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin® Professional software v.5.3 (Pharsight Corp., Sunnyvale, CA, USA). The calculated parameters were: the amount of unchanged excreted drug in urine over a dosing interval, percentage of the dose excreted unchanged in urine, AUC_{tau}, C_{max} in plasma; trough C_{min} in plasma total body total body clearance (CL), renal clearance (CLR); time to maximum drug concentration in plasma (C_{max}); terminal

elimination of half-life ($t_{1/2}$), volume of distribution in steady state (Vd_{ss}) and volume of distribution based on terminal phase (V_z).

3.12 PROCESSING OF SPECIMENS FOR MICROBIOLOGICAL ANALYSES

Samples from feces (Paper I, II, III and IV) and saliva (Paper IV) were suspended in pre-reduced peptone yeast extract medium, diluted ten-fold and inoculated on non-selective and selective agars as described by Nord et al. [181]. Aerobic agar plates were incubated for 24 h at 37°C and anaerobic plates for 48 h at 37°C in anaerobic jars (GasPak™; BBL, Cockeysville, MD, USA). Following incubation, different colony types were counted and isolated in pure culture. All isolates were identified according to Gram-reaction and colony morphology, followed by biochemical tests to genus level [46]. The anaerobic microorganisms were identified by gas chromatographic analysis [46, 182]. *C. difficile* strains were further characterized by the cell cytotoxicity neutralization assay, PCR ribotyping and a multiplex real-time polymerase chain reaction (PCR) (Xpert® C. difficile Assay; Cepheid, San Francisco, CA, USA) as recently described [183]. The lower limit of detection for microorganisms was 10^2 CFU/g feces or 10^2 CFU/ml saliva.

3.13 ANTIBIOTIC SUSCEPTIBILITY TESTS

The minimum inhibitory concentrations (MICs) of ceftobiprole (Paper I), ciprofloxacin (Paper II), telavancin (Paper III) and doxycycline (Paper IV) were determined for isolated strains from agar plates containing antibiotic. Using the agar dilution method, MICs were determined for strains isolated from ceftobiprole (4 mg/l) agar plates; ciprofloxacin (1mg/l) agar plates; telavancin (2 mg/l) agar plates; or doxycycline (4 mg/l) agar plates [184-186]. The final inoculum was 10^4 CFU/spot for aerobic bacteria and 10^5 CFU/spot for anaerobic bacteria. Inoculated plates were incubated for 24 h (aerobic bacteria) and 48 h (anaerobic bacteria). Reference strains were *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *B. fragilis* ATCC 25285 and *C. difficile* ATCC 700057. The strains were considered resistant according to breakpoints used in clinical and laboratory standards institute (CLSI) recommendations (Paper I, III and IV) or according to break-off points used in the European committee on antimicrobial susceptibility testing (EUCAST) guidelines (Paper II) [184-186]. The MIC was defined as the lowest concentration of drug that inhibited growth completely. MIC_{50} and MIC_{90} values corresponded to the concentrations that inhibited the growth of 50% and 90% of the strains tested, respectively. All antimicrobial agents were dissolved and diluted according to the companies' instructions.

3.14 SAFETY AND ADVERSE EVENTS

A safety assessment was conducted for all volunteers at baseline and at every following visit. Safety parameters were the adverse events (AEs). A physical examination was performed at each study visit. An adverse event was any event that impaired the wellbeing of a subject during the period of observation in the clinical study, including illness or accident. Other safety variables were: abnormalities at physical examination, vital signs and concomitant medications. Abnormalities that did not constitute an exclusion criterion and that were judged as not clinically significant were also recorded. Systolic and diastolic blood pressure and heart rate were measured at all visits.

The investigator was responsible for the necessary acute medical treatment of any adverse event during the trial and ensured that appropriate medical care was maintained thereafter. All findings were reported on an 'adverse event' page in the case report form and in the subject's medical records. AE incidences were summarized for all AEs and for related AEs by system organ class (SOC) and preferred time (PT) based on the medical dictionary for regulatory activities (MedDRA) dictionary (version 13.0).

3.15 STATISTICAL METHODS

Results were calculated for the values estimated for saliva and feces samples as log number of microorganisms per ml of saliva or per gram of feces. Feces, saliva and plasma concentrations done by bioassays were calculated from standard curves. For the pharmacokinetic analyses fecal, plasma and urine concentrations calculations were done by the Wilcoxon signed-rank test and Mann-Whitney U-test. IBM SPSS Statistics 20 (Armonk, NY, USA) software was used to calculate the percentiles 50 and 90 of the MIC results. In general, descriptive statistics were used to summarize both the microbiological and pharmacokinetic data.

4 RESULTS

4.1 EFFECT OF CEFTOBIPROLE ON THE NORMAL HUMAN INTESTINAL MICROFLORA (PAPER I)

4.1.1 Ceftobiprole concentrations in plasma and feces

All the volunteers (6 males and 6 females) finished the study successfully. Plasma ceftobiprole concentrations are shown in Table 1. Concentrations in samples taken 10 min after completion of infusion were as follows: Day 1, 14.7–23.6 mg/l (mean 19.4 mg/l); Day 4, 15.9–24.5 mg/l (mean 20.5 mg/L); and Day 7, 15.9–23.9 mg/l (mean 20.3 mg/l). No ceftobiprole was detected in plasma on Days −1, 10, 14 and 21. No measurable fecal concentrations were found on Days −1, 2, 4, 7, 10, 14 and 21.

Subject	Time (Days)						
	-1	2	4	7	10	14	21
1	ND	19.5	22.0	18.0	ND	ND	ND
2	ND	14.7	17.3	15.9	ND	ND	ND
3	ND	20.4	23.1	20.7	ND	ND	ND
4	ND	15.6	21.8	20.8	ND	ND	ND
5	ND	22.9	23.1	21.9	ND	ND	ND
6	ND	15.3	16.9	20.1	ND	ND	ND
7	ND	20.9	18.7	19.9	ND	ND	ND
8	ND	22.5	21.3	21.7	ND	ND	ND
9	ND	21.4	22.7	21.9	ND	ND	ND
10	ND	18.1	15.9	18.6	ND	ND	ND
11	ND	23.6	24.5	23.9	ND	ND	ND
12	ND	18.1	18.5	19.7	ND	ND	ND
Range	-	14.7-23.6	15.9-24.5	15.9-23.9	-	-	-
Mean	-	19.4	20.5	20.3	-	-	-

ND – Not detected

Table 1. Ceftobiprole plasma concentrations 10 min after completion of infusion in 12 volunteers receiving 500 mg doses of ceftobiprole intravenously every 8 h for 7 days.

4.1.2 Effect of ceftobiprole on the aerobic intestinal microflora

The effect of ceftobiprole on the aerobic intestinal microflora is shown in Figure 1. Mean counts of *E. coli* decreased by ca. 1.5 log CFU/g of feces from study Day −1 to study Day 7, with recovery to baseline counts on Day 21. Mean values for Enterobacteriaceae did not change from study Day −1 to study Day 21. Mean numbers of enterococci decreased 1.0 log CFU/g of feces from Day −1 to Day 7 and then increased 2 log CFU/g of feces to Day 14; on Day 21 the numbers of enterococci were recovered to baseline. The numbers of *Candida albicans* were within the normal variation. Changes in the aerobic intestinal microflora (≤2 log CFU/g of feces) were not significant.
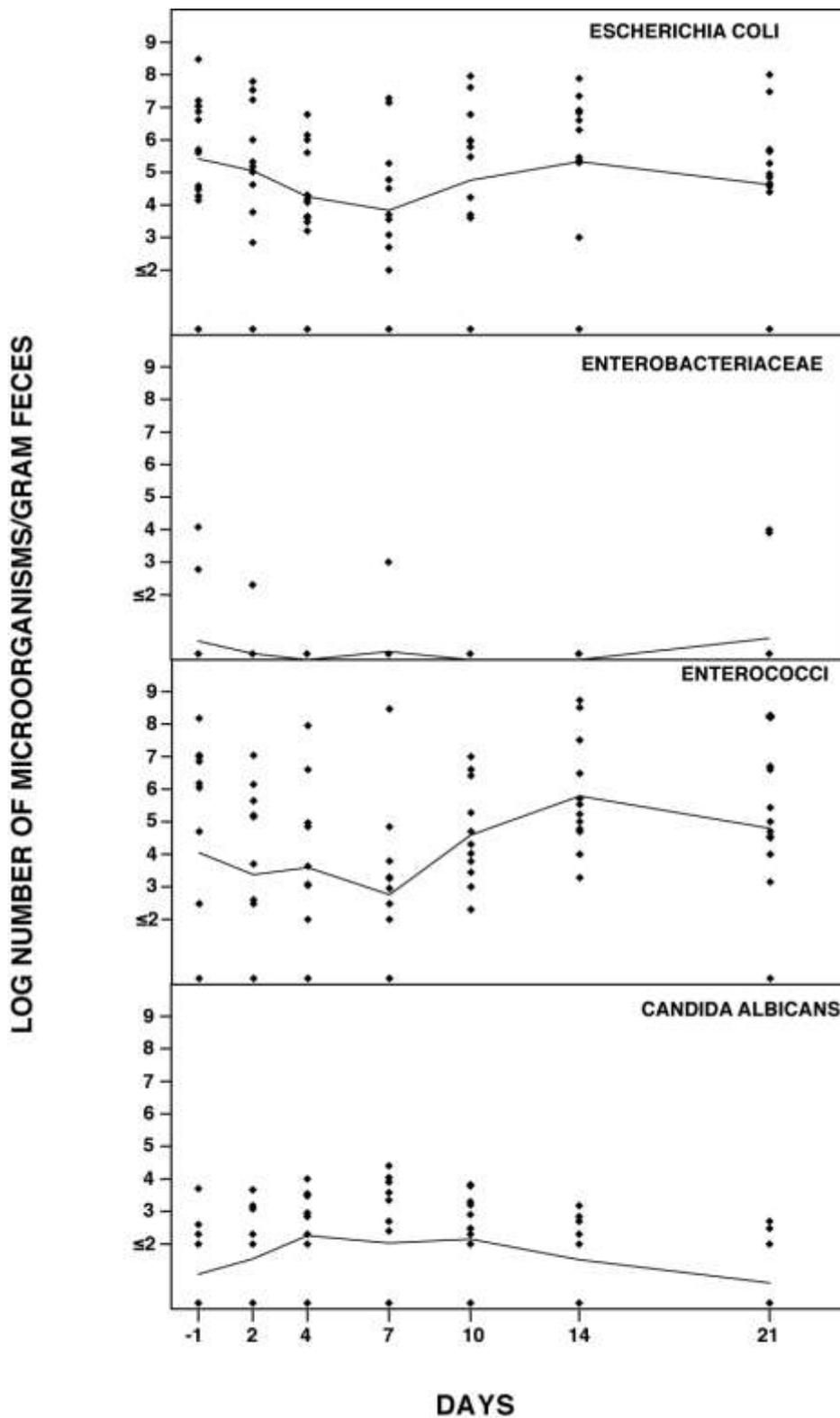
Figure 1. Effect of ceftobiprole administration on the aerobic intestinal microflora of 12 healthy volunteers. Solid line represents mean value of logarithmic number of microorganisms/g of feces.

4.1.3 Effect of ceftobiprole on the anaerobic intestinal microflora

The effect of ceftobiprole on the anaerobic intestinal microflora is shown in Figure 2. There were no changes in the numbers of lactobacilli and bifidobacteria from Day −1 to Day 21. Counts of clostridia increased from Day 2 to Day 7 by 1.5 log CFU/g of feces

and then returned to baseline counts. The numbers of *Bacteroides* were only influenced on Day 2, with a decrease of 0.5 log CFU/g of feces. All alterations were within the normal variation. No *C. difficile* strains were found.
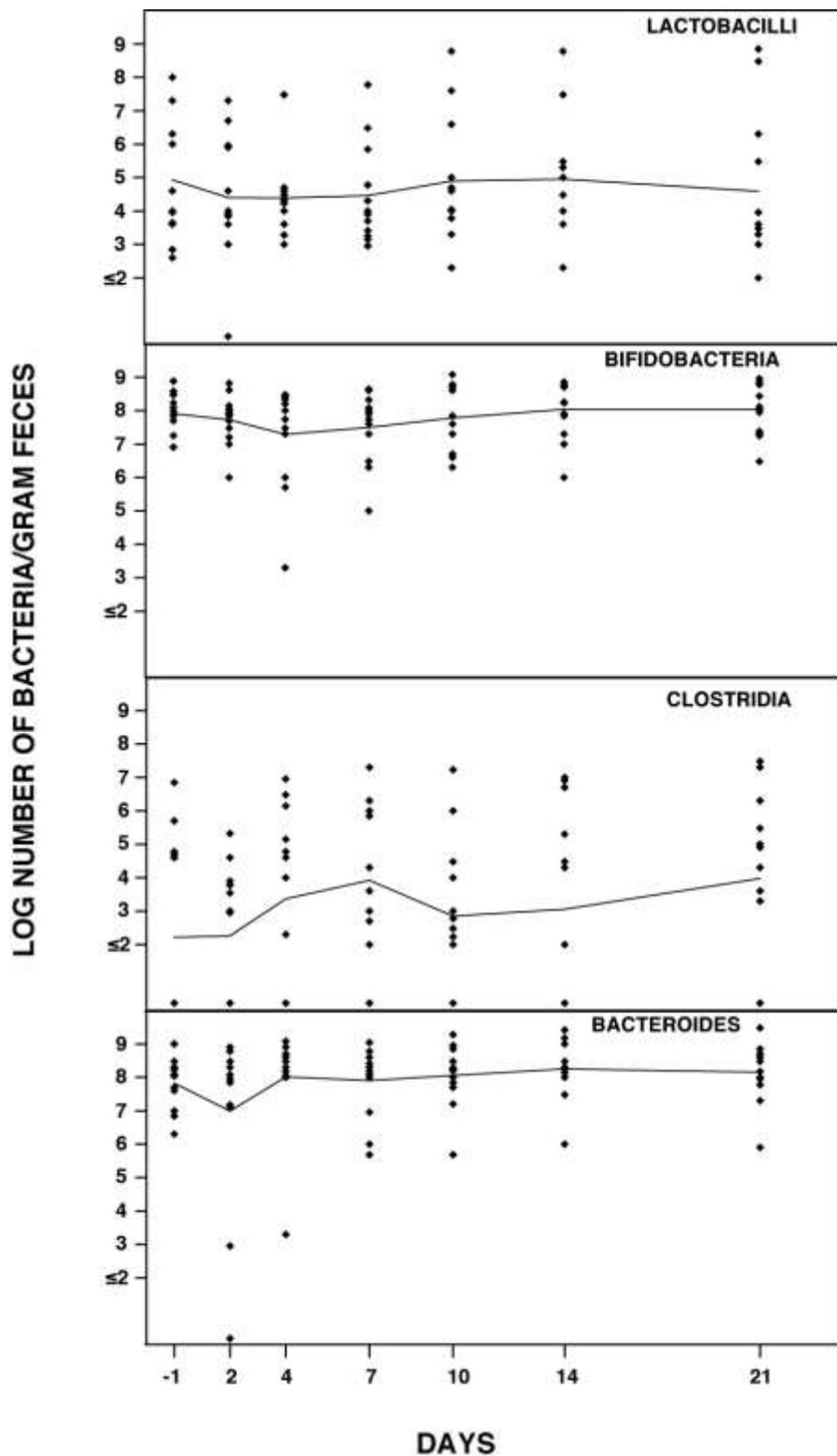

Figure 2. Effect of ceftobiprole administration on the anaerobic intestinal microflora of 12 healthy volunteers. Solid line represents mean value of logarithmic number of bacteria/g of feces.

4.1.4 Ceftobiprole susceptibility tests

No new colonizing aerobic and anaerobic bacteria resistant to ceftobiprole (MIC ≥ 4 mg/l) were found.

4.1.5 Safety and tolerability

There were 10 volunteers with 28 adverse events, all mild in severity. Four events of common cold, one event of very mild swelling of the lower lip, one event of vasovagal reaction in relation to i.v. cannula insertion and one event of muscle pain were considered to be unrelated to the study drug. Other adverse events were considered to be possibly related to ceftobiprole and included infusion-site reactions in three volunteers, with pain, mild swelling and thrombophlebitis in one volunteer. Mild rash was seen in three volunteers and vaginal candida infection in two volunteers. Two volunteers had two events of headache each. One volunteer had two events of mild diarrhea. Nausea was seen in one volunteer. During study drug infusion, five volunteers noticed mild taste alterations. No volunteer had potentially clinically significant changes in post-baseline vital sign values. No volunteers had potentially clinically significant changes in post-baseline hematology, chemistry or urine analysis based both on normal ranges and on percent change from baseline. There were no significant changes in ECG parameters from baseline to post baseline in any volunteer.

4.2 COMPARATIVE EFFECTS OF THE IMMEDIATE AND THE EXTENDED RELEASE FORMULATIONS OF CIPROFLOXACIN ON THE INTESTINAL MICROFLORA (PAPER II)

4.2.1 Eligible and non-eligible volunteers

For the study, 36 volunteers were screened and randomized. Eighteen volunteers received extended release formulation ciprofloxacin and 18 volunteers received immediate release formulation ciprofloxacin. In the extended release formulation ciprofloxacin group, one volunteer provided limited fecal material and two volunteers had non-conclusive ciprofloxacin concentrations in feces. All three volunteers were therefore excluded. In the immediate release formulation ciprofloxacin group, one volunteer provided limited fecal material, three volunteers had non-conclusive ciprofloxacin concentrations and one volunteer deviated from the protocol. All five volunteers were therefore excluded.

4.2.2 Ciprofloxacin concentrations in feces

The volunteers receiving the extended release formulation ciprofloxacin (Volunteers number 1, 2, 6, 9, 10, 12, 14, 16, 17, 27, 28, 29, 35, 37, 41) had mean concentration of ciprofloxacin 453 mg/kg on visit 2, the median concentration of ciprofloxacin was 432 mg/kg and the standard deviation was 164 mg/kg.

In the immediate release formulation ciprofloxacin group volunteers (Volunteers number 3, 5, 7, 8, 11, 13, 15, 18, 30, 32, 33, 40, 42) the mean concentration of ciprofloxacin on visit 2 was 392 mg/kg, the median concentration of ciprofloxacin was 304 mg/kg and the standard deviation was 231 mg/kg. The mean and median concentrations of ciprofloxacin in the feces were 61 mg/kg and 128 mg/kg higher in the

extended release formulation group as compared to the immediate release formulation group. No ciprofloxacin was detected in feces on visits 1, 3 and 4 in both groups.

4.2.3 Effect of ciprofloxacin agents on the intestinal aerobic and anaerobic microflora

The impact of the extended release formulation ciprofloxacin on the numbers of *E. coli*, Enterobacteriaceae, enterococci and *B. fragilis* in the intestinal microflora is shown in Figure 3 (Filled circles and dotted line). The numbers of *E. coli* were significantly suppressed while the enterococci decreased moderately.
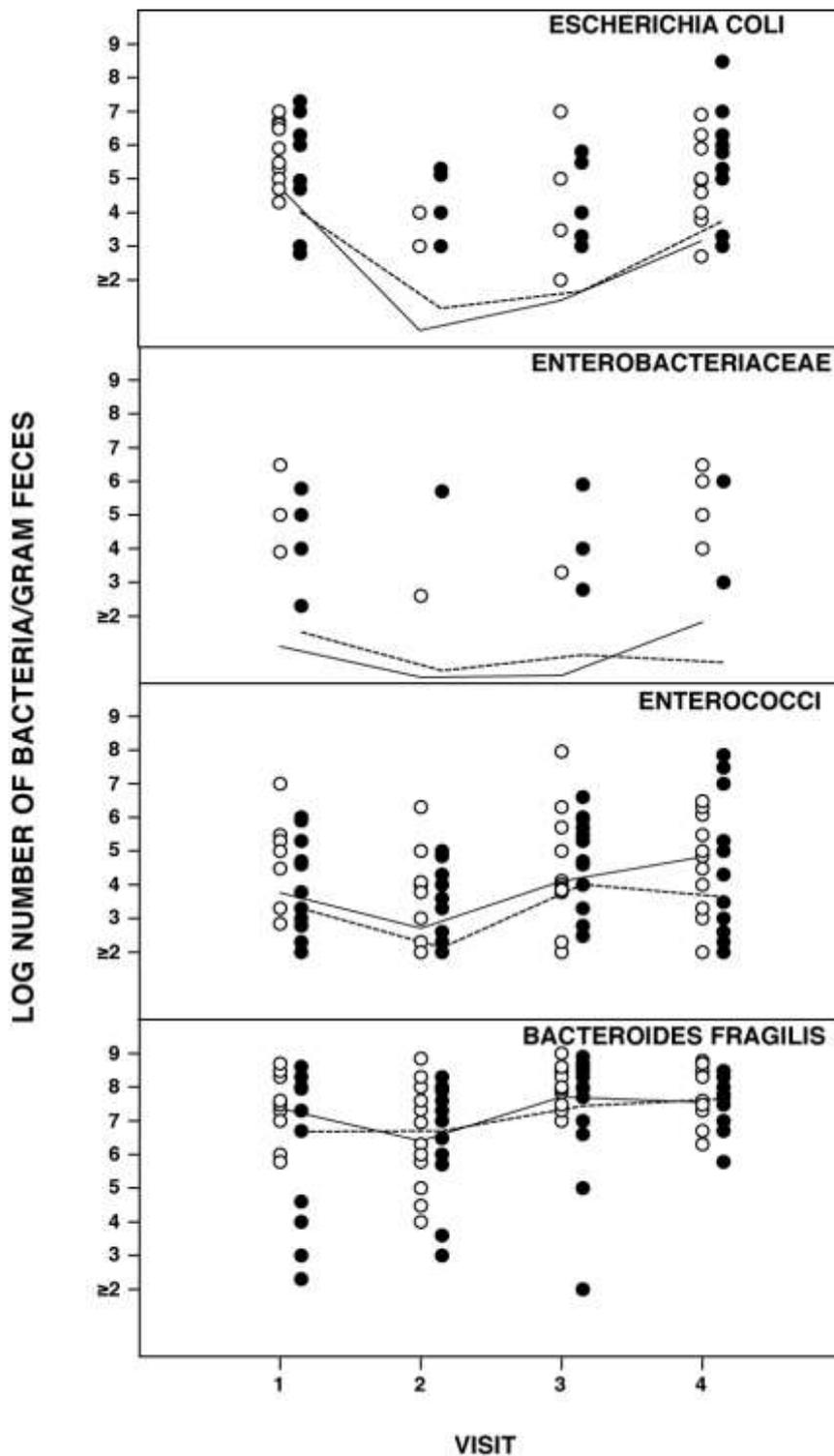
Figure 3. Effect of the extended release formulation (Filled circles and mean dotted line) ciprofloxacin and the immediate release formulation (Empty circles and mean solid line) ciprofloxacin administration on the intestinal microflora.

No significant effects were observed on the Enterobacteriaceae (*Klebsiella* and *Enterobacter* species) and *B. fragilis*. No *C. difficile* strains or toxins were detected in the extended release treatment group. The fecal flora was normalized 2 weeks after end of treatment.

In the volunteers receiving the immediate release formulation ciprofloxacin, similar findings were observed in the intestinal microflora (Figure 3 Empty circles and solid line). The numbers of *E. coli* decreased significantly on visit 2 while the numbers of Enterobacteriaceae (*Klebsiella, Enterobacter, Citrobacter* and *Pseudomonas* species), enterococci and *B. fragilis* were moderately suppressed. *E. coli* and Enterobacticeae were normalized in the fecal microflora 2 weeks after end of treatment. Enterococci and *B. fragilis* were normalized in the fecal microflora 1 week after end of treatment. One non-toxigenic *C. difficile* strain was detected from volunteer number 7 on visit 3 in the immediate release treatment group.

4.2.4 Colonization with new resistant strains

In the extended release ciprofloxacin group, one volunteer became colonized with resistant *E. coli* strains. In addition, six volunteers were colonized with resistant *E. faecium* and three volunteers were colonized with resistant *E. faecalis* strains. In the immediate release ciprofloxacin group, three volunteers were colonized with resistant *E. faecium* and three volunteers were colonized with *E. faecalis*.

4.2.5 Adverse effects and tolerability

No adverse effects or serious adverse effects were reported during the study. Both extended and immediate release formulation ciprofloxacin were safe and well tolerated in both study groups.

4.3 EFFECT OF TELAVANCIN ON HUMAN INTESTINAL MICROFLORA (PAPER III)

4.3.1 Telavancin pharmacokinetics in plasma and urine

All the volunteers (6 males and 7 females) finished the study successfully. In plasma (Figure 4) evaluation of C_{min} values indicated that steady state was achieved by Day 4 without any further increases. The plasma concentration of telavancin increased until the end of infusion (1 h). The mean ($\pm$ standard deviation) C_{max} obtained amounted to 80.3 ± 9.9 µg/ml. After infusion was stopped, the concentration initially dropped rapidly (distribution phase), but eventually declined with a mean $t_{1/2}$ of 6.51 ± 0.93 h (Fig. 4). Mean CL and Vd_{ss} were low and amounted to 1.26 ± 0.15 L/h and 11.6 ± 1.4 L/h, respectively. For V_z, a slightly higher mean value of 11.8 ± 1.5 L/h was obtained. The mean area under the concentration–time curve over a dosing interval (AUC_{tau}) amounted to 545 ± 65 h µg/ml. Telavancin was extensively excreted in the urine, with a mean CLR of 0.812 ± 0.165 L/h. A mean amount of 436 ± 75 mg telavancin was recovered from urine, corresponding to 64.4% (11.6%) of the administered dose.
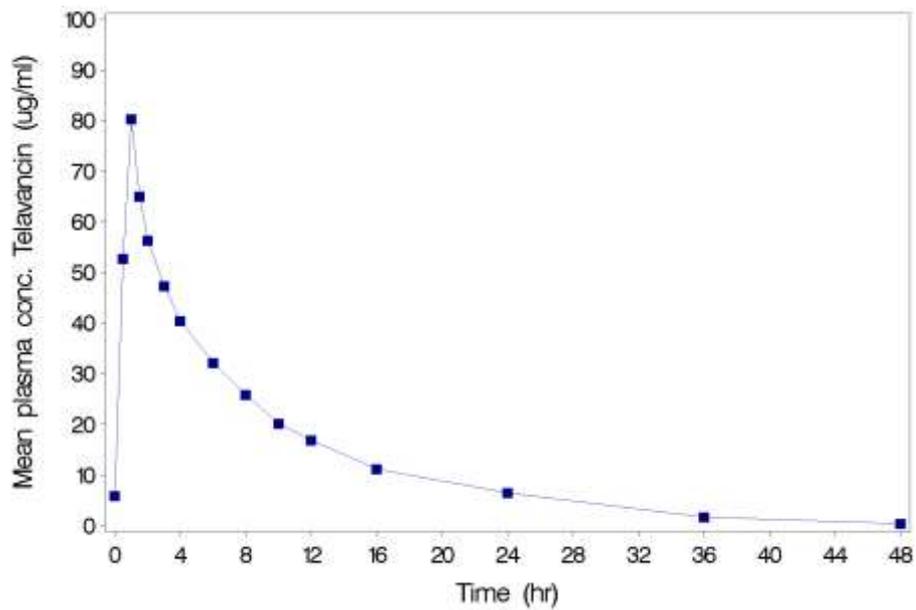
Figure 4. Mean steady-state plasma concentration versus time profile of telavancin after the last dose (Day 7).

4.3.2 Telavancin concentrations in feces

No measurable concentrations (mg/kg) of telavancin were found in the fecal samples on Days −1 (pre-dose), 2, 5, 7, 9, 14 and 21.

4.3.3 Effect of telavancin on the aerobic intestinal microflora

The effect of telavancin on the aerobic intestinal microflora is shown in Figure 5. The mean numbers of enterococci were within the normal variations (1 log CFU/g feces). No significant effects (>2 log CFU/g feces) on the mean numbers of *E. coli* and other Enterobacteriaceae species were observed during or after the administration of telavancin. The mean numbers of *C. albicans* in the intestinal microflora was not changed within the study period. Changes in the aerobic intestinal microflora were not significant.
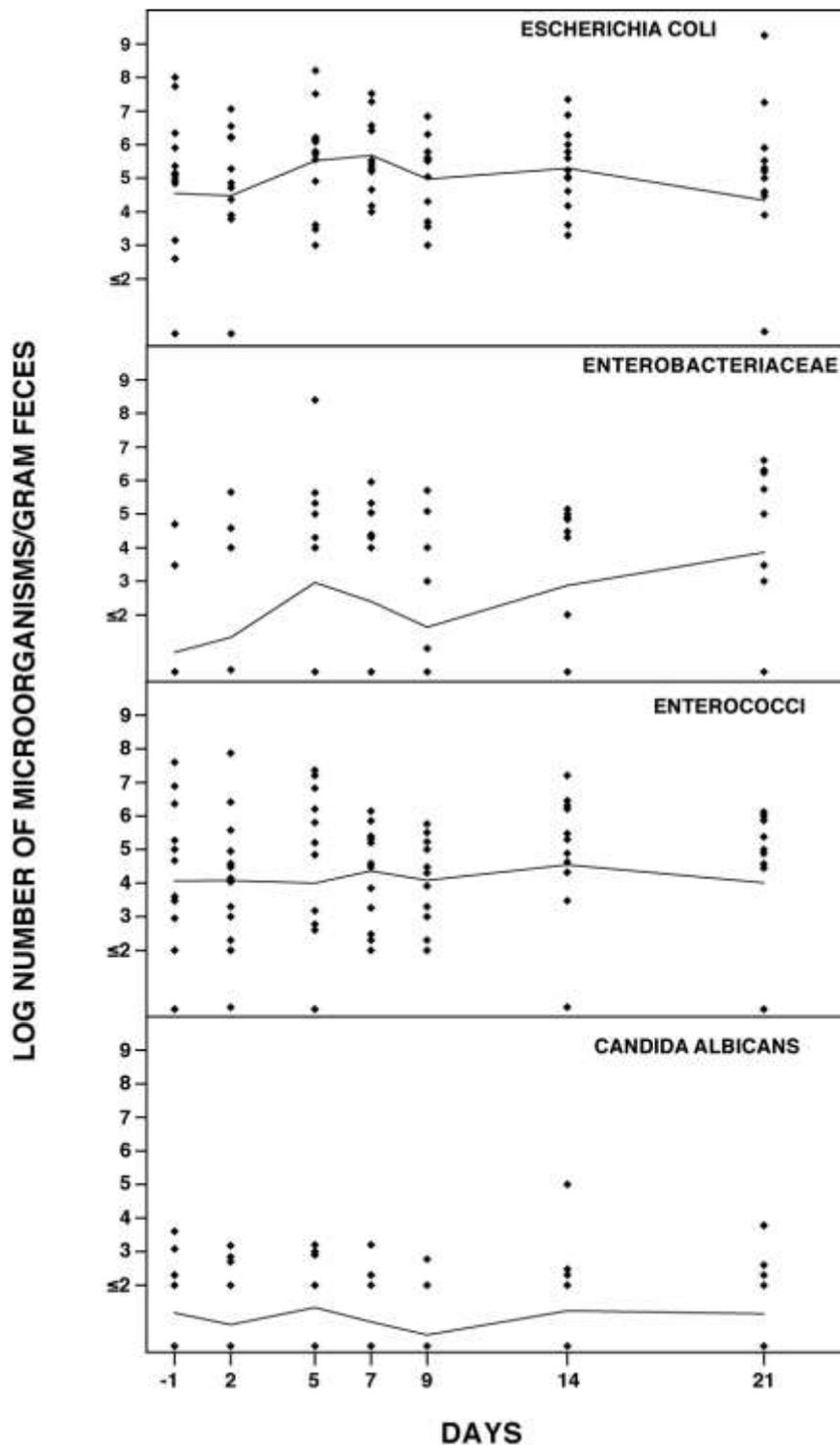
Figure 5. Effect of telavancin administration on the aerobic intestinal microflora of 13 healthy volunteers. Solid line represents mean value of logarithmic number of microorganisms/g feces.

4.3.4 Effect of telavancin on the anaerobic intestinal microflora

Figure 6 shows the effect of telavancin on the anaerobic intestinal microflora. No significant effects (>2 log CFU/g feces) on the mean numbers of lactobacilli, bifidobacteria and *Bacteroides* spp. were observed before, during or after the administration of telavancin. Mean numbers of clostridia species decreased by 1.5 log

CFU/g feces from Day 1 to Day 7, with recovery of baseline counts on Day 21. No *C. difficile* strains or toxins were detected. The changes in the anaerobic intestinal microflora were not significant.
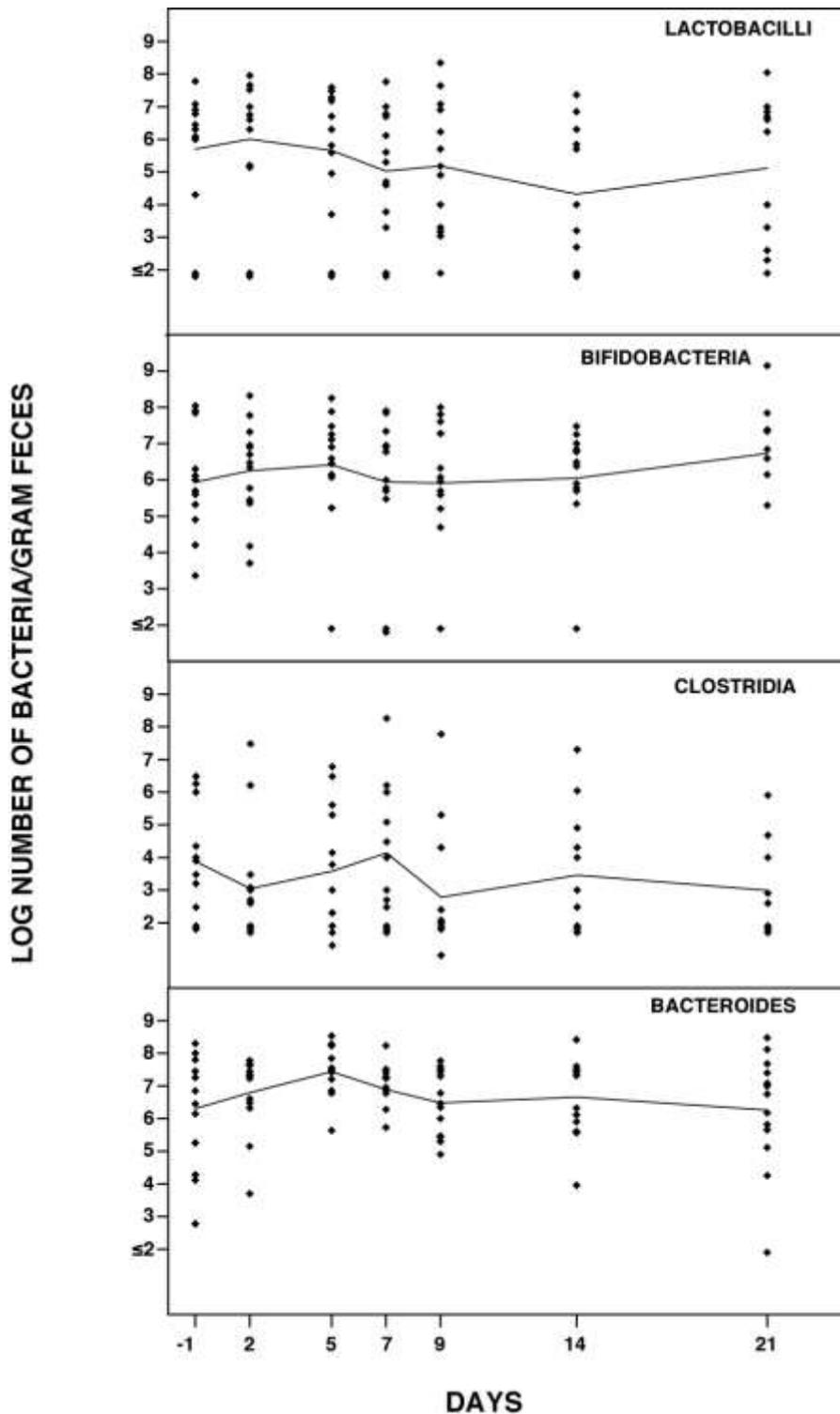

Figure 6. Effect of telavancin administration on the anaerobic intestinal microflora of 13 healthy volunteers. Solid line represents mean value of logarithmic number of bacteria/g feces.

4.3.5 Telavancin susceptibility tests

Telavancin MICs were evaluated for the Gram-positive isolates. No new colonizing aerobic or anaerobic Gram-positive bacteria resistant to telavancin (MIC $\geq$ 2 mg/l) were found.

4.3.6 Safety data

The relevant treatment-emergent adverse events (TEAEs) were considered and recorded by the investigator. Taste abnormality was reported by eight volunteers. Headache, nausea and urine abnormality were reported by three volunteers, sleepiness was reported by one volunteer, mild gastrointestinal disorders were reported by eight volunteers. During the study no volunteers died and there were no serious TEAEs. None of the adverse events resulted in a volunteer's discontinuation of the study. Results from the clinical laboratory were unremarkable and did not identify any increased risk of renal dysfunction following multiple doses of telavancin.

4.4 ECOLOGICAL IMPACT OF DOXYCYCLINE AT LOW DOSE ON NORMAL OROPHARYNGEAL AND INTESTINAL MICROFLORA (PAPER IV)

4.4.1 Eligible and non-eligible volunteers

In the doxycycline group, one volunteer was excluded from the study because of an episode of vestibular neuritis. Another volunteer in the doxycycline group was excluded due to a flexor tendon rupture in the right hand after an accident with a kitchen knife and subsequent antibiotic prophylaxis (cloxacillin 2 g orally, one dose) given to cover surgical suturing of the flexor tendon. However, all the samples from this volunteer were analyzed. In the placebo group, one volunteer with a UTI received pivmecillinam 200 mg orally b.i.d. for 7 days and provided only two samples. Another volunteer had received ciprofloxacin 500 mg orally b.i.d. for 7 days for the treatment of a UTI during the visits at Weeks 16 and 20. These volunteers were excluded.

4.4.2 Doxycycline concentrations in plasma, saliva and feces

Plasma doxycycline concentrations in the volunteers receiving doxycycline are shown in Table 2. The concentrations in samples taken after dosing were as follows: baseline visit, 0.20–0.61 mg/l (mean 0.47 mg/l); 4-week visit, 0.30–1.04 mg/l (mean 0.68 mg/l); 8-week visit, 0.43–1.49 mg/l (mean 0.72 mg/l); and 16-week visit, 0.32–1.12 mg/l (mean 0.75 mg/l). No doxycycline was detected in plasma at the 20-week visit. No doxycycline was detected in the plasma samples in the placebo group at the five visits.

Volunteer No.	Concentration (mg/l)				
	Baseline[1]	Week 4	Week 8	Week 16	Week 20
5	0.33	0.64	0.57	0.96	ND[2]
6	0.45	0.60	0.52	0.53	ND
8	0.58	0.65	0.68	1.03	ND
9	0.57	1.04	0.69	1.04	ND
11	0.42	0.80	0.70	0.35	ND
12	0.61	0.72	1.38	0.84	ND
14	0.20	1.04	0.86	-[4]	ND
18	0.49	0.70	0.66	1.12	ND
19	0.51	1.03	1.49	0.94	ND
22	0.37	0.41	0.43	0.63	ND
23	0.61	0.30	0.80	0.84	ND
25	0.57	0.64	0.68	0.85	-[3]
31	0.38	0.69	0.50	0.32	ND
32	0.47	0.43	0.47	0.56	ND
35	0.50	0.68	0.68	0.45	ND
36	0.39	0.70	0.66	0.71	ND
Range	0.20-0.61	0.30-1.04	0.43-1.49	0.32-1.12	ND
Mean	0.47	0.68	0.72	0.75	ND
Median	0.49	0.68	0.68	0.84	ND
SD	0.11	0.21	0.30	0.26	ND

[1]Baseline plasma sample taken 2 h after administration of doxycycline; [2]ND – Not Detected; [3]- Sample missing; [4]- Not reported

Table 2. Doxycycline plasma concentrations in 16 volunteers receiving 40 mg doxycycline capsule once daily for 16 weeks.

No doxycycline was detected in the saliva samples at the five visits in the volunteers receiving doxycycline and the volunteers receiving placebo. Fecal doxycycline concentrations in the volunteers receiving doxycycline are presented in Table 3. The concentrations were as follows: baseline visit, 0 mg/kg; 4-week visit, 0–3.71 mg/kg (mean 0.95 mg/kg); 8-week visit, 0–1.85 mg/kg (mean 0.51 mg/kg); 16-week visit, 0–4.10 mg/kg (mean 0.98 mg/kg); and 20-week visit, 0 mg/kg. No doxycycline was detected in the fecal samples in the placebo group during the five visits.

Volunteer No.	Concentration (mg/kg)				
	Baseline	Week 4	Week 8	Week 16	Week 20
5	ND[1]	1.16	0.73	0.40	ND
6	ND	ND	1.00	2.39	ND
8	ND	0.41	0.35	ND	ND
9	ND	0.86	0.33	0.46	ND
11	ND	0.75	ND	0.25	ND
12	ND	0.65	ND	ND	ND
14	ND	ND	1.25	1.87	ND
18	ND	0.41	0.46	1.03	ND
19	ND	2.67	ND	4.10	ND
22	ND	0.82	0.29	0.26	ND
23	ND	3.71	0.59	0.89	ND
25	ND	2.22	1.85	1.86	-[2]
31	ND	0.38	ND	0.29	ND
32	ND	ND	0.28	0.38	ND
35	ND	0.30	0.42	0.64	ND
36	ND	0.92	0.54	0.93	ND
Range	ND	0 - 3.71	0 - 1.85	0 - 4.10	ND
Mean	ND	0.95	0.51	0.98	ND
Median	ND	0.70	0.39	0.55	ND
SD	ND	1.05	0.51	1.09	ND

[1]ND – Not Detected; [2]- Sample missing

Table 3. Doxycycline fecal concentrations in 16 volunteers receiving 40 mg doxycycline capsule once daily for 16 weeks.

4.4.3 Effect of doxycycline on the oropharyngeal microflora

The effect of doxycycline on the aerobic oropharyngeal microflora is shown in Figure 7 There were no significant changes (>2 log CFU/ml) in the numbers of *Streptococcus salivarius*, *Streptococcus mitis*, *Neisseria,* micrococci, *Candida* spp. or other microorganisms during the 16 weeks of doxycycline administration. The aerobic microflora was normal at Week 20.
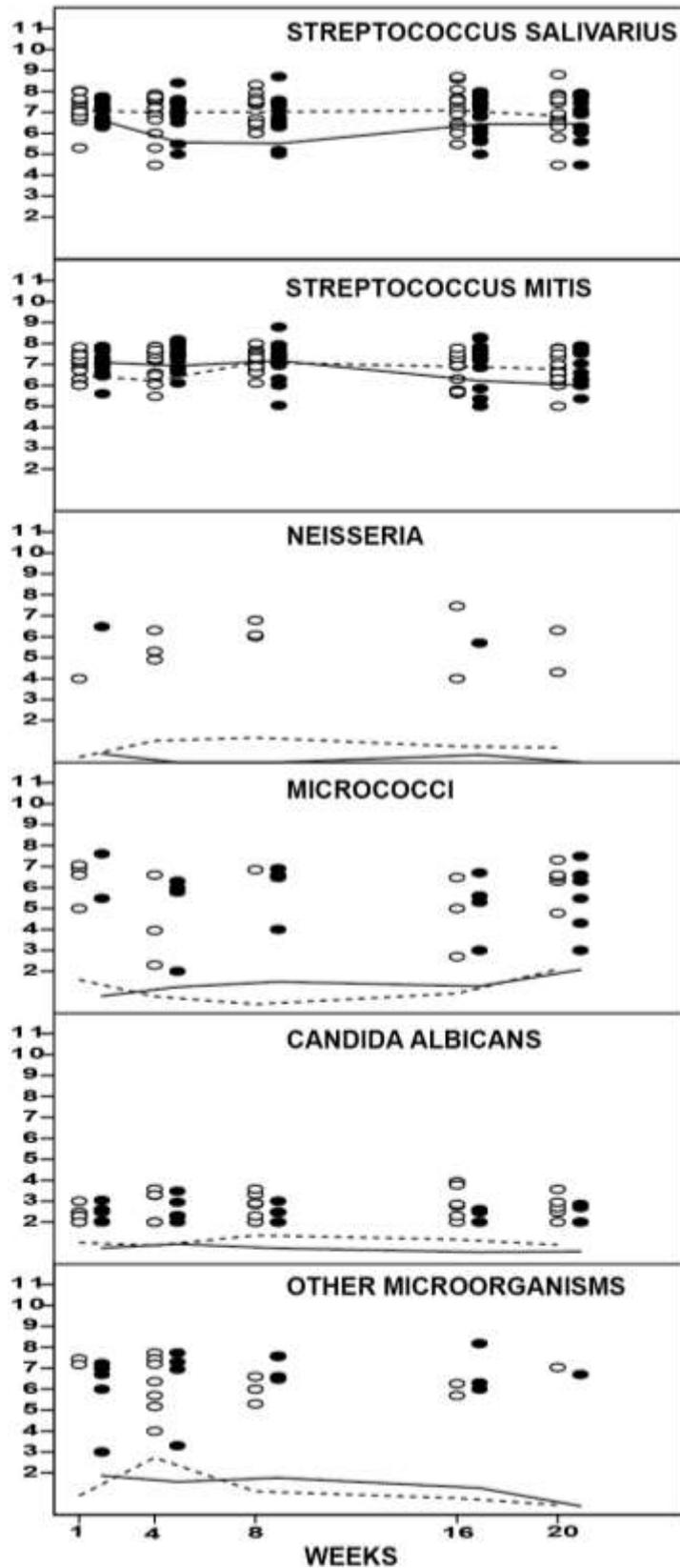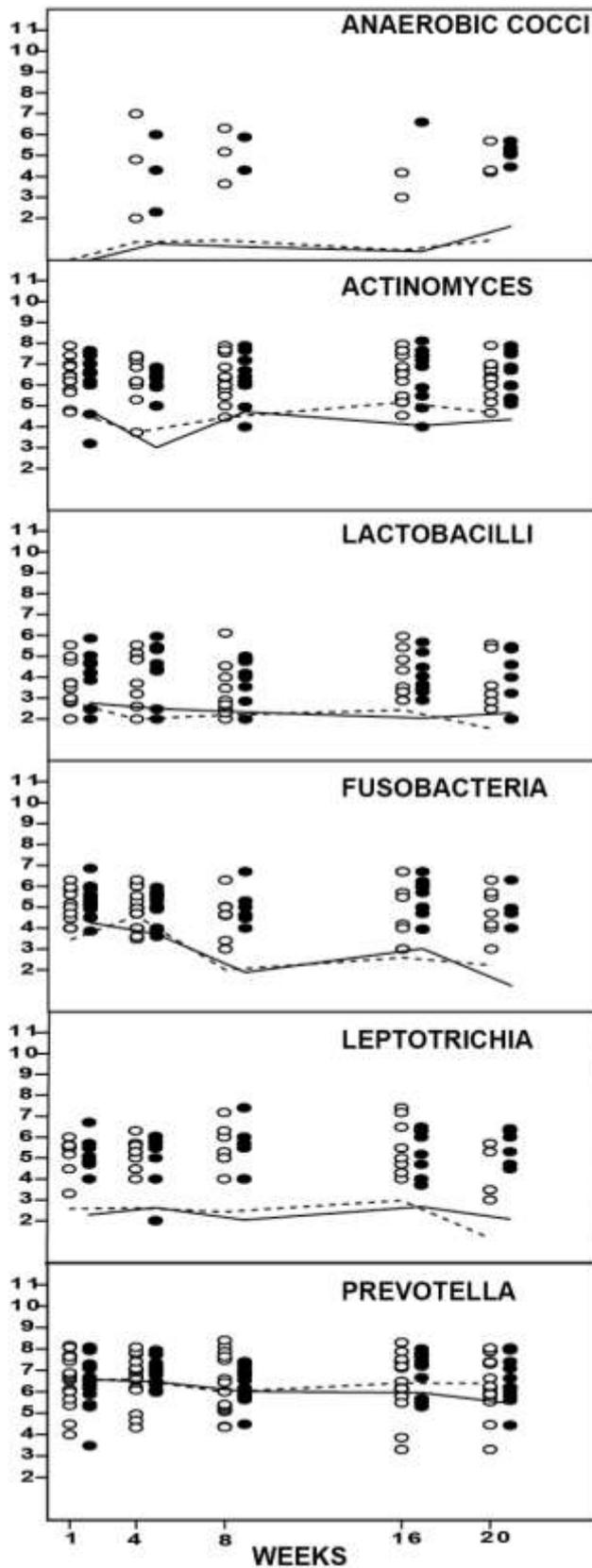
Figure 7. Effect of administration of doxycycline (— ● —) or placebo (– – ○ – –) on the aerobic oropharyngeal microflora. Log numbers of microorganisms are represented as symbols, with the mean value as lines.

Figure 8. Effect of administration of doxycycline (— ● —) or placebo (– – ○ – –) on the anaerobic oropharyngeal microflora. Log numbers of microorganisms are represented as symbols, with the mean value as lines.

Figure 8 shows the effect of doxycycline on the anaerobic oropharyngeal microflora. There were no significant changes (>2 log CFU/ml) in the numbers of anaerobic cocci, actinomyces, lactobacilli, leptotrichia and prevotella during the 16-week administration of doxycycline. Fusobacteria decreased at Week 8.

4.4.4 Effect of placebo on the oropharyngeal microflora

The effect of placebo on the aerobic oropharyngeal microflora is presented in Figure 7. The numbers of *S. salivarius, S. mitis, Neisseria,* micrococci, *Candida* spp. and other microorganisms were not significantly changed (>2 log CFU/ml) during the 20-week period.
The effect of placebo on the anaerobic oropharyngeal microflora is shown in Figure 8. The numbers of anaerobic cocci, actinomyces, lactobacilli and prevotella were not significantly changed (>2 log CFU/ml) during the 20-week period. Fusobacteria decreased at Week 8 and leptotrichia decreased at Week 20.

4.4.5 Effect of doxycycline on the intestinal microflora

Figure 9 presents the effect of doxycycline on the aerobic intestinal microflora. There were changes (2 log CFU/g) in the numbers of enterococci and *E. coli* during the 16 weeks of doxycycline administration. Other microorganisms such as other enterobacteria, *Candida* spp. and other microorganisms were not affected. The aerobic microflora was normal at Week 20.
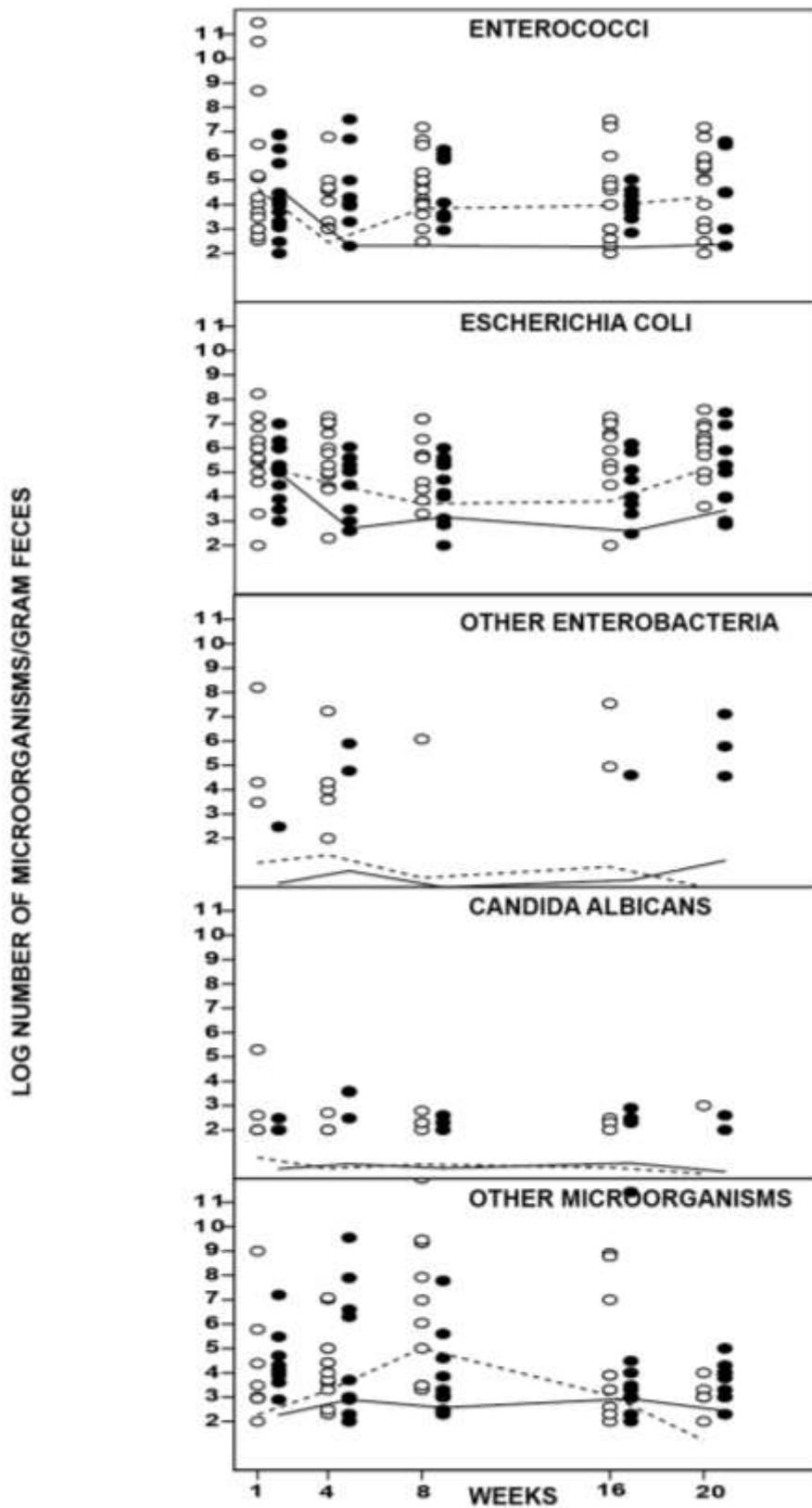
Figure 9. Effect of administration of doxycycline (— ●——) or placebo (– – ○ – –) on the aerobic intestinal microflora. Log numbers of microorganisms are represented as symbols, with the mean value as lines.

Figure 10 presents the effect of doxycycline on the anaerobic intestinal microflora. There were no significant changes (>2 log CFU/g) in the numbers of lactobacilli, bifidobacteria, clostridia and *Bacteroides* during the 16 weeks of doxycycline administration. No *C. difficile* strains were isolated. At Week 20 the anaerobic microflora was normal.
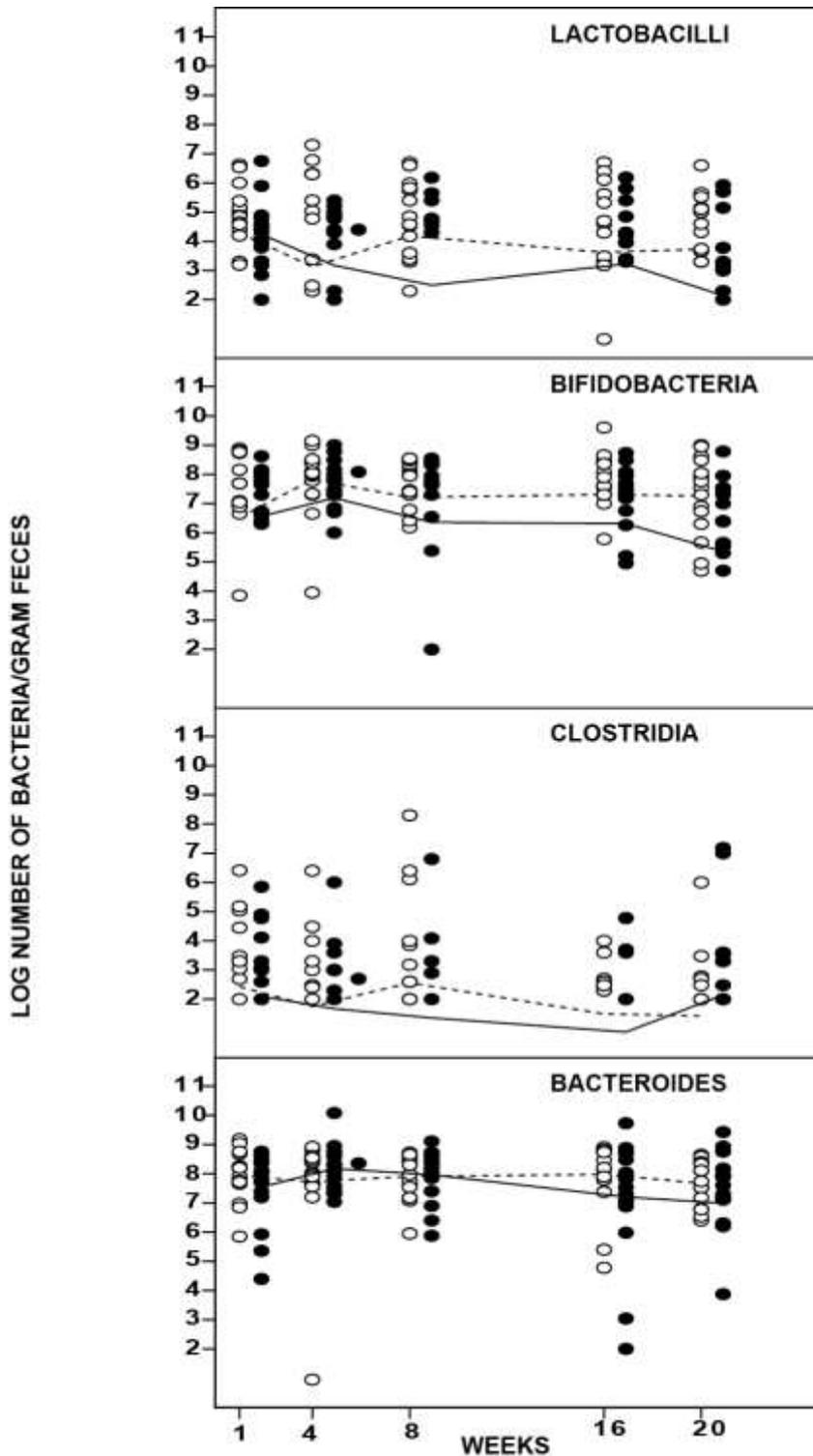

Figure 10. Effect of administration of doxycycline (— ● —) or placebo (– – ○ – –) on the anaerobic intestinal microflora. Log numbers of microorganisms are represented as symbols, with the mean value as lines.

4.4.6 Effect of placebo on the intestinal microflora

Figure 9 shows the effect of placebo on the aerobic intestinal microflora. The number of enterococci was changed (>2 log CFU/g) during the initial 4-week period. There was a minor change in the number of *E. coli* during the 20-week period. The numbers of other enterobacteria and *Candida* spp. were not changed. The numbers of other microorganisms were significantly increased during the initial 8-week period.

Figure 10 shows the effect of placebo on the anaerobic intestinal microflora. The numbers of lactobacilli, bifidobacteria, clostridia and *Bacteroides* were not significantly changed (>2 log CFU/g) during the 20-week period. No *C. difficile* strains were isolated.

4.4.7 New colonizing doxycycline-resistant microorganisms in the oropharyngeal and intestinal microflora

New colonizing doxycycline-resistant microorganisms are defined as microorganisms growing at a doxycycline MIC ≥ 4 mg/l that were not present at the baseline visit. In the oropharyngeal microflora, resistant Gram-positive cocci were isolated in the doxycycline group but not in the placebo group. There were no other marked differences in the isolation frequency between the two groups. In the anaerobic oropharyngeal microflora, significantly more doxycycline-resistant Gram-positive rods, veillonella and prevotella were found in the doxycycline group than in the placebo group (data not shown).

In the intestinal microflora, *E. faecalis*, *E. faecium* and Gram-positive rods were found significantly more in the doxycycline group than in the placebo group. In the anaerobic intestinal microflora, more doxycycline-resistant anaerobic cocci, bifidobacteria and Gram-positive rods were isolated in the doxycycline group than in the placebo group (data not shown).

4.4.8 Safety and tolerability

One hundred AEs were reported in 33 volunteers, of which the majority were mild and transient. No serious AEs were observed and four AEs were classified as related to treatment. These involved two episodes of diarrhea in one volunteer and one episode of genital candida infection in another volunteer; both of the volunteers were in the placebo group. In the doxycycline group in one volunteer flushing was reported. The most common AEs were headache (38 episodes in 16 volunteers), and nasopharyngitis (28 episodes in 19 volunteers). Less common AEs that were pyrexia (3 episodes in 3 volunteers, with 2 subjects in the doxycycline group), oropharyngeal pain (3 episodes in 2 volunteers, 1 in each treatment group), diarrhea (3 episodes in 2 volunteers, 1 in each treatment group), nausea (2 episodes in 2 volunteers, both in the placebo group) and UTI (2 episodes in 2 volunteers, both in the placebo group).

5 DISCUSSION

Antibiotics are one of the fundamental revolutions of modern medicine [22, 79, 80, 187]. Antibiotic consumption not only fortifies modern medicine, but has taken enormous modifications to human health [22, 72-74, 79, 80, 187]. The developing problem of antibiotic resistance is a severe warning to comprehensive public health [22, 72-74, 79, 80, 187]. The growing occurrence of resistant and multi-resistant bacterial strains worldwide is placing a major problem on healthcare systems and civilization [22, 72-74, 79, 80, 187]. "Antimicrobial resistance: no action today and no cure tomorrow" was the theme of World Health Day 2011 [188]. The condition is provoked by a significant weakening in research and development into antibacterial agents [22, 187].

The extensive use of antibiotics both in humans and animals has caused the development of many resistant bacteria and subsequently limited their use in therapy [79, 80, 187, 189] Resistance is undoubtedly not limited to one antibiotic and has been reported amongst most classes of antibacterials [79, 80, 187, 189]. Administration of antibacterial agents can cause disturbances in the ecological balance between the host and microorganisms [6, 7]. These changes are dependent on the spectrum of activity, dose, route of administration, pharmacokinetic and pharmacodynamic properties and in vivo inactivation of the agent [6, 7]. Secretion of an agent by intestinal mucosa or bile may have an impact on the intestinal microflora leading to antibiotic resistance [6, 7].

5.1 CEFTOBIPROLE (PAPER I)

For the treatment of complicated skin infections and pneumonia, ceftobiprole is a promising antimicrobial agent [190]. The effect of ceftobiprole on the normal intestinal microflora has not been studied earlier. No fecal concentration of ceftobiprole was found and the minor effect on the intestinal microflora is thus explained by these results. Ceftobiprole is mainly eliminated by renal excretion [105]. No new colonizing ceftobiprole-resistant aerobic and anaerobic bacteria in the normal intestinal flora were recovered, probably due to less selective pressure for the emergence of colonization by resistant microorganisms. CDI is an adverse event mainly associated with antibiotic treatment and prophylaxis [11]. Broad-spectrum antibiotics such as cephalosporins, fluoroquinolones and amoxicillin are most often involved in causing the CDI [36, 123]. In the paper I, no clinical *C. difficile* infection was observed, probably due to lack of biological activity of ceftobiprole in the intestine. Ceftobiprole is reported to be well tolerated with good safety, which was also observed in paper I [191]. Based on the findings in paper I, ceftobiprole has a favorable ecological profile. However, when ceftobiprole is used in hospitalized patients with serious infections and pre-existing *C. difficile* strains, the risk of development of CDI should be monitored.

5.2 CIPROFLOXACIN (PAPER II)

Ciprofloxacin is a widely used fluroquinolone for the treatment of UTIs with high bactericidal activity against uropathogens and has a well-established clinical efficacy [192]. The impact of ciprofloxacin on the human intestinal microflora has been studied before and measurable concentration of ciprofloxacin in feces had been detected [193-

197]. The anaeerobic and aerobic bacteria in the fecal flora were suppressed markedly during the prophylactic period as well as throughout treatment period [6, 193]. The intestinal microflora was almost normal within 2 weeks after the treatment by ciprofloxacin [6]. The concentrations of ciprofloxacin in the intestinal mucosa and feces were in excess of the MICs for most of the anaerobic and aerobic bacteria [6, 193]. In paper II, the effects of the two formulations of ciprofloxacin on the normal microflora were compared. Measurable concentrations of ciprofloxacin in feces were detected in both groups. The aerobic fecal flora was suppressed during the treatment in both study groups and the microflora was normal 2 weeks after the end of the treatment. Both formulations had minor effects on the intestinal normal anaerobic microflora. No toxigenic *C. difficile* strains or toxins were detected in this study. Compared with other fluoroquinolones, ciprofloxacin has lower impact on the normal microflora and in causing CDI [36, 123, 198]. Based on the results from paper II, on the microbiological data on the intestinal microflora as well as on the bioassays for antibiotic concentrations in the fecal samples, no major differences could be observed between the new extended release formulation and the immediate release formulation ciprofloxacin.

5.3 TELAVANCIN (PAPER III)

The impact of telavancin on the normal intestinal microflora has not been studied previously. Telavancin is a semisynthetic derivative of vancomycin and is recommended for the treatment of adult patients with complicated skin and skin-structure infections caused by Gram-positive bacteria [140, 141, 199, 200]. Most often described adverse reactions have been reported to be mild and reversible, with taste disturbance, headache, nausea, vomiting and procedural site pain [130]. Telavancin was well tolerated in the study III and is excreted primarily by renal elimination, with 60–70% of the dose excreted unchanged in the urine and <1% in the feces. Renal dysfunction has been found more frequently with telavancin than vancomycin [130, 199, 201]. Prolongation of corrected QT (QTc) interval has been reported for telavancin, but no clinically significant ECG changes have been seen [140]. Owing to its $t_{1/2}$ of ca. 6.5 h with low intersubject variability, a steady state of telavancin was already achieved by Day 4 in all volunteers. Telavancin showed low variability in C_{max} and AUC_{tau} between volunteers, resulting in a consistent and predictable exposure. In paper III, the main route for elimination was via renal excretion of unchanged telavancin, which accounted for 64.4% of its elimination. No fecal concentration of telavancin was found, which probably explains the lack of an effect on the intestinal microflora. No new colonizing telavancin-resistant anaerobic and aerobic Gram-positive bacteria in the normal flora were recovered, probably due to less selective pressure for the emergence of colonization by resistant microorganisms. No toxigenic *C. difficile* strains were detected in the subjects during or after treatment with telavancin. Based on the results from paper III, the microbiological data on the intestinal microflora as well as the results of the bioassays for antibiotic concentrations in fecal samples, telavancin has a favorable ecological profile.

5.4 DOXYCYCLINE (PAPER IV)

Extensive use of tetracyclines both in humans and animals has caused the development of many resistant bacteria and subsequently limited the use of tetracyclines in the treatment of infections [151, 153, 154]. Tetracyclines have many other interesting properties not related to their antibiotic activity [144, 156, 157]. Tetracyclines have independent anti-inflammatory effects at subantimicrobial doses [144, 156, 157]. A large amount of literature has provided evidence for the anti-inflammatory properties of tetracyclines as well as in the management of acne and rosacea [158, 159, 161-163, 202]. The traditional tetracycline dose has an effect on antibiotic susceptibility and resistance development on the host microflora [169, 170]. Subantimicrobial doxycycline, which has the benefit of fewer AEs compared with higher doses, has raised questions about potential changes in antibiotic susceptibility of the host microflora, an event known to occur with higher-dose doxycycline [176, 177]. Many studies have shown that a long-term subantimicrobial doxycycline dose does not contribute to changes in antibiotic susceptibility and resistance of the host microflora [176, 177]. In the study IV, it was found that a subantimicrobial doxycycline dose (40 mg) had a minor ecological effect on the oropharyngeal and intestinal microflora. The oropharyngeal and intestinal microflora in the doxycycline group had more resistant Gram-positive cocci, Gram-positive rods, veillonella, prevotella, *E. faecalis* and *E. faecium* than the placebo group. The clinical significance of this finding is not apparent and more studies will be needed with a lower dose of doxycycline (20 mg) in order to maintain the anti-inflammatory effects without any ecological impact on the normal microflora.

6 CONCLUSION

This thesis shows that intravenous administration of antibiotics (ceftobiprole and telavancin) has less impact on the intestinal microflora if excreted through urine. As a consequence, there is less disruption of the normal microflora by the antibiotics and low risk to develop CDI. The antibiotic (ciprofloxacin) that had an impact on the intestinal microflora regardless of the formulations of dose release, has potential risk to cause CDI. The sub-antimicrobial dose of antibiotics (doxycycline) has effects on the normal microflora in relation to placebo. The sub-antimicrobial dose of antibiotics has a selective pressure on microflora and it may cause a development of resistance or to increase the frequency of the resistance among commensals.

7 FUTURE PERSPECTIVES OF THE NORMAL FLORA STUDY

For the treatment of infections by antibiotics, the importance is to eradicate the pathogen as quickly as possible with minimal adverse effects on the host and minimal disruption of the normal microflora. This may decrease the frequency of resistance development against antibiotics. Using the best methods to predict the effects of antibiotics on the dominant microflora of healthy humans it is possible to predict the impact of antibiotics by studying the dominant microflora. Antibiotics have effects on the susceptible microflora directly and on the non-susceptible microflora indirectly. There are scopes to develop suitable methods to predict the impacts of antibiotics on the minor microflora and to discover their relation with the dominant microflora as well as the immunological consequences and health for the host. Studying the microbiome using advanced and specific molecular methods can predict the impact on the uncultivable normal microflora. However, the molecular technology does not reflect on the phenotypic properties of the normal microflora. Conventional studies are very important to identify new resistance patterns. By studying the resistance mechanisms, it will be possible to design and invent new drugs that will have less influence on the normal microflora. Additionally, it is important to gain knowledge on the mechanisms or pathways by which the microorganisms become resistant to certain antibiotics and by reversing or inhibiting the pathways or mechanisms the drug can be still active. The molecular studies are using different classification methods to identify normal microflora and are also avoiding minor microflora to present their results. Recent comparison studies show that molecular studies are not superior to culture based studies. Molecular studies are at this moment still very expensive. Therefore, there is still a need for further development of the molecular methods to be comparable with the culture-based methods. A multi-disciplinary approach is needed since the whole bacterial community plays a role in antibiotic resistance. Future research is required to discover additional ways how bacteria communicate with each other, with the environment and with other microorganisms. We do not have suitable methods to know any particular antibiotic concentration at the active site of the microorganisms. We have methods to measure the surrogate concentrations of antibiotics by using blood or epithelial lining fluids. A multi-disciplinary approach is therefore needed to solve the problem. It will eventually help to use appropriate dosages of antibiotics that may show less impact on the normal microflora, new ways to combat the emergence of antibiotic resistance and decrease the risk of resistance development.

We are conducting a collaborative study with six European countries to understand how the administration of standard doses of antibiotics affects the normal microflora during one year. Both molecular and conventional methods are the basis of the study. It will give new insights into limiting resistance development and transmission of antibiotic-resistant bacterial strains. The genetic basis of drug resistance is studied together with the persistence and mode of transmission of antibiotic-resistant strains, biological cost to the microorganism, resistant phenotype and ecological impact.

8 ACKNOWLEDGEMENTS

I would like to thank all the former and present staff at the substrate division for helping me by providing all kinds of media whenever necessary.

I would like to thank to Dr. Shah Jalal, Dr. Erick Amaya, Dr. Samuel Vilchez, Dr. Protim Sarker, Dr. Hongliang Zhong, Dr. Syed Moshfiqur Rahman, Dr. Krizsán Gergely, Dr. Jenny Svärd, Dr. Samir Abdurahman, Salah Shanan and Halime Ekici for giving their valuable time to analyze data and also help me to think scientifically.

I am very thankful to all the former and present post-docs, PhD students and staff at the Department who all supported me, particularly to Roberto, Kerstin, Haihui, Ha, Selina, Alenka, Jessica, Anthony, Sarene, Hong, Yijian, Rozita, Helena, Roberto, Salah, Piotr, Marjan, Amir, Samir, Synthia, Sharon, Monika, Eva, Sonal, Hanna, Omar, Bea, Annica, Markus, Marcus, Fredrik, Sepideh, Lars, Gustaf, Melissa, Johanna, Irene, Marcel, Kasper, Harold, Noel, Simin, Imran, Masoud, Gökce, Masud, Maroof, Kathrin, Catharina, Semin, Maria, Waltteri, Martin, Amanda, Anette, Babilonia, Erwin, João, Shuba, Sophie, Xiaoshan, Kajsa.

I would like to acknowledge the academic, administrative, library and technical support of the Karolinska Institutet and its staff, particularly to Gudrun Rafi for her assistance in different academic and administrative works.

I am equally thankful to all my teachers and friends from Karolinska Institutet, Swedish Language School, Rangpur Medical College, and Cantonment Public School & College in Saidpur.

I am very grateful to my brother Md. Harun-Ur Rashid. He gave me moral support and guided me in different matters. I would like to thank all my friends and family members around the world.

I would like to show my gratitude to Nora and Roffe for making my life in Sweden easier by giving much practical advice. I and my wife enjoyed a lot with Nora in her summer house.

Above all, it is a great pleasure to thank my wife Disha for her personal support and great patience at all times. My parents, sisters and my wife's family have given me their unequivocal support throughout, as always, for which my mere expression of thanks likewise does not sufficient.

9 REFERENCES

[1]   Sullivan AE, C. Nord, CE. Interaction between antimicrobial agents and the oropharyngeal and the intestinal microflora. In: Bryskier A, editor. Antimicrobial Agents. Bryskier A ed. Washington, DC: ASM Press; 2005, p. 1357-70.

[2]   Sullivan Å, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 2001;1:101-14.

[3]   Nord CE, Kager L. The normal flora of the gastrointestinal tract. Neth J Med 1984;27:249-52.

[4]   Tannock GW. Molecular assessment of intestinal microflora. Am J Clin Nutr 2001;73:410S-4S.

[5]   O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep 2006;7:688-93.

[6]   Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect Dis 2001;1:101-14.

[7]   Rashid MU, Weintraub A, Nord CE. Effect of new antimicrobial agents on the ecological balance of human microflora. Anaerobe 2012;18:249-53.

[8]   Nord CE, Edlund C. Impact of antimicrobial agents on human intestinal microflora. J Chemother 1990;2:218-37.

[9]   Nord CE, Heimdahl A. Impact of different antimicrobial agents on the colonisation resistance in the intestinal tract with special reference to doxycycline. Scand J Infect Dis Suppl 1988;53:50-8.

[10]   Nord CE, Heimdahl A, Kager L, Malmborg AS. The impact of different antimicrobial agents on the normal gastrointestinal microflora of humans. Rev Infect Dis 1984;6 Suppl 1:S270-5.

[11]   Nord CE, Kager L, Heimdahl A. Impact of antimicrobial agents on the gastrointestinal microflora and the risk of infections. Am J Med 1984;76:99-106.

[12]   Nord CE, Kager L, Malmborg AS. Effects of antimicrobial prophylaxis on colonization resistance. J Hosp Infect 1988;11 Suppl A:259-64.

[13]   Sullivan A, Edlund C, Svenungsson B, Emtestam L, Nord CE. Effect of perorally administered pivmecillinam on the normal oropharyngeal, intestinal and skin microflora. J Chemother 2001;13:299-308.

[14]   Rylander M, Nord CE, Norrby SR. Comparative in vitro activity of the new oral cephalosporin Bay v 3522 against aerobic and anaerobic bacteria. Eur J Clin Microbiol Infect Dis 1990;9:777-82.

[15]   Ritz M, Lode H, Fassbender M, Borner K, Koeppe P, Nord CE. Multiple-dose pharmacokinetics of sparfloxacin and its influence on fecal flora. Antimicrob Agents Chemother 1994;38:455-9.

[16]   Nord CE. Effect of quinolones on the human intestinal microflora. Drugs 1995;49 Suppl 2:81-5.

[17]   Nord CE, Edlund C. Clinical impact of newer quinolones: influence on normal microflora. J Chemother 1989;1:18-23.

[18]   Nord CE, Heimdahl A. Impact of orally administered antimicrobial agents on human oropharyngeal and colonic microflora. J Antimicrob Chemother 1986;18 Suppl C:159-64.

[19]   Norhagen GE, Engstrom PE, Hammarstrom L, Smith CI, Nord CE. Oral and intestinal microflora in individuals with different immunoglobulin deficiencies. Eur J Clin Microbiol Infect Dis 1990;9:631-3.

[20] Edlund C, Nord CE. Ecological impact of antimicrobial agents on human intestinal microflora. Alpe Adria Microbiol J 1993;3:137-64.

[21] Sullivan A, Fianu-Jonasson A, Landgren BM, Nord CE. Ecological effects of perorally administered pivmecillinam on the normal vaginal microflora. Antimicrob Agents Chemother 2005;49:170-5.

[22] Norrby SR, Nord CE, Finch R. Lack of development of new antimicrobial drugs: a potential serious threat to public health. Lancet Infect Dis 2005;5:115-9.

[23] Freire-Moran L, Aronsson B, Manz C, Gyssens IC, So AD, Monnet DL, et al. Critical shortage of new antibiotics in development against multidrug-resistant bacteria-Time to react is now. Drug Resist Updat 2011;14:118-24.

[24] Yezli S, Li H. Antibiotic resistance amongst healthcare-associated pathogens in China. Int J Antimicrob Agents 2012;40:389-97.

[25] Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, et al. The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. Lancet Infect Dis 2013;13:155-65.

[26] van der Bij AK, Pitout JD. The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. J Antimicrob Chemother 2012;67:2090-100.

[27] Savard P, Perl TM. A call for action: managing the emergence of multidrug-resistant Enterobacteriaceae in the acute care settings. Curr Opin Infect Dis 2012;25:371-7.

[28] Otto M. MRSA virulence and spread. Cell Microbiol 2012;14:1513-21.

[29] Khan AS, Dancer SJ, Humphreys H. Priorities in the prevention and control of multidrug-resistant Enterobacteriaceae in hospitals. J Hosp Infect 2012;82:85-93.

[30] Johnson S. Recurrent *Clostridium difficile* infection: a review of risk factors, treatments, and outcomes. J Infect 2009;58:403-10.

[31] Nord CE. Effect of new quinolones on the human gastrointestinal microflora. Rev Infect Dis 1988;10 Suppl 1:S193-6.

[32] Nord CE. Studies on the ecological impact of antibiotics. Eur J Clin Microbiol Infect Dis 1990;9:517-8.

[33] Nord CE. The effect of antimicrobial agents on the ecology of the human intestinal microflora. Vet Microbiol 1993;35:193-7.

[34] Nord CE, Heimdahl A, Kager L. Antimicrobial agents and the human oropharyngeal and intestinal microflora. Ann Ist Super Sanita 1986;22:883-92.

[35] Nord CE, Heimdahl A, Kager L. Antimicrobial induced alterations of the human oropharyngeal and intestinal microflora. Scand J Infect Dis Suppl 1986;49:64-72.

[36] Spencer RC. The role of antimicrobial agents in the aetiology of *Clostridium difficile*-associated disease. J Antimicrob Chemother 1998;41 Suppl C:21-7.

[37] Levy SB. Antibiotic resistance: an ecological imbalance. Ciba Found Symp 1997;207:1-9; discussion -14.

[38] Nord CE, Kager L, Heimdahl A. Microbiological and clinical aspects on intraabdominal infections. Scand J Gastroenterol Suppl 1984;100:31-4.

[39] Thomas C, Stevenson M, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile*-associated diarrhoea: a systematic review. J Antimicrob Chemother 2003;51:1339-50.

[40] Lai KK, Melvin ZS, Menard MJ, Kotilainen HR, Baker S. *Clostridium difficile*-associated diarrhea: epidemiology, risk factors, and infection control. Infect Control Hosp Epidemiol 1997;18:628-32.

[41] Louie TJ. How should we respond to the highly toxogenic NAP1/ribotype 027 strain of *Clostridium difficile*? CMAJ 2005;173:1049-50.

[42] Edlund C, Hedberg M, Nord CE. Antimicrobial treatment of periodontal diseases disturbs the human ecology: a review. J Chemother 1996;8:331-41.

[43] Edlund C, Bjorkman L, Ekstrand J, Sandborgh-Englund G, Nord CE. Resistance of the normal human microflora to mercury and antimicrobials after exposure to mercury from dental amalgam fillings. Clin Infect Dis 1996;22:944-50.

[44] Mackowiak PA. The normal microbial flora. N Engl J Med 1982;307:83-93.

[45] Gibbons RJ. Adherent interactions which may affect microbial ecology in the mouth. J Dent Res 1984;63:378-85.

[46] Murray PB, EJ. Jorgensen, JH. Landry, ML. Pfaller, MA. Manual of Clinical Microbiology. 9th ed. Washington, DC: ASM Press; 2007.

[47] Davis CP. Normal Flora. In: Baron S, editor. Medical Microbiology. Galveston TX: The University of Texas Medical Branch at Galveston; 1996.

[48] Nord CE, Heimdahl A. Cardiovascular infections: bacterial endocarditis of oral origin. Pathogenesis and prophylaxis. J Clin Periodontol 1990;17:494-6.

[49] Nord CE. Incidence and significance of intraperitoneal aerobic and anaerobic bacteria. Clin Ther 1990;12 Suppl B:9-20.

[50] Mollby R, Aronsson B, Nord CE. Pathogenesis and diagnosis of *Clostridium difficile* enterocolitis. Scand J Infect Dis Suppl 1985;46:47-56.

[51] Fainstein V, Bodey GP, McCredie KB, Keating MJ, Estey EH, Bolivar R, et al. Coagulation abnormalities induced by beta-lactam antibiotics in cancer patients. J Infect Dis 1983;148:745-50.

[52] Kikuchi S, Ando A, Minato K. [Acquired coagulopathy caused by administration of parenteral broad-spectrum antibiotics]. Rinsho Byori 1991;39:83-90.

[53] Sjostedt S, Kager L, Heimdahl A, Nord CE. Microbial colonization of tumors in relation to the upper gastrointestinal tract in patients with gastric carcinoma. Ann Surg 1988;207:341-6.

[54] Bartlett JG. *Clostridium difficile*: clinical considerations. Rev Infect Dis 1990;12 Suppl 2:S243-51.

[55] Kelly CP, Pothoulakis C, LaMont JT. *Clostridium difficile* colitis. N Engl J Med 1994;330:257-62.

[56] Manian FA, Meyer L. CDAD rates. Infect Control Hosp Epidemiol 1995;16:63-5.

[57] McDonald LC, Killgore GE, Thompson A, Owens RC, Jr., Kazakova SV, Sambol SP, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med 2005;353:2433-41.

[58] Maroo S, Lamont JT. Recurrent *Clostridium difficile*. Gastroenterology 2006;130:1311-6.

[59] Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 2006;367:1747-57.

[60] Zaffiri L, Gardner J, Toledo-Pereyra LH. History of antibiotics. From salvarsan to cephalosporins. J Invest Surg 2012;25:67-77.

[61] Wallace WC, Cinat ME, Nastanski F, Gornick WB, Wilson SE. New epidemiology for postoperative nosocomial infections. Am Surg 2000;66:874-8.

[62] Riethmiller S. From Atoxyl to Salvarsan: searching for the magic bullet. Chemotherapy 2005;51:234-42.

[63] Gradmann C. Magic bullets and moving targets: antibiotic resistance and experimental chemotherapy, 1900-1940. Dynamis 2011;31:305-21.

[64] Schwartz RS. Paul Ehrlich's magic bullets. N Engl J Med 2004;350:1079-80.

[65] Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer 2008;8:473-80.

[66] Hare R. New light on the discovery of penicillin. Med Leg J 1970;38:31-41.

[67] Hare R. New light on the history of penicillin. Med Hist 1982;26:1-24.

[68] The Chemical Study of Penicillin: A Brief History. Science 1947;105:653-9.

[69] Fleming A. The story of penicillin. J Am Inst Homeopath 1946;39:154-7.

[70] Florey HW. Penicillin; its development for medical uses. Proc R Inst G B 1946;33:23-30.

[71] Powers JH. Antimicrobial drug development--the past, the present, and the future. Clin Microbiol Infect 2004;10 Suppl 4:23-31.

[72] Rao GG. Risk factors for the spread of antibiotic-resistant bacteria. Drugs 1998;55:323-30.

[73] Shah D, Dang MD, Hasbun R, Koo HL, Jiang ZD, DuPont HL, et al. *Clostridium difficile* infection: update on emerging antibiotic treatment options and antibiotic resistance. Expert Rev Anti Infect Ther 2010;8:555-64.

[74] Rybak MJ, LaPlante KL. Community-associated methicillin-resistant *Staphylococcus aureus*: a review. Pharmacotherapy 2005;25:74-85.

[75] Wadl M, Heckenbach K, Noll I, Ziesing S, Pfister W, Beer J, et al. Increasing occurrence of multidrug-resistance in Acinetobacter baumannii isolates from four German University Hospitals, 2002-2006. Infection 2010;38:47-51.

[76] Milloy MJ, Wood E. Transmitted antiretroviral-resistant HIV: a coming anarchy? Lancet Infect Dis 2011;11:336-7.

[77] Jones RN. Resistance patterns among nosocomial pathogens: trends over the past few years. Chest 2001;119:397S-404S.

[78] Miethke T. [Increasing Therapeutic Challenges through Multi-Resistant Bacteria in the Hospital.]. Zentralbl Chir 2011.

[79] Appelbaum PC. 2012 and beyond: potential for the start of a second pre-antibiotic era? J Antimicrob Chemother 2012;67:2062-8.

[80] Elhani D. [Does the emergence of antibiotic resistance announce the return of the dark ages?]. Ann Biol Clin (Paris) 2011;69:637-46.

[81] Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 2010;74:417-33.

[82] Fernandez L, Hancock RE. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. Clin Microbiol Rev 2012;25:661-81.

[83] Johnson AP, Woodford N. Global spread of antibiotic resistance: the example of New Delhi metallo-beta-lactamase (NDM)-mediated carbapenem resistance. J Med Microbiol 2013;62:499-513.

[84] Kanerva M. [Gut bacteria and antimicrobial resistance--what to worry about]. Duodecim 2012;128:1755-61.

[85] Karki S, Cheng AC. Impact of non-rinse skin cleansing with chlorhexidine gluconate on prevention of healthcare-associated infections and colonization with multi-resistant organisms: a systematic review. J Hosp Infect 2012;82:71-84.

[86] Lawley TD, Walker AW. Intestinal colonization resistance. Immunology 2013;138:1-11.

[87] Torok ME, Chantratita N, Peacock SJ. Bacterial gene loss as a mechanism for gain of antimicrobial resistance. Curr Opin Microbiol 2012;15:583-7.

[88] Ganguly NK, Arora NK, Chandy SJ, Fairoze MN, Gill JP, Gupta U, et al. Rationalizing antibiotic use to limit antibiotic resistance in India. Indian J Med Res 2011;134:281-94.

[89] Kumar R, Yadav BR, Anand SK, Singh RS. Molecular surveillance of putative virulence factors and antibiotic resistance in *Staphylococcus aureus* isolates recovered from intra-mammary infections of river buffaloes. Microb Pathog 2011;51:31-8.

[90] Amaya E, Reyes D, Paniagua M, Calderon S, Rashid MU, Colque P, et al. Antibiotic resistance patterns of *Escherichia coli* isolates from different aquatic environmental sources in Leon, Nicaragua. Clin Microbiol Infect 2012;18:E347-54.

[91] Moellering RC, Jr. NDM-1--a cause for worldwide concern. N Engl J Med 2010;363:2377-9.

[92] Bhat M, Dumortier C, Taylor BS, Miller M, Vasquez G, Yunen J, et al. *Staphylococcus aureus* ST398, New York City and Dominican Republic. Emerg Infect Dis 2009;15:285-7.

[93] Walsh C, Fanning S. Antimicrobial resistance in foodborne pathogens--a cause for concern? Curr Drug Targets 2008;9:808-15.

[94] Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 2010;8:251-9.

[95] Wright GD. Antibiotic resistance in the environment: a link to the clinic? Curr Opin Microbiol 2010;13:589-94.

[96] Sahoo KC, Tamhankar AJ, Sahoo S, Sahu PS, Klintz SR, Lundborg CS. Geographical variation in antibiotic-resistant Escherichia coli isolates from stool, cow-dung and drinking water. Int J Environ Res Public Health 2012;9:746-59.

[97] Ochman H, Lawrence JG, Groisman EA. Lateral gene transfer and the nature of bacterial innovation. Nature 2000;405:299-304.

[98] Dubey GP, Ben-Yehuda S. Intercellular nanotubes mediate bacterial communication. Cell 2011;144:590-600.

[99] Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. Br J Pharmacol 2008;153 Suppl 1:S347-57.

[100] Li XZ, Nikaido H. Efflux-mediated drug resistance in bacteria. Drugs 2004;64:159-204.

[101] Li XZ, Zhang L, Poole K. Interplay between the MexA-MexB-OprM multidrug efflux system and the outer membrane barrier in the multiple antibiotic resistance of *Pseudomonas aeruginosa*. J Antimicrob Chemother 2000;45:433-6.

[102] Smith R, Coast J. The true cost of antimicrobial resistance. BMJ 2013;346:f1493.

[103] Hebeisen P, Heinze-Krauss I, Angehrn P, Hohl P, Page MG, Then RL. In vitro and in vivo properties of Ro 63-9141, a novel broad-spectrum cephalosporin with activity against methicillin-resistant staphylococci. Antimicrob Agents Chemother 2001;45:825-36.

[104] Jones RN, Deshpande LM, Mutnick AH, Biedenbach DJ. In vitro evaluation of BAL9141, a novel parenteral cephalosporin active against oxacillin-resistant staphylococci. J Antimicrob Chemother 2002;50:915-32.

[105] Murthy B, Schmitt-Hoffmann A. Pharmacokinetics and pharmacodynamics of ceftobiprole, an anti-MRSA cephalosporin with broad-spectrum activity. Clin Pharmacokinet 2008;47:21-33.

[106] Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007;298:1763-71.

50

[107] Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 2006;355:666-74.

[108] Micek ST. Alternatives to vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Clin Infect Dis 2007;45 Suppl 3:S184-90.

[109] Maclayton DO, Hall RG, 2nd. Pharmacologic treatment options for nosocomial pneumonia involving methicillin-resistant *Staphylococcus aureus*. Ann Pharmacother 2007;41:235-44.

[110] Barbour A, Schmidt S, Rand KH, Derendorf H. Ceftobiprole: a novel cephalosporin with activity against Gram-positive and Gram-negative pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA). Int J Antimicrob Agents 2009;34:1-7.

[111] Entenza JM, Hohl P, Heinze-Krauss I, Glauser MP, Moreillon P. BAL9141, a novel extended-spectrum cephalosporin active against methicillin-resistant *Staphylococcus aureus* in treatment of experimental endocarditis. Antimicrob Agents Chemother 2002;46:171-7.

[112] Davies TA, Page MG, Shang W, Andrew T, Kania M, Bush K. Binding of ceftobiprole and comparators to the penicillin-binding proteins of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2007;51:2621-4.

[113] Kosowska K, Hoellman DB, Lin G, Clark C, Credito K, McGhee P, et al. Antipneumococcal activity of ceftobiprole, a novel broad-spectrum cephalosporin. Antimicrob Agents Chemother 2005;49:1932-42.

[114] Arias CA, Singh KV, Panesso D, Murray BE. Evaluation of ceftobiprole medocaril against *Enterococcus faecalis* in a mouse peritonitis model. J Antimicrob Chemother 2007;60:594-8.

[115] Bush K, Heep M, Macielag MJ, Noel GJ. Anti-MRSA beta-lactams in development, with a focus on ceftobiprole: the first anti-MRSA beta-lactam to demonstrate clinical efficacy. Expert Opin Investig Drugs 2007;16:419-29.

[116] Queenan AM, Shang W, Kania M, Page MG, Bush K. Interactions of ceftobiprole with beta-lactamases from molecular classes A to D. Antimicrob Agents Chemother 2007;51:3089-95.

[117] Jones ME. In-vitro profile of a new beta-lactam, ceftobiprole, with activity against methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 2007;13 Suppl 2:17-24.

[118] Ednie L, Shapiro S, Appelbaum PC. Antianaerobe activity of ceftobiprole, a new broad-spectrum cephalosporin. Diagn Microbiol Infect Dis 2007;58:133-6.

[119] Blondeau JM. Current issues in the management of urinary tract infections: extended-release ciprofloxacin as a novel treatment option. Drugs 2004;64:611-28.

[120] Hickerson AD, Carson CC. The treatment of urinary tract infections and use of ciprofloxacin extended release. Expert Opin Investig Drugs 2006;15:519-32.

[121] Sharma PC, Jain A, Jain S, Pahwa R, Yar MS. Ciprofloxacin: review on developments in synthetic, analytical, and medicinal aspects. J Enzyme Inhib Med Chem 2010;25:577-89.

[122] Waugh J, Keating GM. Ciprofloxacin extended release: in the treatment of urinary tract infections and uncomplicated pyelonephritis. Drugs Aging 2004;21:55-64; discussion 5-6.

[123] Appelbaum PC, Hunter PA. The fluoroquinolone antibacterials: past, present and future perspectives. Int J Antimicrob Agents 2000;16:5-15.

[124] Naber KG, Bergman B, Bishop MC, Bjerklund-Johansen TE, Botto H, Lobel B, et al. EAU guidelines for the management of urinary and male genital tract

infections. Urinary Tract Infection (UTI) Working Group of the Health Care Office (HCO) of the European Association of Urology (EAU). Eur Urol 2001;40:576-88.

[125] Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002;113 Suppl 1A:5S-13S.

[126] Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. Antimicrob Agents Chemother 2002;46:2540-5.

[127] Stratton C. Fluoroquinolone antibiotics: properties of the class and individual agents. Clin Ther 1992;14:348-75; discussion 7.

[128] Leadbetter MR, Adams SM, Bazzini B, Fatheree PR, Karr DE, Krause KM, et al. Hydrophobic vancomycin derivatives with improved ADME properties: discovery of telavancin (TD-6424). J Antibiot (Tokyo) 2004;57:326-36.

[129] Higgins DL, Chang R, Debabov DV, Leung J, Wu T, Krause KM, et al. Telavancin, a multifunctional lipoglycopeptide, disrupts both cell wall synthesis and cell membrane integrity in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2005;49:1127-34.

[130] Shaw JP, Cheong J, Goldberg MR, Kitt MM. Mass balance and pharmacokinetics of [14C]telavancin following intravenous administration to healthy male volunteers. Antimicrob Agents Chemother 2010;54:3365-71.

[131] Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171:388-416.

[132] Heyland DK, Cook DJ, Griffith L, Keenan SP, Brun-Buisson C. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. The Canadian Critical Trials Group. Am J Respir Crit Care Med 1999;159:1249-56.

[133] Safdar N, Dezfulian C, Collard HR, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. Crit Care Med 2005;33:2184-93.

[134] Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS. Epidemiology and outcomes of health-care-associated pneumonia: results from a large US database of culture-positive pneumonia. Chest 2005;128:3854-62.

[135] Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992-2003. Clin Infect Dis 2006;42:389-91.

[136] Levine DP. Vancomycin: a history. Clin Infect Dis 2006;42 Suppl 1:S5-12.

[137] Rubinstein E, Corey GR, Stryjewski ME, Kanafani ZA. Telavancin for the treatment of serious gram-positive infections, including hospital acquired pneumonia. Expert Opin Pharmacother 2011;12:2737-50.

[138] Draghi DC, Benton BM, Krause KM, Thornsberry C, Pillar C, Sahm DF. In vitro activity of telavancin against recent Gram-positive clinical isolates: results of the 2004-05 Prospective European Surveillance Initiative. J Antimicrob Chemother 2008;62:116-21.

[139] Mendes RE, Moet GJ, Janechek MJ, Jones RN. In vitro activity of telavancin against a contemporary worldwide collection of *Staphylococcus aureus* isolates. Antimicrob Agents Chemother 2010;54:2704-6.

[140] Saravolatz LD, Stein GE, Johnson LB. Telavancin: a novel lipoglycopeptide. Clin Infect Dis 2009;49:1908-14.

[141] Rubinstein E, Lalani T, Corey GR, Kanafani ZA, Nannini EC, Rocha MG, et al. Telavancin versus Vancomycin for Hospital-Acquired Pneumonia due to Gram-positive Pathogens. Clin Infect Dis 2011;52:31-40.

[142] Bahrami F, Morris DL, Pourgholami MH. Tetracyclines: drugs with huge therapeutic potential. Mini Rev Med Chem 2012;12:44-52.

[143] Laugier P, Daguet J. Acne conglobata successfully treated with aureomycin. Bull Soc Fr Dermatol Syphiligr 1951;58:74-5.

[144] Sapadin AN, Fleischmajer R. Tetracyclines: nonantibiotic properties and their clinical implications. J Am Acad Dermatol 2006;54:258-65.

[145] Zhanel GG, Homenuik K, Nichol K, Noreddin A, Vercaigne L, Embil J, et al. The glycylcyclines: a comparative review with the tetracyclines. Drugs 2004;64:63-88.

[146] Saikali Z, Singh G. Doxycycline and other tetracyclines in the treatment of bone metastasis. Anticancer Drugs 2003;14:773-8.

[147] Eady EA, Cove JH. Is acne an infection of blocked pilosebaceous follicles? Implications for antimicrobial treatment. Am J Clin Dermatol 2000;1:201-9.

[148] Joshi N, Miller DQ. Doxycycline revisited. Arch Intern Med 1997;157:1421-8.

[149] Barden TC, Buckwalter BL, Testa RT, Petersen PJ, Lee VJ. "Glycylcyclines". 3. 9-Aminodoxycyclinecarboxamides. J Med Chem 1994;37:3205-11.

[150] Sum PE, Lee VJ, Testa RT, Hlavka JJ, Ellestad GA, Bloom JD, et al. Glycylcyclines. 1. A new generation of potent antibacterial agents through modification of 9-aminotetracyclines. J Med Chem 1994;37:184-8.

[151] Sum PE, Sum FW, Projan SJ. Recent developments in tetracycline antibiotics. Curr Pharm Des 1998;4:119-32.

[152] Chopra I. Glycylcyclines: third-generation tetracycline antibiotics. Curr Opin Pharmacol 2001;1:464-9.

[153] Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 2001;65:232-60 ; second page, table of contents.

[154] Tuckman M, Petersen PJ, Projan SJ. Mutations in the interdomain loop region of the tetA(A) tetracycline resistance gene increase efflux of minocycline and glycylcyclines. Microb Drug Resist 2000;6:277-82.

[155] Tally FT, Ellestad GA, Testa RT. Glycylcyclines: a new generation of tetracyclines. J Antimicrob Chemother 1995;35:449-52.

[156] Golub LM, Ramamurthy N, McNamara TF, Gomes B, Wolff M, Casino A, et al. Tetracyclines inhibit tissue collagenase activity. A new mechanism in the treatment of periodontal disease. J Periodontal Res 1984;19:651-5.

[157] Golub LM, Ramamurthy NS, McNamara TF, Greenwald RA, Rifkin BR. Tetracyclines inhibit connective tissue breakdown: new therapeutic implications for an old family of drugs. Crit Rev Oral Biol Med 1991;2:297-321.

[158] Baldwin HE. Tricks for improving compliance with acne therapy. Dermatol Ther 2006;19:224-36.

[159] Conde JF, Yelverton CB, Balkrishnan R, Fleischer AB, Jr., Feldman SR. Managing rosacea: a review of the use of metronidazole alone and in combination with oral antibiotics. J Drugs Dermatol 2007;6:495-8.

[160] Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, et al. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. J Biol Chem 1997;272:31504-9.

[161] Krakauer T, Buckley M. Doxycycline is anti-inflammatory and inhibits staphylococcal exotoxin-induced cytokines and chemokines. Antimicrob Agents Chemother 2003;47:3630-3.

[162] Marks R. The enigma of rosacea. J Dermatolog Treat 2007;18:326-8.

[163] Preshaw PM, Hefti AF, Bradshaw MH. Adjunctive subantimicrobial dose doxycycline in smokers and non-smokers with chronic periodontitis. J Clin Periodontol 2005;32:610-6.

[164] Berman B, Zell D. Subantimicrobial dose doxycycline: a unique treatment for rosacea. Cutis 2005;75:19-24.

[165] Bhatia ND, Del Rosso JQ. Optimal management of papulopustular rosacea: rationale for combination therapy. J Drugs Dermatol 2012;11:838-44.

[166] Caton JG, Ciancio SG, Blieden TM, Bradshaw M, Crout RJ, Hefti AF, et al. Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planing in patients with adult periodontitis. J Periodontol 2000;71:521-32.

[167] Preshaw PM, Hefti AF, Jepsen S, Etienne D, Walker C, Bradshaw MH. Subantimicrobial dose doxycycline as adjunctive treatment for periodontitis. A review. J Clin Periodontol 2004;31:697-707.

[168] Walker C, Puumala S, Golub LM, Stoner JA, Reinhardt RA, Lee HM, et al. Subantimicrobial dose doxycycline effects on osteopenic bone loss: microbiologic results. J Periodontol 2007;78:1590-601.

[169] Maibach H. Second-generation tetracyclines, a dermatologic overview: clinical uses and pharmacology. Cutis 1991;48:411-7.

[170] Moller JK, Bak AL, Stenderup A, Zachariae H, Afzelius H. Changing patterns of plasmid-mediated drug resistance during tetracycline therapy. Antimicrob Agents Chemother 1977;11:388-91.

[171] Heimdahl A, Nord CE. Influence of doxycycline on the normal human flora and colonization of the oral cavity and colon. Scand J Infect Dis 1983;15:293-302.

[172] Heimdahl A, Nord CE, Borthen L. Impact of phenoxymethylpenicillin, erythromycin, clindamycin and doxycycline on Streptococcus salivarius in the oropharynx. J Antimicrob Chemother 1984;13:505-9.

[173] Thomas J, Walker C, Bradshaw M. Long-term use of subantimicrobial dose doxycycline does not lead to changes in antimicrobial susceptibility. J Periodontol 2000;71:1472-83.

[174] Thomas JG, Metheny RJ, Karakiozis JM, Wetzel JM, Crout RJ. Long-term sub-antimicrobial doxycycline (Periostat) as adjunctive management in adult periodontitis: effects on subgingival bacterial population dynamics. Adv Dent Res 1998;12:32-9.

[175] Walker C, Bradshaw M. The effect of oral doxycycline 100 mg once-daily form 14 days on the nasopharyngeal flora of healthy volunteers: a preliminary analysis. 26th Anniversary Fall Clinical Dermatology Conference. Las Vegas (NV); 2007, p. 18-27.

[176] Walker C, Preshaw PM, Novak J, Hefti AF, Bradshaw M, Powala C. Long-term treatment with sub-antimicrobial dose doxycycline has no antibacterial effect on intestinal flora. J Clin Periodontol 2005;32:1163-9.

[177] Walker C, Thomas J, Nango S, Lennon J, Wetzel J, Powala C. Long-term treatment with subantimicrobial dose doxycycline exerts no antibacterial effect on the subgingival microflora associated with adult periodontitis. J Periodontol 2000;71:1465-71.

[178] Nord CE, Sillerstrom E, Wahlund E. Effect of tigecycline on normal oropharyngeal and intestinal microflora. Antimicrob Agents Chemother 2006;50:3375-80.

[179] Rashid M, Weintraub A, Nord CE. Comparative effects of the immediate and the extended release formulations of ciprofloxacin on normal human intestinal microflora. J Chemother 2011;23:145-9.

[180] Backstrom T, Panagiotidis G, Beck O, Asker-Hagelberg C, Rashid MU, Weintraub A, et al. Effect of ceftobiprole on the normal human intestinal microflora. Int J Antimicrob Agents 2010;36:537-41.

[181] Nord CE, Rasmanis G, Wahlund E. Effect of dalbavancin on the normal intestinal microflora. J Antimicrob Chemother 2006;58:627-31.

[182] Nord CE. Diagnosis of anaerobic infections by gas-liquid chromatography. Acta Pathol Microbiol Scand Suppl 1977:55-9.

[183] Huang H, Weintraub A, Fang H, Nord CE. Comparison of a commercial multiplex real-time PCR to the cell cytotoxicity neutralization assay for diagnosis of *Clostridium difficile* infections. J Clin Microbiol 2009;47:3729-31.

[184] CLSI. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard-Eighth Edition. 2012.

[185] CLSI. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. In: Institute CaLS, editor. Approved standard- Seventh edition (M7-A7) ed: Wayne, PA: Clinically and Laboratory Standards Institute; 2006.

[186] CLSI. Methods for antimicrobial susceptibility testing of anaerobic bacteria. In: Institute CaLS, editor. Approved standard- Seventh edition (M11-A7) ed: Wayne, PA: Clinically and Laboratory Standards Institute; 2007.

[187] Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother 2008;62 Suppl 1:i1-9.

[188] WHO. http://www.who.int/dg/speeches/2011/WHD_20110407/en/index.html. 2011.

[189] Someya Y, Yamaguchi A, Sawai T. A novel glycylcycline, 9-(N,N-dimethylglycylamido)-6-demethyl-6-deoxytetracycline, is neither transported nor recognized by the transposon Tn10-encoded metal-tetracycline/H+ antiporter. Antimicrob Agents Chemother 1995;39:247-9.

[190] Cereda RF, Azevedo HD, Girardello R, Xavier DE, Gales AC, Group I-ACBS. Antimicrobial activity of ceftobiprole against gram-negative and gram-positive pathogens: results from INVITA-A-CEFTO Brazilian study. Braz J Infect Dis 2011;15:339-48.

[191] Schirmer PL, Deresinski SC. Ceftobiprole: a new cephalosporin for the treatment of skin and skin structure infections. Expert Rev Anti Infect Ther 2009;7:777-91.

[192] Fourcroy JL, Berner B, Chiang YK, Cramer M, Rowe L, Shore N. Efficacy and safety of a novel once-daily extended-release ciprofloxacin tablet formulation for treatment of uncomplicated urinary tract infection in women. Antimicrob Agents Chemother 2005;49:4137-43.

[193] Brismar B, Edlund C, Malmborg AS, Nord CE. Ciprofloxacin concentrations and impact of the colon microflora in patients undergoing colorectal surgery. Antimicrob Agents Chemother 1990;34:481-3.

[194] Brismar B, Edlund C, Malmborg AS, Nord CE. Ecological impact of antimicrobial prophylaxis on intestinal microflora in patients undergoing colorectal surgery. Scand J Infect Dis Suppl 1990;70:25-30.

[195] Edlund C, Nord CE. Suppression of the oropharyngeal and gastrointestinal microflora by ciprofloxacin: microbiological and clinical consequences. Scand J Infect Dis Suppl 1989;60:98-103.

[196] Ljungberg B, Nilsson-Ehle I, Edlund C, Nord CE. Influence of ciprofloxacin on the colonic microflora in young and elderly volunteers: no impact of the altered drug absorption. Scand J Infect Dis 1990;22:205-8.

[197] Wistrom J, Gentry LO, Palmgren AC, Price M, Nord CE, Ljungh A, et al. Ecological effects of short-term ciprofloxacin treatment of travellers' diarrhoea. J Antimicrob Chemother 1992;30:693-706.

[198] Adams DA, Riggs MM, Donskey CJ. Effect of fluoroquinolone treatment on growth of and toxin production by epidemic and nonepidemic clostridium difficile strains in the cecal contents of mice. Antimicrob Agents Chemother 2007;51:2674-8.

[199] Stryjewski ME, Graham DR, Wilson SE, O'Riordan W, Young D, Lentnek A, et al. Telavancin versus vancomycin for the treatment of complicated skin and skin-structure infections caused by gram-positive organisms. Clin Infect Dis 2008;46:1683-93.

[200] Rubinstein E, Corey GR, Stryjewski ME, Boucher HW, Daly RN, Genter FC, et al. Telavancin for hospital-acquired pneumonia, including ventilator-associated pneumonia: the ATTAIN studies. Clin Microbiol Infect 2008;14 (S7).

[201] Wong SL, Barriere SL, Kitt MM, Goldberg MR. Multiple-dose pharmacokinetics of intravenous telavancin in healthy male and female subjects. J Antimicrob Chemother 2008;62:780-3.

[202] van Zuuren EJ, Kramer SF, Carter BR, Graber MA, Fedorowicz Z. Effective and evidence-based management strategies for rosacea: summary of a Cochrane systematic review. Br J Dermatol 2011;165:760-81.