Ciprofloxacin

Use and resistance in Community, Nursing Home and Hospital

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Ciprofloxacine

gebruik en resistentie in de bevolking, het verpleeghuis en het ziekenhuis

(met een samenvatting in het Nederlands)

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Promotor: Prof. dr. M.J.M. Bonten

Co-promotor: Dr. M. Tersmette

For my father, and his father

Contents

Chapter 1

General Introduction

(Fluoro)quinolones

History

Quinolones are one of the most frequently used classes of antimicrobial agents worldwide. The development of the quinolones began in 1962, when Lesher et al.[1] made the accidental discovery of nalidixic acid as a by-product of the synthesis of the antimalarial compound chloroquine. Nalidixic acid, the prototype 4-quinolone had adequate activity against Gram-negative aerobes, but the use was limited to the treatment of urinary tract infections because of its modest serum and tissue concentrations. Other shortcomings of nalidixic acid include a lack of activity against *Pseudomonas* spp., poor activity against Gram-positive organisms and a high incidence of adverse effects.[2,3] However the discovery of this compound and its introduction into clinical use in 1967, marks the beginning of 5 decades of quinolone development and use. The advancement of quinolones proceeded over the years as researchers focused on structural modifications that would increase and change antibacterial activity and enhance pharmacokinetic properties. The main quinolone nucleus is a nitrogen-containing, 8-membered heterocyclic aromatic quinolone ring.

In the 1970s subsequent derivations, such as pipemidic acid and cinoxacin were discovered, albeit without significant improvements compared to nalidixic acid. Modification of the nalidixic acid structure led to the synthesis of the first fluoroquinolone; norfloxacin (1978), a 6-fluorinated quinolone with enhanced activity against Gram-negative organisms, including *Pseudomonas aeruginosa*. Norfloxacine also had a longer half-life than the previous compounds and less protein binding.[4] However, treatment indications for norfloxacin remained limited to genitourinary infections because of high urinary concentrations but inadequate serum concentrations.

Between 1979 and 1982 a number of fluoroquinolones were patented, including ciprofloxacin in 1981, the first fluoroquinolone suitable for treatment of infections outside the urinary tract. These 'improved' fluoroquinolones had enhanced activity against *Enterobacteriaceae*, *Pseudomonas aeruginosa* and many Gram-positive cocci. The route of administration is usually orally and the compounds are well distributed in tissues and

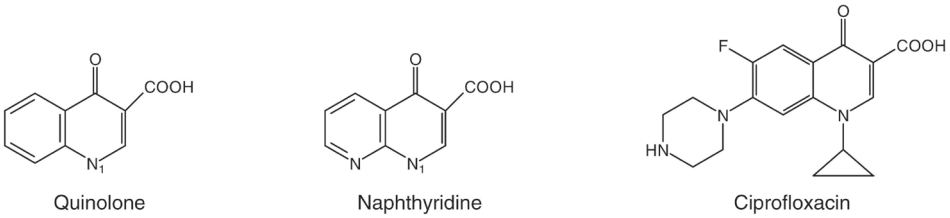

Figure 1. *Chemical structures of quinolone, naphthyridine and ciprofloxacin*

bones. Furthermore, these fluoroquinolones had more favourable toxicological profiles and a lower potential for resistance development than nalidixic acid. Of the improved fluoroquinolones, ciprofloxacin is the most widely used. Newer fluoroquinolones have been developed over the past 10 years, and these agents have better activity against Gram-positive cocci and anaerobes, whilst activity against Gram-negative organisms has been maintained. These fluoroquinolones will not be addressed in this thesis.

Ciprofloxacin

Ciprofloxacin (marketed in the United States in 1987) possesses a broad spectrum of antimicrobial activity, which includes aerobic Gram-negative and Gram-positive organisms. It has lesser activity against staphylococci and borderline or poor activity against enterococci, streptococci and anaerobes. Methicillin-sensitive and -resistant strains of *S. aureus* are usually susceptible to ciprofloxacin. Ciprofloxacin is very active against members of the family Enterobacteriaceae and other Gram-negative organisms, such as *P. aeruginosa*, *Acintobacter* spp., *Haemophilus influenzae*, *Moraxella catarrhalis* and *Neisseria* spp. Its favourable pharmacokinetics allow twice-daily administration, and a low risk for adverse effects. It is usually administered orally, with good absorption and tissue penetrance.

Clinical indications for the use of ciprofloxacin

The spectrum of activity and potency of fluoroquinolones has led to a wide range of indications and frequent use of these agents for both Gram-positive and Gram-negative infections (Table 1).

Table 1. Clinical indications for the use of ciprofloxacin

Urinary tract infections, including prostatitis (Enterobacteriaceae, Pseudomonas)
Gonnorrhoea
Bone and joint infections (*S. aureus*, *Salmonella*)
Bacterial (gastro-) enteritis (*Salmonella*, *Shigella*, *Campylobacter*)
Typhoid fever
Complicated intra-abdominal infections
Legionella pneumophila infection
Inhalational anthrax (after exposure)
Skin and skin-structure infections (diabetic foot)
Prophylaxis in neutropenic haematologic patients

Mechanisms of action

The bactericidal effect of fluoroquinolones results from induced bacterial apoptosis.[5] In order to exert their action, it has been proposed that fluoroquinolones bind to a specific target site on bacterial DNA.[6,7] Once inside the cell, the activity of quinolones stems primarily from the formation of ternary complexes with DNA and type II topoisomerases, DNA gyrase and topoisomerase IV, two enzymes that are essential for supercoiling, relaxing and knotting of DNA (figure 2).[8-10] DNA gyrase is a tetrameric protein comprised of two A and two B subunits (GyrA and GyrB).[11] The A subunits (encoded by *gyrA* gene) are involved in breakage and reunion of DNA, while subunits B (encoded by *gyrB* gene) are responsible for introducing negative supercoils into the DNA strand.[12] Topoisomerase IV is comprised of two *parC* and two *parE* subunits, encoded for by *parC* and *parE* genes.[13] Topoisomerase IV separates the daughter chromosomes following the

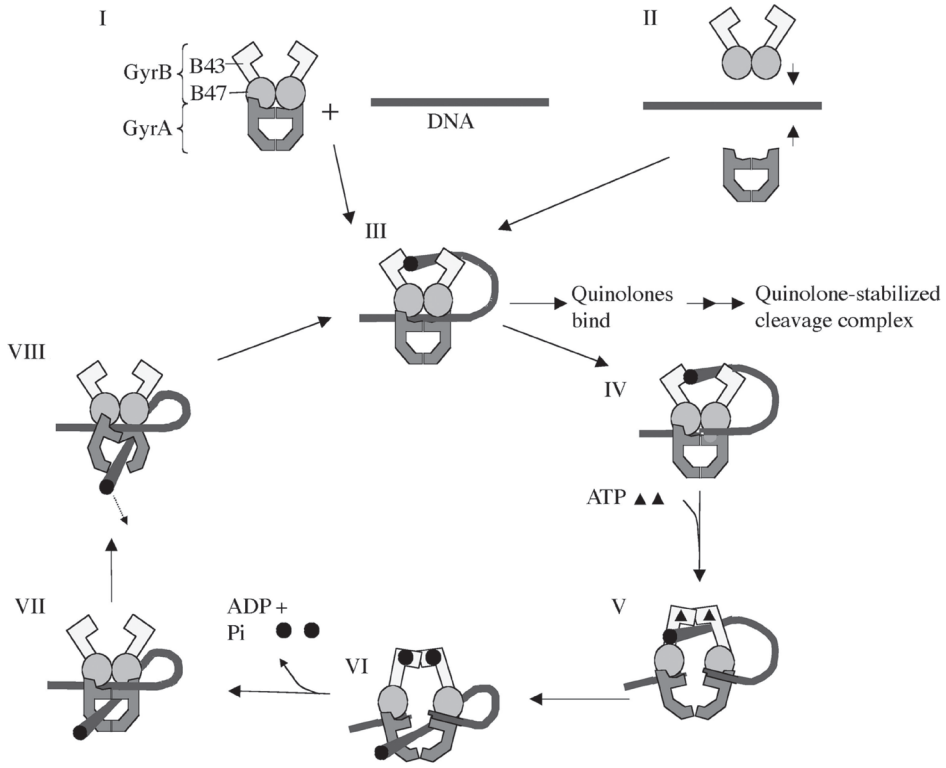

Figure 2. *Schematic illustration of the DNA gyrase supercoiling cycle, showing the point of action of quinolones. The domains of gyrase are shown in different grey shades with the C-terminal (DNA-wrapping) domain of gyrA omitted for clarity. From: Hawkey et al.* [14]

replicationprocess. Quinolones exert their antibacterial effects by binding to complexes of DNA and topoisomerase II or IV, which leads to interference with DNA repair, replication processes as well as transcription, and ultimately to bacterial cell death. In Gram-negative bacteria, gyrase is more susceptible to inhibition by quinolones than is topoisomerase IV, whereas in Gram-positive bacteria, topoisomerase IV is the primary target.

Resistance to ciprofloxacin

Antimicrobial resistance has reached pandemic proportions, with increasing incidences worldwide. The World Health Organization has identified antimicrobial resistance as one of the greatest threats to human health.[15] It is obvious that antibiotic use is associated with development of resistance. The general principle can be expressed in a simple verb: " The more you use it, the sooner you loose it".[16] Rapid induction and selection of antibiotic resistance is one of the most important arguments against the use of ciprofloxacin.[17] For instance, in Pneumococci the process of acquisition of resistance against beta-lactam antibiotics took decades, whereas acquisition of resistance to quinolones seems to be relatively easy.[18] Fluoroquinolone-resistant *N. gonorrhoeae* emerged during the 1990s. In the Netherlands *N. gonorrhoeae* has reached an ever increasing and alarmingly high level of resistance against ciprofloxacin of 52% in 2009 (46 % in 2006).[19]

Mechanisms of resistance

Three main mechanisms of quinolone resistance are (1) alteration in the drug target; (2) mutations that reduce drug accumulation; (3) plasmids that produce the Qnr protein, which protects the quinolone targets from inhibition. The main mechanism of quinolone resistance is the accumulation of mutations in the bacterial enzymes targeted by fluoroquinolones: DNA gyrase and DNA topoisomerase IV.[20]

1. Alterations in drug target

Resistance to quinolones most commonly arises stepwise as a result of spontaneous mutations usually accumulating in the genes encoding DNA gyrase and topoisomerase IV.[5] As wild-type organisms are highly susceptible, multiple mutations are generally required for clinically important resistance. The first step in mutational resistance in the drug target usually occurs by an amino acid change in the primary enzyme target, inducing a rise of MIC. Once a first-step mutation has reduced the susceptibility of DNA gyrase in a Gram-negative organism, additional mutations in *gyrA* or mutations in *gyrB* or *parC* are more likely to occur further augmenting resistance. Resistance mutations occur first in *gyrA* in Gram-negative bacteria, and in *parC* in Gram-positive bacteria. Resistance involves amino acid substitutions in a region of the *gyrA* or *parC* subunit termed the "quinolone-resistance-determining region" (QRDR).[21]

2. Efflux resistance mechanisms

Another mechanism of fluoroquinolone resistance is increased drug efflux via the AcrAB efflux pump.[22,23] To reach their targets fluoroquinolones must cross the cell wall and cytoplasmic membrane. Gram-negative bacteria can regulate membrane permeability by altering expression of outer membrane porin proteins that form channels for passive diffusion. Both Gram-negative and Gram-positive bacteria have non-specific, energy-dependent efflux systems, some of which are inducible by mutation. Target alterations and efflux activation are often found together in resistant clinical isolates.

3. Plasmid mediated resistance

Plasmid-mediated resistance to quinolones was discovered in a clinical isolate of *K. pneumoniae* from Alabama in 1998 that could transfer low-level resistance to quinolones to *E.coli* and to other Gram-negative bacteria.[24] The plasmid-mediated quinolone resistance gene was named "*qnr*" and is already broadly distributed globally.[25-30] Qnr proteins are capable of protecting DNA gyrase from quinolones and have been in circulation for at least 20 years.[31] Since the first description of *qnrA1* 5 different *qnr* families have been discovered: *qnrA*, *qnrS*, *qnrB*, *qnrC*, and *qnrD*.[32]

Development of resistance to ciprofloxacin

Fluoroquinolones have gained broad acceptance in hospitalized and community patients and their use is increasing.[33] Liberal and inappropriate use of antibiotics is considered to be an important reason for development of antibiotic resistance.[15,34,35] Reducing the selective pressures of antibiotic usage by the judicious prescription of antibiotics aiming at optimal selection, dose, and duration, should prevent or delay the emergence of resistant strains.[36,37] Also for fluoroquinolones antibiotic resistance is increasing.[15,38,39] In patients receiving ciprofloxacin therapy, emergence of ciprofloxacin resistant bacteria can occur as the result of; i) clonal spread from another patient colonized with ciprofloxacin resistant bacteria ii) selection of pre-existing ciprofloxacin resistant strains under selective pressure, iii) *de-novo* resistance mutation in ciprofloxacin susceptible strains. Acquisition of *de novo* resistance mutations by indigenous susceptible strains during use of ciprofloxacin is demonstrated in chapter 6.

In several studies use of fluoroquinolones is an important risk factor in the emergence of resistance in hospital settings and nursing homes.[40-44] In Europe significant differences exist in the quantity of antibiotic consumption in humans, the Netherlands being the country with lowest consumption (10.0 Defined Daily Dose (DDD) per 1000 inhabitants daily versus 32.2 DDD per 1000 inhabitants daily in France, with the highest rate of consumption).[15,45] This low consumption is, among others, due to the restrictive prescription habits in Dutch primary care physicians, and the close collaboration between

clinicians and medical microbiologists. Nevertheless, also in Dutch hospitals the use of for example ciprofloxacin is increasing.[45] Although the DDD/100 bed days of the St. Antonius Hospital figures were beneath the average of the Netherlands in 2007, the use of ciprofloxacin more than doubled from 1996 till 2004 (2.31 DDD/100 bed days to 5.72 DDD/100 bed days respectively) (Chapter 3).[46]
Up to 50% of antibiotic usage in hospitals has been judged inappropriate.[36,37,47,48] Concern about the appropriate choice of antibiotics is growing, especially the overuse of newer, broad-spectrum agents.[49,50] In comparison with infections caused by susceptible bacteria, infections caused by resistant bacteria are associated with higher incidences of mortality and morbidity, prolonged hospital stay and an increased financial burden.[44,51-53] Rapid increases in resistance to fluoroquinolones are observed for a broad range of bacterial species. Emergence of resistance is observed for common bacterial agents of respiratory infections, particularly *S. pneumoniae*[54,55] and *Haemophilus influenzae*.[56] *S. pneumoniae* can become resistant to quinolones during monotherapy with these drugs.[57] Fluoroquinolone resistance has become a problem in the treatment of *gonorrhea*[58,59] and enteric infections due to *Salmonella*, *Shigella*, or *Campylobacter* species.[60-62] In *Enterobacteriaceae* the recently discovered plasmid-borne quinolone resistance gene (Qnr) mediates horizontal transfer of fluoroquinolone resistance between strains, facilitating the selection of higher-level resistance, thus contributing to the alarming increase in resistance to quinolones.[21,24,60,63]

Infection-control

Nosocomial transmission between patients may occur directly, or indirectly through healthcare workers or instruments.[52,64,65] To control and limit antimicrobial resistance and the transmission of resistant micro-organisms in hospitals and nursing homes both restrictive antimicrobial use and adequate infection control measures are important.

Outline of the thesis

The central theme of this thesis is ciprofloxacin: its use and epidemiological, clinical and molecular aspects of ciprofloxacin resistance have been studied. **Chapter 2** addresses use of ciprofloxacin in the community for *Campylobacter* infections. It describes a nationwide epidemiological analysis of *Campylobacter* infections in the Netherlands. **Chapters 3 and 4** deal with the use of ciprofloxacin in the hospital setting. In **Chapter 3** the effects of an intervention study on the use of ciprofloxacin (antibiotic stewardship) is described. **Chapter 4** describes a randomized, double-blind, placebo controlled trial to evaluate the effects of single dose ciprofloxacin prophylaxis (as compared to single dose co-trimoxazol

or placebo) before urinary catheter removal on the occurrence of significant bacteriuria and urinary tract infection. In **chapter 5** the prevalence of ciprofloxacin resistant Gram-negatives and extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* among residents in 3 nursing homes and one hospital in the Netherlands is assessed. The prevalence of intestinal carriage with ciprofloxacin resistant Gram-negatives and extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* was assessed in a retrospective study (from 2005-2010) and in a cross-sectional point-prevalence study.
In **Chapter 6** molecular aspects of ciprofloxacin resistance were studied in clinical *E.coli* isolates from patients admitted to a hematology-oncology service where fluoroquinolone prophylaxis during neutropenia was recommended as the standard of care. In **Chapter 7**, the results and conclusions of this thesis are summarized and discussed.

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Chapter 2

Regional and seasonal differences in incidence and antibiotic resistance of *Campylobacter* from a nationwide surveillance study in The Netherlands: an overview of 2000-2004

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Abstract

Campylobacter is the most common cause of bacterial gastroenteritis worldwide. This study describes regional and seasonal differences among culture-proven *Campylobacter* infections in The Netherlands in 2000-2004. Data were used from two ongoing projects in The Netherlands, covering 3 million and 8 million inhabitants, respectively, for surveillance of infectious diseases. The incidence of *Campylobacter* infection was highest in the south of The Netherlands (55.7 / 100 000 vs. an average of 39.1 / 100 000 in other regions). The incidence in urbanised areas was 41.9 / 100 000 vs. 32.4 / 100 000 in rural areas. High stable rates of resistance to fluoroquinolones (35%) were observed. Resistance to erythromycin increased from 1.9% (in 2001) to 2.7% (in 2004). The highest rates of resistance to erythromycin were found in the south. Resistance rates increased with increasing urbanisation, most obviously for fluoroquinolones (35.9% urban vs. 27.10% rural). An inverse relationship was observed between the incidence of infection (high in summer, low in winter) and resistance to both fluoroquinolones and macrolides. Resistance to fluoroquinolones was higher in travel-related infections (54%) than in endemic infections (33%). Differences in regional incidence and resistance rates of *Campylobacter* infections were found. Foreign travel appeared to be associated with higher resistance rates. Given the high fluoroquinolone resistance rate, empirical treatment of severe, microbiologically confirmed, *Campylobacter* infection with a fluoroquinolone should be discouraged, pending susceptibility testing.

Introduction

Infections with *Campylobacter* spp. are the most frequent cause of bacterial gastroenteritis in industrialised and developing countries worldwide.[1-5] The number of infections has risen several-fold during the past two decades. A consensus seems to have emerged that this rise is caused largely by increased consumption of poultry meat.[6,7] Most often, infections with *Campylobacter* spp. cause a selflimiting diarrhoeal illness and need not be treated with antimicrobial agents, although *Campylobacter* infections may be followed by severe complications.[8] Antimicrobial therapy is necessary for patients with severe and prolonged enteritis (particularly neonates and elderly individuals), suspected septicaemia, other invasive extra-intestinal manifestations, and patients with a severe underlying illness (e.g., hypo- or agammaglobulinaemia or human immunodeficiency virus infection). Drugs of choice include ciprofloxacin[9] for early empirical treatment of adults, especially for travel-related disease, and erythromycin for treatment following microbiological confirmation.[1,10]

During the 1990s, resistance to fluoroquinolones among *Campylobacter* spp. increased significantly in The Netherlands and other European countries.[11,12] The increase in quinolone resistance has been associated with the introduction of fluoroquinolones into veterinary medicine during the late 1980s and early 1990s[11-13], especially for poultry. A previous regional surveillance study in The Netherlands demonstrated an increase in fluoroquinolone-resistant *Campylobacter*s, from 15% in 1992 to >30% in 2003.[14] This was an alarming increase, since, among other reasons, patients infected with ciprofloxacin-resistant *Campylobacter* strains may have a six- fold increased risk of invasive illness or death compared with patients infected with ciprofloxacin-susceptible *Campylobacter* strains.[4] The present study describes a nationwide epidemiological analysis of culture-proven *Campylobacter* infections in The Netherlands during 2000-2004.

Patients and methods

Data from two ongoing projects for surveillance of infectious diseases in The Netherlands were used.

Laboratory surveillance system (LSI)

Since 1995, 15 regional public health laboratories have reported weekly to the National Institute for Public Health and the Environment (Bilthoven, The Netherlands) concerning the total number of first isolates of *Campylobacter* spp., as well as other bacterial pathogens, and the total number of faecal samples under investigation. These 15 laboratories are situated in all regions of The Netherlands and cover c. 50% of the 16 million inhabitants. Since 2002, information concerning the species and antibiotic resistance against fluoroquinolones, tetracyclines and erythromycin, and data concerning patient age, gender, place of residence and recent foreign travel, have been reported periodically for each first isolate.

Infectious disease surveillance information system

In 1994, the National Institute for Public Health and the Environment developed a computer-based centralised data system called the ‘infectious diseases surveillance information system’ (ISIS). ISIS consists of a steadily expanding network of sentinel laboratories. Early in 2000, seven laboratories participated, covering 2.6 million inhabitants. During the year 2000, another three laboratories joined, and since 2001, ten more laboratories, situated in the middle and south of The Netherlands have participated, covering 3.3 million inhabitants. Participating laboratories transmit electronically all new results of microbiological investigations to ISIS on a daily basis. In contrast to most

surveillance systems, both positive and negative laboratory results are recorded. All test results are anonymously and uniquely coded for each patient. Data accompanying the laboratory results include information from the laboratory request form, e.g., age, gender, place of residence, type of material sampled, type of investigation, test results, whether the request came from a general practitioner, specialist or outpatient clinic, and information concerning the strain and its antibiotic resistance. Information concerning foreign travel is not available from ISIS. For laboratory test results, a *Campylobacter* infection was recorded if a faecal culture was positive for *Campylobacter* . In order to include only one isolate per disease episode, only the first positive sample from each patient during a 6-month period was included in the analyses.

Analyses

Results are presented for the period 2000-2004. Analysis was performed at the genus level only, but previous results indicate that 94% of isolates were *Campylobacter* jejuni.[15-17] Demographical information, e.g., age and gender, was obtained from ISIS for individuals with either positive or negative test results. Age groups were categorised, on the basis of definitions used previously[18], as 0-4, 5-14, 15-29, 30-44, 45-59 and ≥ 60 years. Positive and negative test results were analysed separately according to age and gender. Geographical information was most complete for LSI surveillance, since the laboratories participating in LSI covered all geographical regions of The Netherlands. For each year, population size by municipality was obtained from Statistic Netherlands, and incidences were calculated, with the appropriate denominators adjusted for the degree of coverage. Incidence and resistance rates were analysed according to the level of urbanisation[14], defined on a scale from 1 (large cities, >2500 residences / km^2) to 5 (rural municipalities, <500 residences / km^2). Each urbanisation class represented c. 20% of the Dutch population.

In the ISIS database, seasonal variation in the incidence of *Campylobacter* infection was analysed for 2000-2004, and susceptibility to antibiotics was analysed for 2001-2004. Susceptibility data have been complete since 2001. Recent foreign travel was reported only in the LSI project, and these data were used to study variation in antibiotic susceptibility among *Campylobacter* isolates from individuals with and without a history of recent foreign travel. Susceptibilities of *Campylobacter* spp. were determined for fluoroquinolones (ciprofloxacin, ofloxacin and/or norfloxacin), macrolides (erythromycin or clarithromycin) and tetracyclines (tetracycline or doxycycline).

Statistics

Differences among rates were judged by their relative risk (RR), using a 95% CI.

Results

Demographics

During the LSI project in 2000-2004, 16 706 *Campylobacter*s were reported, whereas 4501 of the 73 325 samples tested in the ISIS project were positive for *Campylobacter*. During 2004, >13 000 faecal samples were tested for *Campylobacter* in the laboratories participating in ISIS, of which 885 (6.3 %) were positive. Female patients were tested more frequently for *Campylobacter* than were males (1.2:1), but male patients tested for faecal pathogens had positive *Campylobacter* cultures more often than did females (6.9 % vs. 5.4 % ; RR 1.27; 95 % CI 1.21-1.34).

Patients aged ≥60 years were tested most often, but were found to be positive in only 2.9% of cases (Figure 1). The highest percentage of positive cultures (11.6%) was observed in the group aged 15-29 years. The highest *Campylobacter* rates of resistance against macrolides, fluoroquinolones and doxycycline ⁄ tetracycline (4.7% , 39% and 21% , respectively) were found in the group aged 45-59 years. RRs of infection with resistant *Campylobacter* spp. for the group aged 45-59 years compared with the group aged 0-4 years were 1.86 (95% CI 0.99-3.47) for macrolides, 1.38 (95% CI 1.18-1.62) for fluoroquinolones, and 1.33 (95% CI 0.95-1.86) for doxycycline ⁄ tetracycline.

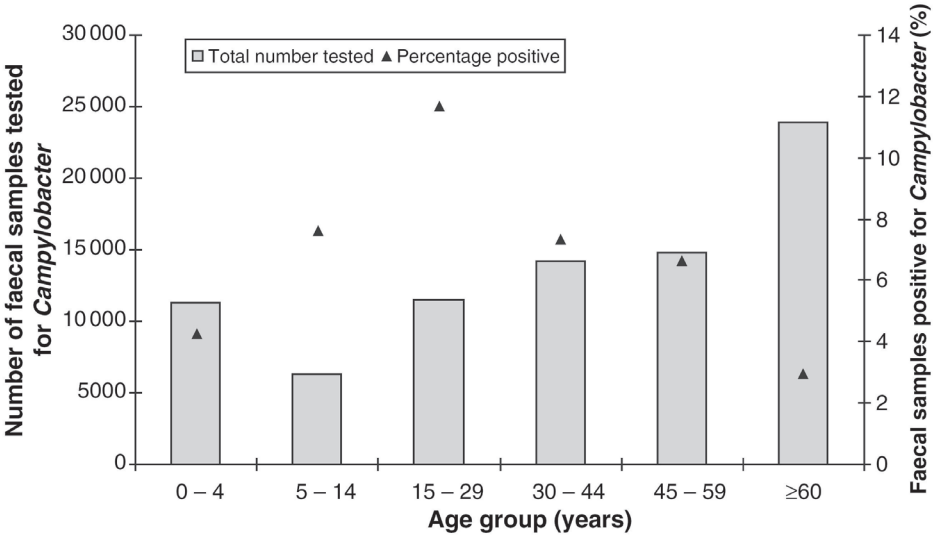

Figure 1. *Campylobacter isolation rate according to age group, 2000-2004 (source: ISIS).*

Geographical data

The incidence of *Campylobacter* infections was highest in the south of The Netherlands, with 49.1 / 100 000 in the south vs. an average of 37.5 / 100 000 in other regions of the country (Table 1). Resistance to macrolides was twice as high in the south (2.1 %) as in the north (1.1 %).

Table 1. Incidence and resistance rate of *campylobacter* infections per region 2002-2004 (excluding travel related infections) Source: LSI

Region	Incidence (/100.000)	Fluoroquinolon resistance (%)	Doxycycline/ tetracycline resistance (%)	Erythromycine resistance (%)
North	37,5	30,1%	16,0%	1,1%
Central	34,3	33,7%	22,6%	1,7%
South	49,1	29,8%	12,8%	2,1%
Total	37,3	32,1%	18,9%	1,6%

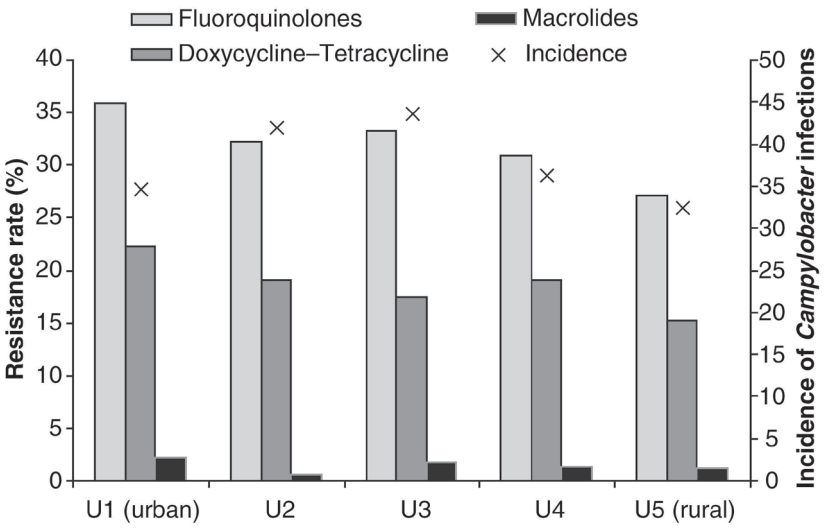

Figure 2. *Resistance and incidence (per 100 000 inhabitants/ year) related to urbanisation level (excluding travel-related infections). Level of urbanisation was defined on a scale from 1 (large cities, >2500 residences /km2) to 5 (municipalities in the country, <500 residences /km2). Data from 2002-2004 are combined (source: LSI).*

The incidence of *Campylobacter* infection was lower in rural than in urban or urbanised areas (32.4 / 100.000 for scale U5 vs. 41.9 / 100 000 for scale U2). Resistance rates increased with increasing urbanisation; this was most apparent for fluoroquinolones (35.9 % for scale U1 vs. 27.1 % for scale U5) (Figure 2).

Temporal and seasonal trends

In both surveillance systems, the yearly incidence, as well as the percentage of positive *Campylobacter* cultures, was stable during 2000-2004 (41.5 / 100 000 and 6.1%, respectively), with an incidental decrease during the year 2003 (33.3 / 100 000 and 5.3% , respectively). Antibiotic resistance to the antibiotics investigated was relatively stable during 2001-2004. Resistance levels were high for fluoroquinolones (34-36%) and tetracyclines (18-24%). Evidence of an increase in resistance was observed for erythromycin (1.9 % in 2001 vs. 2.7 % in 2004) and clarithromycin (1.4 % in 2001 vs. 4.0 % in 2004). Analysis of fluoroquinolone and macrolide resistance among *Campylobacter* isolates in the ISIS project demonstrated an inverse seasonal pattern, with higher incidence and lower resistance rates in summer, and lower incidence and higher resistance rates in winter (Figure 3). The percentage of positive *Campylobacter* cultures was higher in the winter than in the summer (8.2% vs. 4.3 %). This inverse relationship was confirmed in the LSI project.

Association with foreign travel

Resistance to fluoroquinolones was considerably higher for travel-related infections (54%) than for endemic infections (33%) (RR 1.63; 95% CI 1.51- 1.76) (Table 2). The incidence of travel-related *Campylobacter* infections was highest in cities with an urbanisation level of 2-3 (RR 1.54; 95% CI 1.51-1.76; 11.2% of the cases vs. 9.9 %), resulting in a higher overall level of resistance to fluoroquinolones in this category.

Table 2. Antibiotic resistance rates for endemic and travel related *Campylobacter* infections 2002-2004 *Source: LSI*

	Endemic			Travel related		
Antibiotics	Tests	Resistant	% Resistant	Tests	Resistant	% Resistant
Fluoroquinolones	6520	2179	33,4%	645	351	54,4%
Doxycycline/ Tetracycline	4897	960	19,6%	487	132	27,1%
Erythromycin	5739	94	1,6%	568	12	2,1%

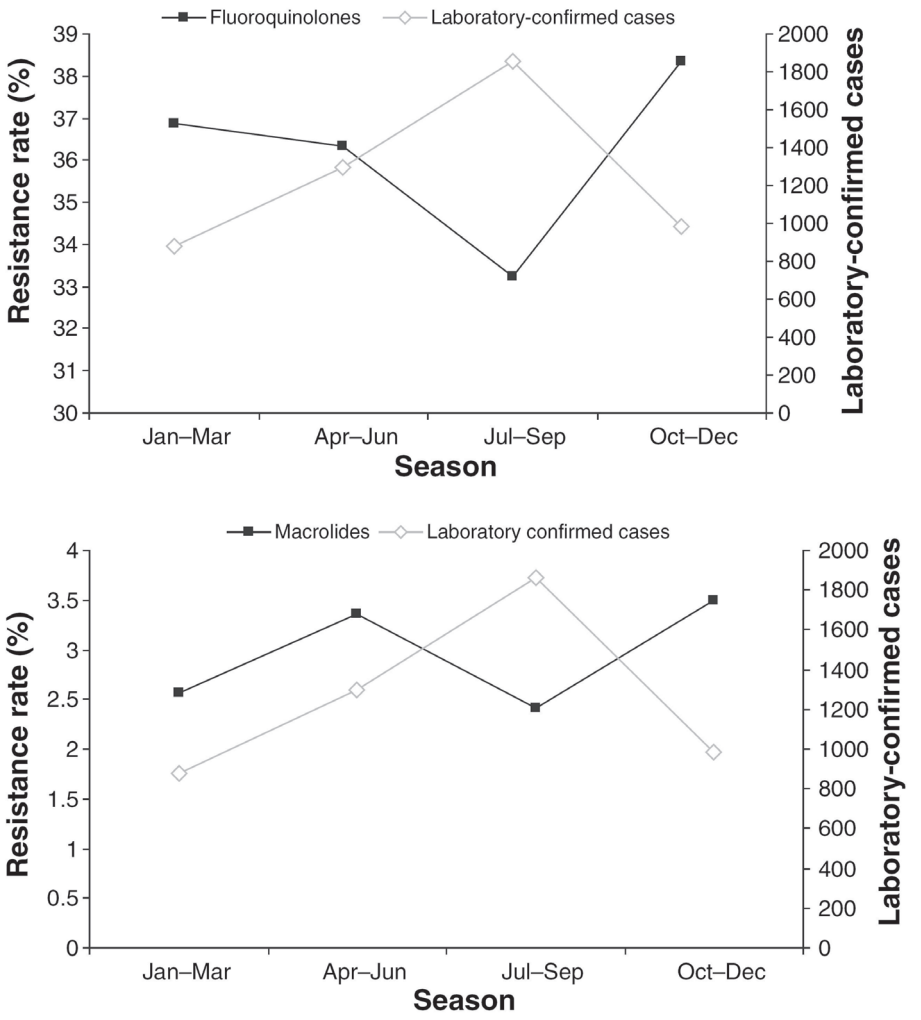

Figure 3. *Laboratory-confirmed cases of Campylobacter infection and resistance rates / month, 2001-2004 (source: ISIS).*

Discussion

This study provided a nationwide epidemiological analysis of culture-proven *Campylobacter* infection in The Netherlands during 2000-2004. The elderly (aged ≥ 60 years) were tested most frequently, but were found to be positive in only a minority of cases. This is probably because of the more frequent occurrence of severe non- infectious diarrhoea among the elderly, especially in hospital settings. In contrast, patients in the group aged 15-29 years were, if tested for *Campylobacter*, found to be positive in almost 12% of cases, probably reflecting a higher threshold of symptoms before visiting a general practitioner in this age category, especially for males.

In the present analysis, three factors were found to influence the incidence and resistance of campylobacteriosis in The Netherlands, i.e., the region, degree of urbanisation and season. The incidental decrease in the incidence of *Campylobacter* for 2003 (33.3 / 100 000 vs. 41.5 / 100 000 in other years) might be explained by a temporary reduction in the sale of (expensive) poultry meat during and after the epidemic of avian influenza among poultry in The Netherlands during 2003.[17]

Both the incidence and the rates of macrolide resistance for *Campylobacter* were highest in the south of The Netherlands. Although no clear explanation exists, differences in the preparation of food, the origin of poultry for consumption and the frequency of antibiotic treatment may all play a role. Resistance to erythromycin appeared to be increasing, particularly in the south of The Netherlands (>3.2% in some regions). No macrolide-resistant strains were detected in *C. jejuni* isolates from Dutch poultry (assumed to be an important source of *Campylobacter*) until 2005.[14] Human infections with *C. jejuni* strains resistant to macrolides are supposedly caused either by consumption of contaminated imported products or by host selection as a result of concomitant antibiotic treatment for other infections. Indeed, a recent surveillance study revealed a 20% resistance rate for erythromycin among *Campylobacter* isolates in contaminated imported poultry meat (D. Mevius, personal communication).

The level of urbanisation also had an effect on the incidence and resistance rates, with lower incidence rates in rural than in urbanised areas, and increasing resistance rates with increasing levels of urbanisation, both including and excluding travel-related infections (Figure 2). An explanation for the higher incidence of *Campylobacter* in urbanised areas may be the presumed higher consumption of ready-to-eat foods. Furthermore, several case-control studies have found an increased risk associated with consumption of chicken in restaurants as opposed to at home.[19-22] Interestingly, Ethelberg et al.[23] associated residence in areas with a low population density in Denmark with an increased risk of infection. This increased risk in rural areas was associated primarily with children, and was explained in terms of increased exposure to farm animals and

natural environments in the countryside as compared with the city.
Seasonal variation in the incidence of *Campylobacter* infection and resistance to ofloxacin has been described by Talsma et al.[13] for one region. A similar inverse relationship was found in the present study throughout the country, for both fluoroquinolones and macrolides. Presumably, different sources of *Campylobacter* (e.g., swimming water) predominate during summer, and are associated with lower rates of fluoroquinolone and macrolide resistance.[24] An alternative explanation for seasonal variation in resistance to erythromycin and fluoroquinolones may be selective pressure resulting from antibiotic therapy for respiratory infections, prescribed most frequently during the winter.
Resistance to fluoroquinolones has increased dramatically during the last decade in poultry (an important source of *Campylobacter*s) and humans (15% in 1992 vs. 35% in 2004).[5,14] Several studies have proposed a causal relationship between veterinary use of fluoroquinolones and the increase in quinolone-resistant *Campylobacter* infections in humans.[2,25] Engberg et al.[5] identified three factors associated independently with an increased risk of a quinolone-resistant *Campylobacter* infection in Denmark, i.e., foreign travel, eating fresh poultry meat other than chicken and turkey, and swimming.[5] Foreign travel was also found to be associated with higher resistance rates in the present study. The relatively high rate of consumption of antibiotics, including fluoroquinolones, in southern Europe and large parts of Asia and the Americas, compared with north-west Europe, is the most likely explanation for this observation. In severely-ill patients with suspected *Campylobacter* infection, especially infants and elderly patients, the use of antibiotics may be indicated. The high frequency of fluoroquinolone resistance suggests that patients (particularly those with a history of foreign travel) in whom severe *Campylobacter* infection has been diagnosed should be treated with a macrolide unless the results of susceptibility testing indicate otherwise.

Acknowledgements

Participating laboratories that contributed data on Campylobacters to ISIS were: Roermond, St Laurentius Hospital; s-Hertogenbosch, Bosch Medisch Centrum; Weert, St Jans Gasthuis; Terneuzen Public Health Laboratory, Zeeland; Goes, Public Health Laboratory, Zeeland; Nijmegen, Canisius Wilhelmina Hospital; Tilburg, St Elisabeth Hospital; Rotterdam, Department of Microbiology, AZR; Spijkenisse, Ruwaard van Putten Hospital; and Nieuwegein, St Antonius Hospital. Participating laboratories that contributed data on Campylobacters to LSI were: the Public Health Laboratories in Amsterdam, Arnhem, Deventer, Apeldoorn, Eindhoven, Enschede, Den Haag, Goes, Terneuzen, Groningen, Haarlem, Heerlen, Leeuwarden, Nijmegen, Rotterdam, Gouda, Tilburg and Nieuwegein.

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Chapter 3

Optimizing use of ciprofloxacin: a prospective intervention study

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Abstract

Objectives

Antimicrobial resistance to ciprofloxacin is increasing. The objective of the present study was to reduce the number of inappropriate prescriptions and improve the quality of ciprofloxacin prescriptions by means of educational intervention.

Methods

In a teaching hospital five units of the Departments of Internal Medicine, Gastro-Enterology, Surgery, Urology and Pulmonary Diseases, selected because of a high rate of ciprofloxacin prescription, participated in a prospective intervention study. The quantity and the quality of prescriptions was reviewed before and after educational intervention and during follow-up.

The quality of each ciprofloxacin prescription was independently evaluated by two medical microbiologists. During the intervention period, a medical microbiologist discussed the appropriateness of prescribing ciprofloxacin with prescribing clinicians, and educational presentations were given to clinicians of participating units. Regression analysis was used to analyse trends in time-series data.

Results

The number of ciprofloxacin prescriptions decreased from 81 prescriptions/1000 admissions before intervention, to 32 prescriptions/1000 admissions after intervention, a significant reduction of 60.5%. Appropriate prescriptions significantly increased. Significantly fewer inappropriate prescriptions were prescribed after intervention and during follow up. At this time, 23 ciprofloxacin prescriptions/1000 admissions were prescribed, a total reduction of 71.3% compared to baseline.

Conclusions

In a hospital with relatively low baseline ciprofloxacin consumption, intervention by direct consultation of a medical microbiologist and educational presentations led to 3-4 fold, sustained reduction of the use of ciprofloxacin and significant improvement in quality of ciprofloxacin prescriptions. Close collaboration between clinicians and medical microbiologists can provide a major contribution to the prudent hospital use of antimicrobial agents.

Introduction

Fluoroquinolones have excellent *in vitro* activity against a wide range of both Gram-negative and Gram-positive organisms and can be prescribed orally, with excellent bioavailability. Fluoroquinolones have gained broad acceptance in hospitalized and community patients and their use is increasing.[1] Resistance to antibiotics is becoming an increasingly important worldwide problem. Liberal and inappropriate use of antibiotics is considered to be the most important reason for development of antibiotic resistance.[2,3] It has been estimated that up to 50% of antibiotic usage in hospitals is inappropriate.[4-8] Reducing the selective pressures of antibiotic usage by judicious antibiotic prescription, will prevent or delay the emergence of resistant strains.[6,8] In addition, antibiotic resistance to fluoroquinolones is increasing.[9,10] Studies have shown that clinical use of fluoroquinolones is an important risk factor for the emergence of resistance in a hospital setting.[10]

In Europe, significant differences exist in the quantity of antibiotic consumption. The Netherlands has relatively low consumption in hospitals.[9] This low consumption is, among other reasons, due to close collaboration between clinicians and medical microbiologists. Nevertheless, ciprofloxacin use is also significantly increasing in Dutch hospitals.[9] Although the DDD/100 bed days at our hospital is below the national average, the use of ciprofloxacin more than doubled from 1996 till 2004 (2.31 DDD/100 bed days to 5.72 DDD/100 bed days respectively) (Figure 1a). The aim of this study was to evaluate the short and long term impact of educational intervention between 09/2004 and 03/2006, on not only quantitative rates of ciprofloxacin prescription but the quality of these prescriptions as well.

Materials and methods

Setting

The St. Antonius Hospital Nieuwegein is a 584-bed Dutch teaching hospital and a tertiary referral centre. This prospective intervention study, with a before and after design, took place in selected units of the Departments of Internal Medicine, Gastro-Enterology, Surgery, Urology and Pulmonary diseases.

Study design

The study was set up as a before and after study. The study comprised three periods and a follow-up: (1) a 3-month pre-intervention observation period (baseline data) from September 2004 to November 2004, (2) a 3-month intervention period from May 2005 to

July 2005 and (3) a 3-month post-intervention period from September 2005 to November 2005. A 3-month follow up was performed from January-March 2006.
Ciprofloxacin use in every hospital nursing unit was analyzed quantitatively by calculating the number of prescriptions per 1000 admissions over the period September 2004 - November 2004. Units with the highest rate of ciprofloxacin prescriptions were selected for intervention, i.e. the Departments of Urology, Surgery, Internal Medicine, Gastro-Enterology, and Pulmonary diseases.
During the two observation periods and follow up period, all ciprofloxacin prescription were registered and reviewed. Clinical and laboratory data were collected to enable accurate assessment of prescribed ciprofloxacin. Prescribers were unaware of the data collection process.

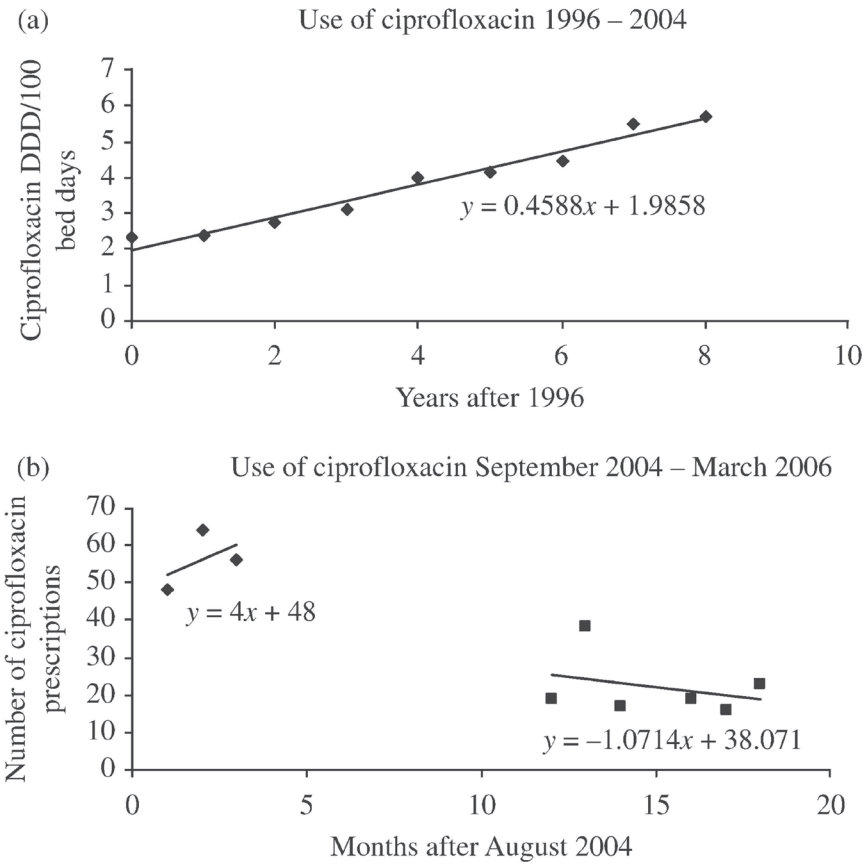

***Figure 1**. (a) Use of ciprofloxacin in St Antonius Hospital, 1996-2004. (b) Use of ciprofloxacin in participating units in September 2004-March 2006.*

A quality evaluation of each ciprofloxacin prescription was performed by two medical microbiologists (B.M. de J. and M.T.) independently. They placed individual prescriptions into categories using well-defined criteria. We adapted the classification that was developed by Kunin et al in 1973 [11] and restyled by Gijssens et al. to a comprehensive evaluation system.[12] In short, prescriptions can be judged appropriate (category I) or unjustified (category V), or the records can be insufficient for evaluation (catergory VI). Other prescriptions are placed in categories II, III, and IV, indicating inappropriate use; incorrect dose (IIa), incorrect interval (IIb), incorrect route (IIc), duration too long (IIIa), or duration too short (IIIb) or better alternative due to higher efficacy (IVa), lower toxicity (IVb), lower cost (IVc), or narrower spectrum (IVd). Inappropriate prescriptions can be allocated to several subcategories at the same time.

Intervention strategies

During the intervention period physicians prescribing ciprofloxacin were interviewed by telephone, by a medical microbiologist (for each single ciprofloxacin prescription), to discuss the indication of the use of ciprofloxacin using the guidelines of the hospital antimicrobial committee as a reference. Furthermore educational lectures on the proper use of ciprofloxacin were given to physicians of participating units.

Statistical Analyses

Chi-square tests and Student's t-tests were applied to establish systematic differences. Agreement between the two independent reviewers was assessed by κ-coefficients.[13] Regression analysis was used to evaluate trends, as recommended by The Cochrane Effective Practice and Organisation of Care Group (EPOC). [5]

Results

The patient populations of the first, the third and the follow-up phase were comparable in terms of sex and age. Mean age was 66 years (SD= 14.8), with a range of 21 to 92 years. Before and after intervention there was no significant difference in sex and age ($p = 0.59$ and $p = 0.53$; respectively).

Quantity of ciprofloxacin prescriptions

A significant increase was observed in ciprofloxacin use before intervention (1996-2004) (Figure 1a) ($y = 0.4588x + 1.9858$, for slope: 95%CI 0.4 - 0.5). In 2004 the use ciprofloxacin in our hospital was 5.72 DDD/100 bed days. From September 2004 - November 2004 the general average of ciprofloxacin prescriptions in our hospital was 62 per 1000 admissions.

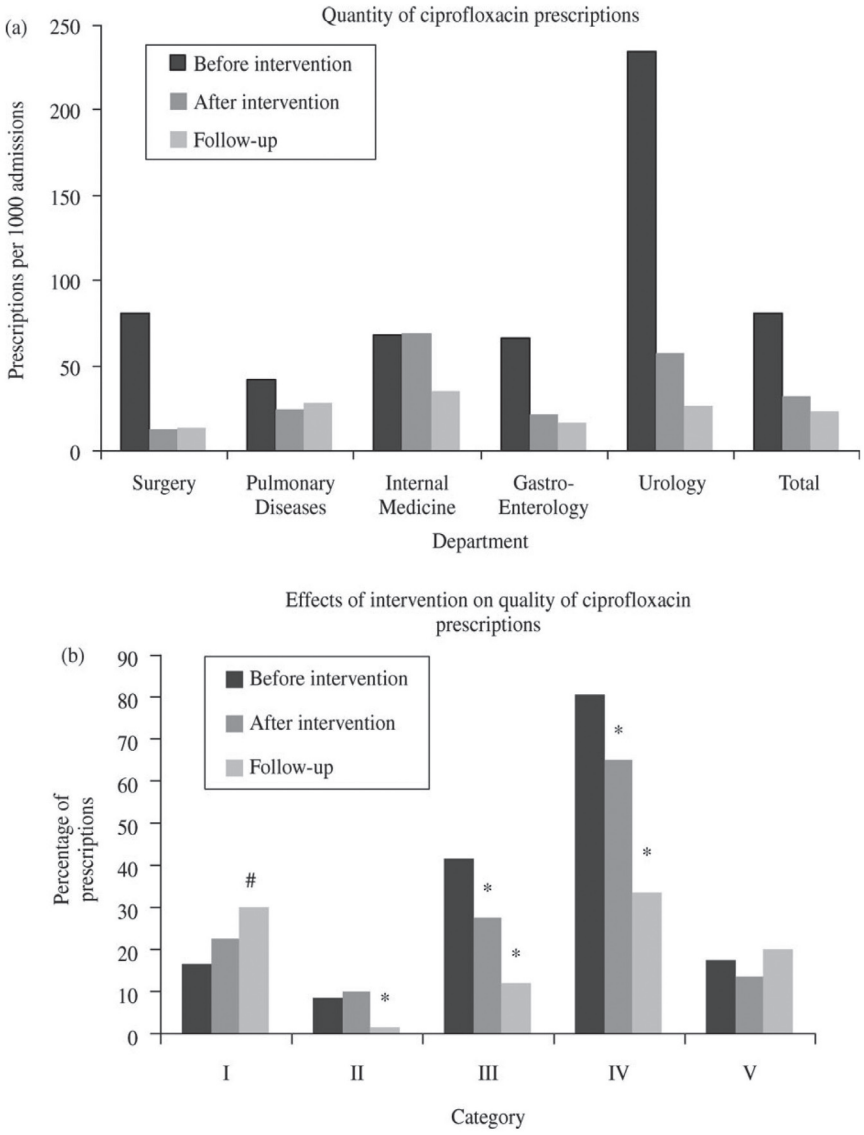

Figure 2. *(a) Number of ciprofloxacin prescriptions per 1000 admissions, before and after intervention and during follow-up. (b) Effects of intervention on quality of ciprofloxacin prescriptions before and after intervention and during follow-up. Categories of quality of prescription: I, appropriate; II, inappropriate; III, duration; IV, alternative; V, unjustified. # Significant increase ($P < 0.05$) in number of prescriptions compared with baseline. *Significant decrease ($P \leq 0.01$) in number of prescriptions compared with baseline.*

On the five selected wards the average number of ciprofloxacin prescriptions was 81 per 1000 admissions. In our study period (September 2004 to march 2006) a significant decrease in ciprofloxacin use was demonstrated (Figures 1b and 2a). After intervention there was a trend towards decreasing prescriptions per 1000 admissions, y = -1,6786x + 32,869 (for slope: 95%CI -7.6 - 4.3).

Figure 2a shows the number of prescriptions per 1000 admissions before and after intervention as well as in the follow-up period for all selected wards. In the first observation period before intervention, 168 prescriptions (81 prescriptions per 1000 admissions) in total were administered in the selected wards. In the second observation period after intervention, there were 74 prescriptions (32 prescriptions per 1000 admissions) in total, a significant reduction of 60.5% (95% CI 50.6-70.4). The highest reduction after intervention was observed in the Departments of Surgery and Urology (83.9% and 75.6%, respectively). Five months after intervention during follow up, 58 prescriptions of ciprofloxacin were prescribed (23 prescriptions/1000 admissions), a total significant reduction of 71.3% (95%CI 62.9-79.8) compared to baseline. The highest long term reduction was observed in the Department of Urology (88.9%). We did not systematically analyse the number of prescriptions of other antibiotics during the study period. In overviews of antibiotic use for the years 2004-2006, we find no evidence for consistent increases in the use of other antibiotics attributable to the restriction of ciprofloxacin use. In particular, consumption of third-generation cephalosporins or carbapenems remained relatively low.

Clinical indications

For each clinical indication, with the exception of sepsis, there was an absolute decrease in the number of prescriptions. Most of the prescriptions prior to intervention concerned inappropriate prophylaxis (32.1%), mainly for removal of urinary catheters. This indication decreased significantly to 14.9% after intervention to only 1.8% during follow-up ($p < 0.01$). A relative increase was observed for clinical indications Respiratory tract infection and Gastro-intestinal tract infection ($p < 0.05$).

Quality of ciprofloxacin prescriptions

Before the intervention, a relevant microbiological investigation was performed in 53.6% of the prescriptions before prescribing ciprofloxacin. After intervention there was a significant increase to 75.7% of prescriptions ($p = 0.01$, chi-square).

The agreement between the two independent reviewers during all phases was substantial to a comparable degree ($\kappa = 0.62$ before intervention, versus $\kappa = 0.68$, after intervention, versus $\kappa = 0.54$ in the follow-up phase) [13]. Figure 2b show the effects of the intervention for the two reviewers combined. Three hundred prescriptions were evaluated (categories II, III and IV could be assigned simultaneously to the same prescription). The proportion

of not evaluable prescriptions (category VI) was 5.7%. The proportion of appropriate prescriptions for the two reviewers combined increased significantly ($p < 0.05$) during follow-up compared to baseline. A significant decrease ($p \leq 0.01$) was observed in the number of inappropriate prescriptions (categories II, III and IV) after intervention and during follow-up compared to baseline.

Discussion

Compared to national and international standards, the pre-study prescription rate of ciprofloxacin was low in our hospital. Nevertheless, this intervention resulted in a 3-4 fold reduction and a significant improvement in the quality of prescriptions. A possible explanation for the increase in appropriateness of prescriptions might be better adherence to hospital guidelines and the significant increase in microbiological investigation before prescribing ciprofloxacin.

Thirty-two percent of the prescriptions prior to intervention concerned non-evidence-based prophylaxis for the removal of urinary catheters, an inappropriate indication in our hospital guidelines. During follow-up only 1.8% of all prescriptions concerned prophylaxis.

A limitation to this intervention study was the lack of control groups. However, in view of the increasing use of ciprofloxacin in our hospital in the years preceding the study period, it is highly unlikely that the substantial decrease observed in this study is due to chance.

We demonstrate a sustained reduction in the use of ciprofloxacin and improvement in the quality of ciprofloxacin prescription, achieved by educational intervention and close collaboration within a hospital between medical microbiologists and clinicians. Given the relatively low baseline consumption of ciprofloxacin in this study it should be worthwhile to adopt this combined approach in other hospital settings.

Acknowledgments

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Chapter 4

Single-dose antibiotic prophylaxis for urinary catheter removal does not reduce the risk of urinary tract infection in surgical patients: a randomized double-blind placebo-controlled trial

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Abstract

We conducted a double-blind, placebo-controlled randomized trial to assess the effect of single-dose prophylaxis using co- trimoxazole (960 mg) (n = 46) or ciprofloxacin (500 mg) (n = 43) vs. placebo (n = 51) before urinary catheter removal on significant bacteriuria (SBU) (primary outcome) and urinary tract infection (UTI) in surgical patients with scheduled bladder drainage for 3-14 days. SBU was determined directly after catheter removal, and UTI 12-14 days after catheter removal. After 12-14 days, incidences of SBU were 19%, 19% and 33% for patients receiving ciprofloxacin, co-trimoxazole and placebo, respectively (p = ns), and incidences of UTI were 3%, 0% and 3% for patients receiving ciprofloxacin, co-trimoxazole and placebo, respectively (p = ns).

Introduction

Urinary tract infections (UTIs) account for about 40% of nosocomial infections and about 80% of these infections are associated with urinary catheters.[1] Opinions diverge with respect to the use of prophylactic antibiotics upon catheter removal.[2] A single dose of antibiotics at the time of catheter removal was as effective in preventing UTI as a 10-day course in patients with asymptomatic bacteriuria, and both strategies were more effective than no therapy.[3] In our hospital it was common practice to use 3 days of ciprofloxacin therapy when removing a urinary catheter, starting 1 day before catheter removal. Because of the absence of scientific evidence and the threat of development of antibiotic resistance we investigated the effects of a single-dose antibiotic regimen, before removing urinary catheters, on the occurrence of significant bacteriuria (SBU) and UTI.

Methods

Patients scheduled to undergo major surgery, such as an abdominal operation or hip surgery, were recruited from January 2005 until December 2006 from general surgical wards within a large teaching hospital. Urological and gynaecological patients were excluded. Patients with a urethral catheter in situ for at least 3 days (72 h) were eligible. Exclusion criteria were: age <18 years, pregnancy, impaired renal or hepatic function (serum creatinine >150 mmol/L, serum transaminases >75 IU/L), fever, UTI, antibiotic use ≤ 48 h before urinary catheter removal, allergy to co-trimoxazole or ciprofloxacin, pathology of the urogenital tract and inability to give informed consent.

Randomization was achieved with permuted blocks of 12 numbers. The co-trimoxazole, ciprofloxacin and placebo were packaged into identical opaque containers, with the trial name and number, and number of randomization. Medication was delivered per patient directly from the pharmacy department to the ward. The investigators were unaware of the medication the patient was given. For control reasons the empty bottles had to be returned to the pharmacy department. The study was approved by the regional and local ethics committees. Written informed consent was obtained from all patients.

Patients were randomly assigned to receive either a single dose of an antibiotic orally (co-trimoxazole (960 mg) or ciprofloxacin (500 mg)) or placebo 2 h before catheter removal. Midstream urine (MSU) samples of the first urine after catheter removal were collected. Twelve to fourteen days after catheter removal, patients were sent a questionnaire regarding UTI symptoms (dysuria, frequency and fever recorded in the 14 days after catheter removal) and follow-up MSU samples were collected.
Standard laboratory methods were used for quantitative urine culture and the isolation, identification and susceptibility testing of organisms. Antimicrobial susceptibility was tested using the disk diffusion technique according to the National Committee for Clinical Laboratory Standards. The primary outcome measure was SBU, defined as ≥ 10 4 colony-forming units (cfu)/mL (one isolate). In the case of >2 uropathogens, SBU was defined as ≥ 10 5 cfu/mL in the presence of pyuria (WBC count of ≥ 6 per high-power field).[4] UTI was defined as SBU at the time of follow-up in combination with symptoms or signs referable to UTI (frequency (>8 times a day) and/or dysuria, fever) in the 14 days after catheter removal. Statistical analysis of numerical data was performed with SPSS software (version 12.0.1). Univariate analyses using chi-squared tests or the Fisher exact test were conducted to assess differences in the occurrence of SBU and UTI. The magnitude of differences was estimated by rate difference with 95% confidence intervals. To detect a difference in SBU between groups of 33% with a significant level of 0.05 and statistical power of 80%, 31 patients per group were required. This trial was registered at http://www.clinicaltrials.gov with the registration number NCT00126698.

Results

Eligibility for study participation was assessed in 147 patients, of whom 140 were randomized and 115 were analysed (Figure 1). The three study groups were comparable in terms of age, gender and number of catheter days (Table 1). At catheter removal, 35% (15/43) of placebo patients had SBU compared with 9% (3/33) of patients receiving ciprofloxacin prophylaxis (p 0.01 as compared with placebo) and 27% (9/ 34) of patients

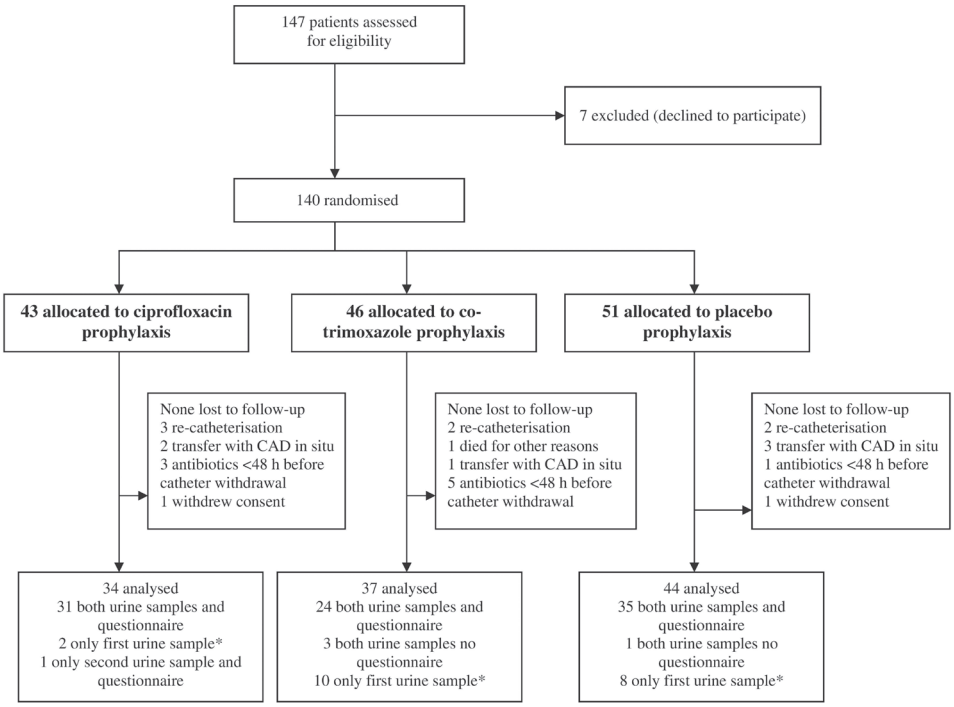

Figure 1. *Flow-chart of recruitment, randomization and follow-up of the trial.*

receiving co-trimoxazole prophylaxis (p 0.47 as compared with placebo).
Two weeks after catheter removal, there was no significant difference in SBU between patients who received placebo vs. patients who received antibiotic prophylaxis. UTI was found in one male patient (2.9%) in the placebo group, vs. one male patient (3.2%) in the ciprofloxacin group (p 0.99) and no patient in the co-trimoxazole group (p 0.99) (Table 1). Prostatitis, pyelonephritis or other upper UTI were not observed, and no short-term mortality was observed due to complications of UTI.

Bacteria isolated from urine samples of patients receiving placebo at catheter removal were mostly *E. coli* (44%) (12/ 27) and *Enterococcus faecalis* (29%) (8/27). Bacteria isolated from urine samples of patients who had received antibiotics at catheter removal were mostly *E. faecalis* (57% (4/7) and 45% (5/11) for ciprofloxacin and co-trimoxazole, respectively). In the placebo group, eight of 44 isolated microorganisms (18%) were resistant to ciprofloxacin. Four of the isolated microorganisms (9%) were resistant to

Table 1. Patient characteristics and effects of antibiotic prophylaxis on pyuria, SBU and UTI

Characteristics	Placebo (N=44)	Co-trimoxazole 960 mg (N=37)	P-value		Ciprofloxacin 500mg (N=34)	P-value	
Age mean (yr)	65.6	69.3	ns		69.3	ns	
Age median (range)	74.5 (41-91)	68.0 (30-91)	ns		71.5 (22-89)	ns	
Female/male (%)	36/64	46/54	ns		56/44	ns	
Median no catheter days (mean)	4.5 (6.5)	5.0 (6.5)	ns		6.0 (6.6)	ns	
Analysis of urine after catheter removal	**Placebo**	**Co-trimoxazole**	**P-value**	**Rate difference (%) (95% CI)**	**Ciprofloxacin**	**P-value**	**Rate difference (%) (95% CI)**
Pyuria	16/43 (37%)	9/34 (27%)	ns	11 (-13 ; +34)	5/33 (15%)	0.04	22 (0.4 ; +44)
Significant Bacteriuria	15/43 (35%)	9/34 (27%)	ns	8 (-15 ; +32)	3/33 (9%)	0.01	26 (6 -;+46)
Analysis of urine 2 weeks after catheter removal	**Placebo**	**Co-trimoxazole**	**P-value**	**Rate difference (%) (95% CI)**	**Ciprofloxacin**	**P-value**	**Rate difference (%) (95% CI)**
Pyuria	5/36 (14%)	3/27 (11%)	ns	3 (-17 ; +22)	2/31 (7%)	ns	7 (-10 ; +25)
Significant Bacteriuria	12/36 (33%)	5/27 (19%)	ns	15 (-10 ; +39)	6/13 (19%)	ns	14 (-10 ; +38)
Symptomatic UTI	1/36 (3%)	0/24 (0%)	ns	3 (-6 ; +12)	1/31 (3%)	ns	-0.4 (-12 ; +11)

co-trimoxazole. In the ciprofloxacin group, ciprofloxacin resistance was found in four of 14 isolated microorganisms (29%). Resistance to co-trimoxazole was found in five of the isolated microorganisms (36%). In the co-trimoxazole group, ciprofloxacin resistance was found in four of 16 isolated microorganisms (25%). Resistance to co-trimoxazole was found in seven of the isolated microorganisms (44%). Two weeks after catheter removal, no significant difference in resistance to ciprofloxacin and/or co-trimoxazole was observed between placebo and prophylaxis groups.

Discussion

The only outcome difference observed was the lower SBU and pyuria rate 2 h after catheter removal in patients that had received ciprofloxacin, as compared with those that had received placebo. Unfortunately, urine samples were not obtained before administration of antibiotic prophylaxis and, therefore, a pre-existing lower prevalence of bacteriuria in the ciprofloxacin group cannot be excluded. Yet, as allocation to study group occurred randomly we concur that this difference results from chance events. Two hours after catheter removal, the prevalence of SBU and pyuria tended to be higher among patients that received co-trimoxazole than among those that had received ciprofloxacin, although statistical significance was not reached. The difference in bacteriuria after catheter removal between patients treated with either ciprofloxacin or co-trimoxazole might be explained by a shorter time to peak concentration, which is ½-2 h for ciprofloxacin and 1- 4 h for co-trimoxazole.

Apart from not obtaining urine cultures before administration of antibiotics, our study also suffered from the Hawthorne effect. Despite daily surveillance at the participating wards, inclusion rates of patients declined progressively during the study as the proportion of urinary catheters removed within 3 days after surgery increased. Although the randomized design prevented the occurrence of bias, these changes in practices prolonged the inclusion period. Our study was powered on the results from Harding et al.[3] In that study patients with asymptomatic bacteruria following catheter removal either received 10 days therapy, a single dose of co- trimoxazole, or no treatment at all. The incidence of UTI 14 days after catheter removal among patients receiving placebo was 17% in that study, which was higher than the incidence of 3% observed in the present study. This might be explained by differences in patient populations (e.g. patients undergoing genitourinary surgery were included in Hardings' study and excluded in the present study).
In this study the incidence of UTI within 2 weeks after catheter removal appeared to be low in all groups, although the power of our study is insufficient to exclude a

difference between antibiotic prophylaxis and placebo in this respect. It is improbable that prophylaxis at catheter removal would significantly affect the long-term outcome in this population.[5-12] Therefore, our results do not support antibiotic prophylaxis for urinary catheter removal in non-genitourinary surgical patients.

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Chapter 5

Molecular epidemiology of ESBL-producing and of ciprofloxacin-resistant *Enterobacteriaceae* in nursing home patients

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Submitted

Abstract

Background

Antimicrobial resistance among Gram-negative bacteria is emerging worldwide, both in the community as in health care settings. The role of nursing homes in the dynamics of these bacteria is largely unknown.

Methods

We performed a cross-sectional prevalence study, to assess the prevalence of intestinal carriage with ciprofloxacin resistant Gram-negatives and extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* among residents in 3 nursing homes in the Netherlands, and a retrospective analysis to determine annual prevalences (from 2005-2010) of these bacteria in clinical samples from nursing home patients. ESBL genes were typed by micro-array and clonal relatedness was determined by AFLP.

Results

In the retrospective study, 1079 Gram negative bacteria were isolated from 812 residents in 7 nursing homes in the catchment area of our hospital. Overall prevalence of ciprofloxacin resistance and ESBL was 26% and 7%, respectively, without significant changes in annual figures. In the point-prevalence survey 195 (38%) and 56 (7%) of 513 patients carried ESBL-producing and ciprofloxacin resistant *Enterobacteriaceae*, respectively. Resistance rates were highest in the nursing home with the highest hospital admission and readmission rate. ESBL-genes most belonged to CTX-M-1-group (85%) and CTX-M-9-group (15%). AFLP analysis of 40 ESBL-producing *E. coli* revealed 16 patterns and nine clusters of 2 to 7 patients. Two clusters were identified in two different nursing homes.

Conclusions

Prevalence of ciprofloxacin-resistant and ESBL-producing *Enterobacteriaceae* appeared to be consistently high in nursing home residents over the period 2005-2010. Intestinal carriage with ESBL-producing Gram-negatives was most prevalent in the nursing home with the highest patient transfer rate with our hospital. These findings suggest an important role of patient admission (and readmissions) from nursing homes in the epidemiology of antibiotic-resistant Gram-negative bacteria.

Introduction

Antimicrobial resistance among Gram-negative bacteria is emerging worldwide, both in the community as in hospital settings. Infections caused by *Enterobacteriaceae* resistant to fluoroquinolones and cephalosporines have been associated with longer hospital stay and higher death rates. [1-3] Important determinants of this growing health-care problem are antibiotic use, both in humans as well as the agricultural industry [4-6], cross-transmission of bacteria in hospitals[7,8], and transfer of colonized patients between health-care settings. Relatively little is known about the role of nursing homes in the epidemiology of these bacteria. Nursing homes have been considered important reservoirs of antibiotic resistant bacteria [9-11], as nursing home residents frequently harbour risk factors for colonization and infection by multiresistant bacteria, such as indwelling bladder catheters, malnutrition, immunocompromised status, skin and soft-tissue breakdowns, recurrent urinary tract infections [11-15] and repeated treatment with broad-spectrum antibiotics.[13,16] Furthermore, hospitalization for any of these reasons increases their risk of acquiring colonization with antibiotic resistant Gram-negative bacilli. Yet, re-hospitalization may also facilitate introduction (or reintroduction) of resistant bacteria in hospitals. We determined the prevalence and molecular epidemiology of ESBL-producing and of ciprofloxacin-resistant *Enterobacteriaceae* in a teaching hospital and nursing homes in its immediate catchment area in a point-prevalence and retrospective analysis.

Methods

Setting

The Gelre hospital is a 925-bed teaching hospital with seven nursing homes (including nursing homes A, B and C) in its catchment area. In the retrospective analysis all microbiology cultures performed between January 2005 and January 2011 in these nursing homes were included, through selection on patient location or physician ordering in the computerized records of the department of Medical Microbiology and Infection Prevention of the Gelre Hospitals. All cultures were reviewed for presence of ESBL-producing *Enterobacteriaceae* and ciprofloxacin resistant gram negatives.

In the cross-sectional point-prevalence study rectal swabs were obtained from 513 residents in 3 different nursing homes (A, B and C) in November 2010. All residents, except those treated in hospice departments, were considered eligible for inclusion. Nursing home A is a 320-bed facility, adjacent to the hospital. Nursing homes B (280-bed) and C (200-bed) are physically separated from the hospital. In 2010, 80% of all new admissions to nursing home A came from our hospital, as were 22% and 24% of newly

admitted patients in nursing homes B and C, respectively. In the same year, 191 nursing home residents from nursing home were hospitalized in our hospital, as were[71] and 1 patient from nursing homes B and C, respectively, which corresponds to 0.52 admissions from nursing home A per day.
Rectal swabs were anonymized and screened for ciprofloxacin resistant Gram-negatives and ESBL-producing *Enterobacteriaceae*. The study was approved by the local ethics committee and informed consent was not required. Clinical information of patients was not collected.

Detection of ESBL and ciprofloxacin resistance

Susceptibility testing was performed as recommended by the National Committee for Clinical laboratory Standards (NCCLS). Antimicrobial susceptibilities and confirmation of species were performed by automatic testing with the Vitek GNI card (BioMerieux, Hazelwood, MO.) Rectal swabs obtained in the point-prevalence survey were screened for ciprofloxacin resistant gram-negatives and ESBL-producing *Enterobacteriaceae* by inoculating onto Mac Conkey agar containing 2 mg/l ciprofloxacin and onto Brilliance ESBL Agar (Oxoid).
ESBL was confirmed by E-tests ESBL. The presence of ESBL genes was determined by microarray analysis. 17 This system uses ligation-mediated amplification, combined with detection of amplified products on a microarray to detect the various CTX-M groups (CTX-M group 1, 2, 9, or combined 8/25) and the ESBL-associated single-nucleotide polymorphisms (SNPs) in TEM and SHV variants.

Epidemiological analysis

The genetic relatedness of the ESBL *E. coli* strains was determined by molecular typing using single enzyme Amplified Fragment Length Polymorphism (seAFLP) technique. DNA isolation was carried out on a MagNA Pure LC system (Roche Diagnostics, Rotkreuz, Switzerland) using the bacterial DNA isolation kit III (Roche) according to the manufacturers instructions. Briefly, a single colony from a pure culture of *E. coli* was suspended in 100 µl molecular grade water (Sigma Aldrich Co Ltd., Poole, UK) and incubated for 10 minutes by 95℃. 43,50 µl of this suspension was mixed with 56,5 µl lysis buffer from the bacterial DNA isolation kit III (Roche). The resulting suspension of 100 µl was then added to the MagNA Pure LC sample cartridge. The DNA isolation was carried out using the bacterial DNA isolation kit III protocol. 10 µl of each DNA isolate was digested with HinfI (37 ℃, 30 min). The partially complementary oligonucleotides 5′-GTC TGC AGC ACG CT-3′ and 5′-ATT AGC GTG CTG CAG ACC AG-3′ were used as adapters and ligated to the digested DNA using T4 DNA ligase (37 ℃, 60 min). The ligation reaction was stopped by incubation at 65 ℃ for 20 min. The restriction fragments

were then amplified by PCR using the primer 5′-CTG CAG CAC GCT AAT CAG-3′ in an ABI GeneAmp PCR system 2700 (Applied Biosystems) using the following cycle program: an initial denaturation step at 95 ˚C for 5 min, 33 cycles of denaturation at 94 ˚C for 60 s, annealing at 60 ˚C for 60 s and extension at 72 ˚C for 150 s. All amplification products were fully extended at 72 ˚C for 7 min. Of each amplification product, 15 µL was then analysed on a 2% agarose gel (gel length 16 cm) at 200 V for 90 min. Amplification products were visualised by ethidium bromide staining. Cluster analysis was performed using Bionumerics™ software, version 5.1, with the Pearson product moment correlation coefficient as a similarity measure and UPGMA for grouping. Isolates with banding patterns that were at least 95% similar were considered to be of the same type.

Statistical analysis

Differences among rates were evaluated by using χ 2 analysis. A P value <.05 was considered significant.

Results

Retrospective study

During the 6-year period 2005-2010, 1079 Gram-negative bacteria were cultured in clinical samples from 812 residents in 7 different nursing homes. The number of cultures from nursing homes increased from 108 (from 87 patients) in 2005 to 230 (from 168 patients) in 2010. Urine cultures were most frequent (90%), and *E. coli* accounted for 57% of all isolates (Table 1). Proportions of isolates resistant to ciprofloxacin ranged from 29% in 2005 to 30% in 2010, and overall 38% (232/618) of *E. coli* isolates and 26% (285/1079) of Gram-negative bacteria were resistant to ciprofloxacin. ESBL-producing *Enterobacteriaceae* were detected in samples from 56 patients (7%), and annual proportions ranged from 7% (6/87) of patients in 2005 to 15% (25/168) in 2010. There was no increasing trend in the proportions of ESBL-resistance (7% (6/87), 11% (14/123), 3% (4/129), 2% (3/149), 3% (4/156), 15% (25/168) from 2005 till 2010).

Point-prevalence study

During a 2-week period, 513 residents (82% of all residents in the three participating nursing homes) participated. A total of 236 isolates of Enterobacteriaceae resistant to ciprofloxacin and or producing ESBL (202 (86%) of them *E. coli*) were detected in samples from 208 residents (41%) (Table 1). Forty patients harboured several resistant bacterial species. Ciprofloxacin resistant strains were found in 193 (38%) and ESBL-producing gram-negatives in 37 patients (7%). Co-resistance for ESBL and ciprofloxacin was

observed in 32 isolates (78% of ESBL-producing isolates). Prevalence of ciprofloxacin-resistance was 40% (n = 95), 38% (n=65) and 33% (n=33) in nursing homes A, B and C, respectively. For ESBL-producing *Enterobacteriaceae* prevalences were 10% (n = 24), 6% (n = 10) and 2% (n = 2) in nursing homes A, B and C, respectively (p = 0.01 for nursing home A compared to B and C). We also determined the prevalence of ESBL-

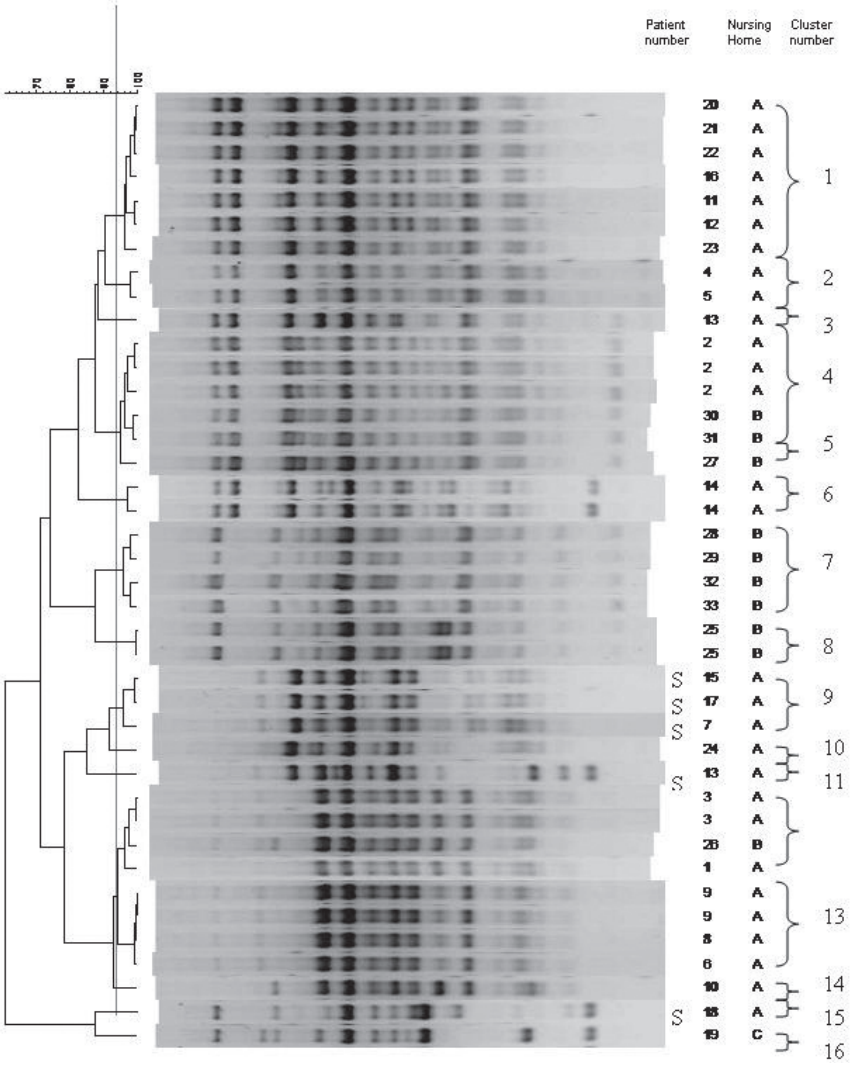

Figure 1. *AFLP patterns and dendrogram of 40 ESBL-containing E. coli. Numbers on horizontal axes indicate 95% similarity as determined by Pearson productmoment correlation coefficient and unweighted pair group method with arithmetic averages. Dotted line depicts 95% similarity coefficient, above which strains were considered to be of identical AFLP type. Numbers to the right indicate AFLP type. S = susceptible to ciprofloxacin. All other strains are resistant to ciprofloxacin.*

producing strains and ciprofloxacin resistance among *Enterobacteriaceae* isolated from blood cultures of hospitalized patients (n=289) in 2009 and 2010. Proportions were 3% (n=9) and 12% (n=34) for ESBL-producers and ciprofloxacin-resistance, respectively, which were both statistically significant lower than the proportions among intestinal carriage in nursing home patients.

Genotypic analysis of ESBL genes and clonal relatedness

The array confirmed the presence of ESBL genes in 41 isolates: 35 (85%) CTX-M-1-group, 6 (15%) CTX-M-9-group. seAFLP of 40 ESBL *E. coli* strains from 33 patients revealed 16 different AFLP clusters of which 1 cluster contained 7 patients (figure 1).

Table 1. Microorganisms cultured in Nursing Homes 2005-2010

Organism	No. of isolates	No. of ciprofloxacin resistant isolates (%)	No. of ESBL-producing isolates (%)
Escherichia coli	618 (57%)	232 (38%)	57 (9.2%)
Proteus species	135 (13%)	10 (7%)	1 (0.7%)
Pseudomonas aeruginosa	121 (11%)	17 (14%)	N/A
Klebsiella species	109 (10%)	12 (11%)	5 (4.6%)
Enterobacter species	34 (3%)	7 (21%)	N/A
Other gram negatives	62 (6%)	7 (11%)	N/A
Total	**1079 (100%)**	**285 (26%)**	**63 (6%)**

Prevalence of Ciprofloxacin resistant and ESBL-producing enterobacteriaceae in isolates from rectal swabs in 513 nursing home residents

Organism	No. of isolates	No. of ciprofloxacin resistant isolates (% of patients)	No. of ESBL-producing isolates (% of patients)
Escherichia coli	202	193	37
Proteus species	20	18	0
Klebsiella species	6	5	2
Enterobacter species	3	0	0
Other gram negatives	5	1	2
Total	**236**	**217 (38%)**	**41 (7%)**

Other clusters contained 4 (n=3), 3 (n=4) and 2 (n=1) patients. Two AFLP types (4 and 12) were found in nursing homes A and B, and 14 genotypes were detected in one nursing home only. The distribution of these AFLP types between nursing home A and B was significantly different (P<0,025, Chi square).

Discussion

Our findings demonstrate a persistently high prevalence of ciprofloxacin-resistant and ESBL-producing *Enterobacteriaceae* (and a high-level of co-resistance) in nursing home residents over the period 2005-2010. The prevalence of intestinal carriage with ESBL-producing Gram-negatives was highest in the nursing home with the highest patient transfer rate between nursing home and hospital, which may partly be explained by the cycle of institutionalization and hospitalization. As only a single rectal swab was obtained in the point prevalence study intermittent carriage may have been missed, and the observed prevalence of ciprofloxacin resistant and ESBL-producing *Enterobacteriacea* could be even higher.
Residence in long-term care facilities is considered a risk factor for colonization or infection with antibiotic-resistant bacteria.[9,10,14,18] Although the point-prevalence of intestinal carriage of ESBL-producing and ciprofloxacin-resistant bacteria was considerable in our study, higher rates have been reported, for instance by Maslow et al who detected fluoroquinolone resistant *E. coli* in 51% of residents in the US[10] and Rooney et al. who reported MDR (fluoroquinolone resistant and ESBl producing) *E. coli* in just over 40% of participating residents in Northern Ireland. [11]
Unfortunately, our study design does not allow determination of the actual place of ESBL-acquisition (hospital, nursing home or community). Yet, the high prevalence of resistant strains in nursing homes A and B, together with the differences in distribution of AFLP clusters and the limited number of ESBL genotypes are compatible with transmission within these nursing homes.

Regardless of the causes of the high prevalence of resistant strains in nursing homes our findings have several implications. Our study suggests an important role of patient admission (and readmissions) from nursing homes in the epidemiology of antibiotic-resistant Gram-negative bacteria. The 0.52 daily hospital admission rate from nursing home A and a 10% prevalence of intestinal ESBL-carriage implies that an ESBL-carrier is admitted to the hospital every 19 days. This admission risk would further increase if ESBL-carriage is associated with a higher likelihood of hospital admission. Vice versa, hospitalization may also increase the risk of acquisition of ESBL-carriage and subsequent introduction in a nursing home.

Transmission of resistant strains within nursing homes should be minimized by adequate prevention measures, such as hand disinfection. Yet, reported compliance rates with hand hygiene before and after patient care among nursing home personnel have been low.[19-21] Regular education of nursing and physician personnel on infection-control measures should be considered.[22,23] The effect of screening nursing home residents for resistant microorganisms at the time of admission in the hospital is now being assessed in an ongoing prospective trial.

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Chapter 6

Molecular analysis of ciprofloxacin resistance and clonal relatedness of clinical *Escherichia coli* isolates from haematology patients receiving ciprofloxacin prophylaxis

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Abstract

Objectives

Widespread use of fluoroquinolones has led to increased levels of resistance in clinical isolates of *Escherichia coli*. We investigated the evolution of ciprofloxacin susceptibility and molecular epidemiology of clinical *E. coli* isolates in haematology patients receiving ciprofloxacin prophylaxis on the population and individual patient level.

Methods

From August 2006 through December 2007 we collected all *E. coli* isolates (n = 404) from surveillance and infection-site cultures from 169 haematology patients receiving ciprofloxacin prophylaxis. Analysis of the A (*gyrA*) gene was performed by denaturing gradient gel electrophoresis (DGGE) in 364 isolates and clonal relatedness was determined by the single-enzyme amplified fragment length polymorphism (seAFLP) technique in 162 isolates. One hundred of these isolates were also subjected to *qnrA* analysis.

Results

The average number of samples per patient was 2.4 (maximum 20) and 122 (30%) of 404 *E. coli* isolates were resistant to ciprofloxacin. In 124 patients only ciprofloxacin-susceptible strains were detected. DGGE revealed 11 different *gyrA* sequence patterns and, based on AFLP analysis, there was evidence of selection of ciprofloxacin-resistant strains under antibiotic pressure, as well as the occurrence of genetically indistinguishable ciprofloxacin-resistant and -susceptible *E. coli* isolates within one patient. Clonal dissemination of ciprofloxacin-resistant *E. coli* was observed, but did not predominate.

Conclusions

The genetic evolution of clinical *E. coli* isolates in haematology patients receiving ciprofloxacin prophylaxis is characterized by selection of ciprofloxacin-resistant strains. However, we did find evidence for de novo resistance mutation in ciprofloxacin-susceptible *E. coli* in individual patients under selective pressure.

Introduction

Widespread use of fluoroquinolones in human and veterinary medicine has led to increased levels of fluoroquinolone resistance in clinical isolates of *Escherichia coli*.[1-6] Fluoroquinolone resistance in *E. coli* has been associated with higher mortality rates due to inappropriate antibiotic treatment.[3,7] The most common mechanism of fluoroquinolone resistance in *E. coli* is chromosomal mutation of the genes encoding the two fluoroquinolone target enzymes (DNA gyrase and topoisomerase IV). These mutations occur most commonly in the *gyrA* and *parC* genes in an area termed the quinolone resistance-determining region (QRDR).[8] Mutations in *parC* are only found in combination with mutations in *gyrA*.[9,10]

In patients receiving ciprofloxacin prophylaxis, emergence of ciprofloxacin-resistant *E. coli* can occur as the result of: (i) clonal spread from another patient colonized with ciprofloxacin- resistant *E. coli* ; (ii) selection of pre-existing ciprofloxacin-resistant strains under selective pressure; or (iii) de novo resistance mutation in ciprofloxacin-susceptible *E. coli*.

Emergence of fluoroquinolone resistance in *E. coli* likely occurs in the gastrointestinal tract, especially in patients receiving fluoroquinolones for selective intestinal decontamination.[11] In these patients, quinolone-resistant *Enterobacteriaceae* may rapidly emerge.[12-14] Colonization usually precedes infection, and massive colonization with fluoroquinolone-resistant *Enterobacteriaceae* is therefore considered a risk factor for so-called breakthrough bacteraemia in cancer patients. In previous clinical and epidemiological studies on fluoroquinolone resistance, either cross-sectional or longitudinal, analyses of resistance were mostly performed at the population level, with only limited characterization of isolates within individual patients.[10,13-17] The aim of this study was to prospectively investigate the molecular evolution of quinolone resistance genes and the genetic relatedness of *E. coli* strains both at the population level and the level of individual patients. For this purpose, all *E. coli* isolates obtained from patients admitted to a haematology/oncology department where fluoroquinolone prophylaxis during neutropenia was the standard of care were investigated.

Patients and methods

Patients

This study was part of common practice and, according to Dutch regulation, did not require permission from the local Medical Ethical Committee. The University Medical Centre of Utrecht is a 1042 bed Dutch academic tertiary-care hospital. It includes a specialized

haematology/ oncology service. The yearly number of admissions to the medical service ranges between 29 000 and 30 000, which includes 380 - 395 admissions of cancer patients to the haematology/oncology service, with, on average, 90 autologous or allogeneic bone marrow transplants each year. Institutional guidelines recommended antibacterial prophylaxis with 500 mg twice daily oral ciprofloxacin when the expected duration of neutropenia was ≥ 7 days. Surveillance cultures were done from all patients at baseline and weekly during admission. If ciprofloxacin-resistant strains were found, ciprofloxacin prophylaxis was replaced by co-trimoxazole. In case of a clinical suspicion of infection, samples were obtained for diagnostic purposes as part of standard clinical practice.

Bacterial isolates

From August 2006 through December 2007 all *E. coli* isolates from surveillance and infection-site cultures were collected for analysis. *E. coli* isolates were identified and their susceptibilities were obtained using automatic methods (BD Phoenix Automated Microbiology System BD Diagnostics, Sparks, MD, USA) and/or the Etest method. Susceptibility to fluoroquinolones other than ciprofloxacin was not tested. The isolates were then stored at -70℃ until molecular studies were performed.

Preparation of target DNA

DNA isolation was carried out on a MagNA Pure LC system (Roche Diagnostics, Rotkreuz, Switzerland) using the bacterial DNA Isolation Kit III (Roche) according to the manufacturer's instructions. Briefly, a single colony from a pure culture of *E. coli* was suspended in 100 µL molecular grade water (Sigma Aldrich, Poole, UK) and incubated for 10 min at 95℃. A total of 43.50 µL of this suspension was mixed with 56.5 µL lysis buffer from the bacterial DNA Isolation Kit III (Roche). The resulting suspension of 100 µL was then added to the MagNA Pure LC sample cartridge. The DNA isolation was carried out using the bacterial DNA Isolation Kit III protocol. DNA was stored at 4℃ until analysis.

Analysis of the *gyrA* gene

PCR amplification

For analysis of the QRDR of the *gyrA* gene, a 196 bp *gyrA* fragment was amplified by PCR using the forward primer 5 ′ -CCGCCCGCCGCGCCCCGCGCCCG-GCCCGCCGCCCCCGCCCC CGT ACT TTA CGC CAT GAACG-3 ′ and the reverse primer 5 ′ -CGA TAG AAC CGA AGT TAC CC-3 ′ . The 41 bp GC clamp was added to the forward primer to prevent complete melting of the DNA fragment during denaturing

gradient gel electrophoresis (DGGE) analysis. Amplification was performed in a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA) using the following protocol: one denaturing step at 94 ℃ for 5 min followed by 25 cycles of 94 ℃ for 0.5 min, 60 ℃ for 0.5 min and 72 ℃ for 0.5 min, then a final extension step of 72 ℃ for 7 min.

DGGE analysis

The PCR product was analysed by DGGE. The DGGE fragment spanned from amino acid 48 to 113. DGGE was performed essentially as described previously.[18] Briefly, PCR products were separated on a 1.5 mm thick vertical gel containing 8% (w/v) polyacrylamide and a linear gradient of the denaturants urea and formamide, increasing from 35% at the top of the gel to 55% at the bottom. This gel was loaded on a DGGE DCODE system (BioRad, Hercules, CA, USA) and run at 60 V for 16 h. After electrophoresis, the gel was stained in ethidium bromide for 30 min, washed in distilled water and viewed under ultraviolet (UV) light.

Sequencing DGGE types

Each DGGE band was sequenced in duplicate in a Big Dye terminator reaction (Applied Biosystems) on an ABI 377 sequencer, following the manufacturer's instructions. To amplify the DNA, the same protocol was used as for DGGE analysis except for the forward primer. The primer used lacked the GC clamp. Obtained sequences were assembled and analysed using the Staden package (http://staden.sourceforge.net/).

Epidemiological analysis

The genetic relatedness of the *E. coli* strains was determined by molecular typing using the single-enzyme amplified fragment length polymorphism (seAFLP) technique. Ten microlitres of each DNA isolate were digested with HinfI (37 ℃, 30 min). The partially complementary oligonucleotides 5 ′ -GTC TGC AGC ACG CT-3 ′ and 5 ′ -ATT AGC GTG CTG CAG ACC AG-3 ′ were used as adapters and ligated to the digested DNA using T4 DNA ligase (37 ℃, 60 min). The ligation reaction was stopped by incubation at 65 ℃ for 20 min. The restriction fragments were then amplified by PCR using the primer 5 ′ -CTG CAG CAC GCT AAT CAG-3 ′ in an ABI GeneAmp PCR System 2700 (Applied Biosystems) using the following cycle program: an initial denaturation step at 95 ℃ for 5 min, 33 cycles of denaturation at 94 ℃ for 60 s, annealing at 60 ℃ for 60 s and extension at 72 ℃ for 150 s. All amplification products were fully extended at 72 ℃ for 7 min. Of each amplification product, 15 µL was then analysed on a 2% agarose gel (gel length 16 cm) at 200 V for 90 min. Amplification products were visualized by ethidium bromide staining. Cluster analysis was performed using Bionumerics software, version 5.1 (Applied Maths NV, Sint-Martens-Latem, Belgium), with the Pearson product

moment correlation coefficient as a similarity measure and UPGMA (unweighted pair group method with arithmetic mean) for grouping. Isolates with banding patterns that were at least 95% similar were considered to be of the same type.

qnrA

The presence of the *qnrA* gene was determined by real-time PCR using the ABI Prism 7000 sequence detection system (Applied Biosystems). Primers to amplify the *qnrA* gene were 5′-GCC GCT GCC GCT TTT A-3′ and 5′-AAT CCT CGA AAC TGG CAT C-3′. To detect the amplification in real time, a TaqMan probe was used: VIC-TCA GTG TGA CTT CAG C-MGB. PCR was performed in 45 cycles using the standard ABI Prism protocol. A clinical isolate (*Enterobacter cloacae*) with CTX-M and *qnrA* was used as positive control.

Statistical analysis

The chi-square test was applied to establish differences.

Results

Patients and bacteria

From August 2006 through December 2007, 404 *E. coli* strains were collected from 352 samples (166 faecal samples, 138 peri-rectal swabs, 15 urine cultures, 14 sputum cultures, 10 throat swabs, 5 nose swabs, 2 blood cultures, 2 pus cultures) from 169 haematology patients receiving ciprofloxacin prophylaxis. The mean number of isolates per patient was 2.4 (range 1 -20) and more than one isolate was obtained from 49% of patients. A total of 122 strains were ciprofloxacin resistant (30%) and 282 strains (70%) were ciprofloxacin susceptible. In 124 patients, only ciprofloxacin-susceptible strains were detected. Some patients had ciprofloxacin-resistant *E. coli* cultured even before the start of ciprofloxacin prophylaxis, possibly because they had ciprofloxacin prophylaxis before our study period. Thirty-six extended-spectrum ß-lactamase (ESBL)-producing *E. coli* were recovered in 21 patients. Eighteen of these 36 strains (from 13 patients) were ciprofloxacin resistant as well.

Analysis of the *gyrA* and *qnrA* genes

DNA was available for molecular analysis from 364 of 404 *E. coli* strains. To investigate whether additional mutations in the *gyrA* gene as well as those described in the literature could be detected, 364 strains were analysed by DGGE. This revealed 11 different *gyrA* patterns, representing 11 different *gyrA* alleles (Table 1). To determine the sequence of the

Table 1. Nucleotide mutations and amino acid substitutions among the different DGGE types of gyrA

	Altered codons and amino acid changes						
DGGE Type	**83**	**85**	**87**	**91**	**100**	**No of strains (%)**	**MIC range (median), in mg/L [no. Tested]**
A	TCG	GTC	GAC	CGC	TAT	45 (12)	0.012-0.032 (0.014) [14]
H	-T- Leu	---	---	---	---	1 (0.3)	0.19 [1]
D	-T- Leu	---	A-- Asn	---	---	32 (9)	4->32(10) [12]
B	---	--T	---	--T	--C	187 (51)	0.004-0.032 (0.020) [16]
C	-T- Leu	--T	---	--T	--C	20(5)	0.004-0.38 (0.25) [11]
E	-T- Leu	--T	A-- Asn	--T	--C	74 (20)	4->32(>32) [14]
Rare DGGE types							
F	---	--T	---	--T	--C	1 (0.3)	0.023 [1]
G	---	--T	---	--T	--C	1 (0.3)	0.023 [1]
I	---	--T	--G Gly	--T	--C	1 (0.3)	0.064 [1]
J	---	---	---	--T	--C	1 (0.3)	0.016 [1]
K	---	---	---	--T	--C	1 (0.3)	0.032 [1]

gyrA alleles, at least two representatives of each allele were sequenced. DNA sequencing and comparison of the sequence of the QRDR of *gyrA* with known DNA sequences of the *E. coli gyrA* gene (accession number x5174) revealed nucleotide changes at amino acid codons 83, 85, 87, 91 and 100 (Table 1). Five different DGGE patterns (C, D, E, H and I) were combinations of the two most common *gyrA* mutations, Ser83Leu and Asp87Asn. In six different patterns (B, C, E, F, G and I) a consistent combination of silent mutations was observed in codons 85, 91 and 100. No other nucleotide changes were found in the region between codon 48 and 113. DGGE types A -E were found frequently,

while F -K were all found once. The most common DGGE pattern in our study was the wild-type allele type B (51%), followed by type E (20%), which had both amino acid changes. Of the 364 isolates, 126 had at least one mutation in the *gyrA* gene, affecting their susceptibility to ciprofloxacin.
DGGE types A - C and F - K were exclusively found in ciprofloxacin-susceptible isolates. None of the ciprofloxacin-susceptible strains had the Asp87Asn mutation, and except for strains with DGGE types C and H, none of the ciprofloxacin-susceptible strains had the Ser83Leu mutation. DGGE types D and E were only present in ciprofloxacin-resistant strains. All resistant strains had the Ser83Leu and Asp87Asn mutations. Table 1 shows the distribution of MICs of the different DGGE types. *qnrA* PCR was performed on the first 100 strains. The *qnrA* gene was not detected in any of these isolates.

Clonal relatedness of ciprofloxacin-resistant and -susceptible *E. coli*

seAFLP was performed to assess the clonal relatedness of 162 *E. coli* strains from 43 patients. These 43 patients were selected because they had *E. coli* with different DGGE types (Table 2). This revealed the occurrence of two different mechanisms of ciprofloxacin-resistant *E. coli* acquisition in individual patients: (i) selection or acquisition of ciprofloxacin-resistant strains unrelated to the susceptible *E. coli* strain in the same patient; and (ii) acquisition of a de novo resistance mutation in ciprofloxacin- susceptible *E. coli* . In 12 of 43 patients (28%), ciprofloxacin-susceptible *E. coli* with *gyrA* types A, B or C were replaced by genetically distinct (different AFLP type) ciprofloxacin-resistant strains with *gyrA* type D or E. This indicates that the emergence of ciprofloxacin-resistant *E. coli* in these patients is the result of acquisition or selection of resistant *E. coli* clones rather than acquisition of *de novo* resistance mutations in indigenous *E. coli* clones. In four patients a switch in DGGE pattern that resulted in a significant decrease in

Table 2: Acquisition of ciprofloxacin resistant *E. coli*

Conversion in DGGE types	**Number of patients** (%)
Absence of conversion of DGGE type	126 (75%)
Conversion of DGGE type without effect on ciprofloxacin susceptibility	27 (16%)
Selection or acquisition of ciprofloxacin resistant strains	12 (7%)
Acquisition of *de novo* resistance mutation	4 (2.4%)
Total	**169 (100%)**

ciprofloxacin susceptibility was noticed in *E. coli* isolates from the same patient that were indistinguishable by AFLP.
For example, in one patient a ciprofloxacin-resistant *E. coli* with *gyrA* DGGE type E was isolated after 4 weeks of ciprofloxacin prophylaxis. Four months later a ciprofloxacin-susceptible *E. coli* with the same AFLP type was isolated carrying *gyrA* DGGE type B. This patient had not received any ciprofloxacin in those 4 months. In another patient, the first recovered *E. coli* was ciprofloxacin resistant with *gyrA* DGGE type E; 5 months later a ciprofloxacin-susceptible *E. coli* carrying *gyrA* DGGE type B with the same AFLP type was isolated. These two patients probably represent cases of reversion of mutated *gyrA* to wild type. At the same time that a ciprofloxacin-susceptible strain was isolated from patient 10, a ciprofloxacin-resistant but genetically distinct *E. coli* with DGGE type E was also isolated, indicating independent selection or acquisition of a second ciprofloxacin-resistant clone.
To quantify the relative contribution of the clonal spread of resistant clones versus endogenous selection of pre-existing resistant clones, all ciprofloxacin-resistant strains (n = 37) from all included patients were compared by AFLP. This revealed nine different AFLP clusters, of which five clusters contained two isolates, two clusters had three isolates and two clusters (5 and 6) had four isolates. Thirteen AFLP types were unique (Figure 1).

Discussion

Fluoroquinolone resistance is emerging as a major type of antibacterial resistance in *E. coli*, with reported resistance rates in haematological populations exceeding 50%.[16] In this study of 404 *E. coli* strains from 169 consecutively studied haematology patients receiving fluoroquinolone prophylaxis the prevalence of ciprofloxacin resistance was 27%. In 100 isolates tested the *qnrA* gene could not be detected. In-depth molecular analysis by a combination of DGGE and DNA sequencing revealed a good correlation between the ciprofloxacin MIC values and different *gyrA* sequence patterns observed, indicating that the main mechanism of ciprofloxacin resistance in the *E. coli* strains of our patient population is the accumulation of Ser83Leu and Asp87Asn mutations in the *gyrA* gene. These mutations in the QRDR have been described previously by Heisig (1993), Ouabdesselam (1995), Oram and Fisher, and Conrad (1996).[19-22] In addition, we identified an Asp87Gly mutation in combination with silent mutations in codons 85, 91 and 100 in susceptible and resistant strains. Asp87Gly in combination with silent mutations has been described before, but not interpreted.[19-23] In total, we found silent mutations in 77% of the strains, mostly in the predominant DGGE types, type B and type E. In these 367 isolates,

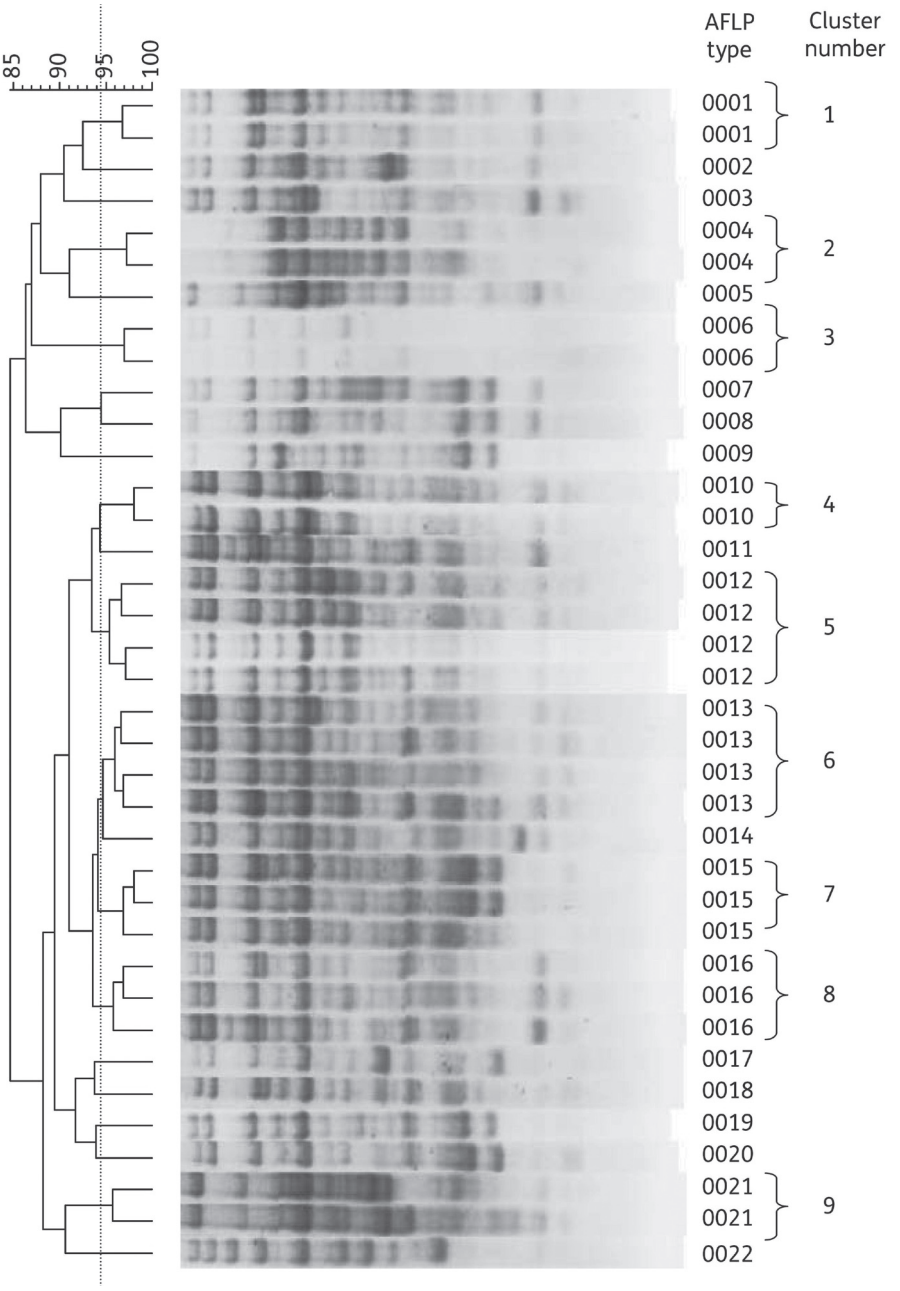

Figure 1. *AFLP patterns and dendrogram of 37 ciprofloxacin-resistant E. coli . Numbers on the horizontal axis indicate percentage similarity as determined by Pearson product moment correlation coefficient and unweighted pair group method with arithmetic averages. Dotted line depicts 95% similarity coefficient, above which strains were considered to be of identical AFLP type. Numbers to the right indicate AFLP type.*

no evidence for additional mutations in the *gyrA* gene was found. We identified two routes for *E. coli* to acquire a *gyrA* resistant genotype: DGGE type A → H → D and DGGE type B → C → E (Table 1). The prevalence ratio DGGE type A/DGGE type B (45/186) is significantly lower than the same ratio for DGGE type D and DGGE type E (32/74) (P = 0.03, chi-square test), which may indicate a relative impediment of genotype B compared with A to evolve into its fully fluoroquinolone-resistant counterpart. The only difference in the *gyrA* fragment analysed between the two sets of genotypes A/H/D and B/C/E (apart from the 83 and 87 mutations) are the three consistently linked silent mutations at codons 85, 91 and 100. These mutations may somehow be related to this difference in rate of mutagenicity of the 83 and 87 codons between genotype A and B.

Molecular typing confirmed previous observations of a limited risk of nosocomial transmission of fluoroquinolone-resistant *E. coli* among haematology/oncology patients.[13,15,24,25] However, the finding that nine AFLP types were shared among 2 -4 patients suggests that occasionally events of cross- transmission have occurred. Twelve of 169 patients (7%) were colonized with distinct (different seAFLP types) susceptible and resistant *E. coli* clones, suggesting selection of pre-existing ciprofloxacin-resistant strains under prophylaxis or acquisition of resistant strains. Ciprofloxacin-susceptible and -resistant *E. coli* with different *gyrA* DGGE types but identical AFLP types were identified in 4 patients (2.3%). This demonstrates acquisition of de novo resistance mutations by indigenous susceptible *E. coli* under ciprofloxacin therapy.

Prophylactic use of antibiotics, like ciprofloxacin, in neutropenic patients remains controversial. In our study we observed that in 45/169 patients (27%), fluoroquinolone-resistant *E. coli* strains can be demonstrated either from the onset or during follow-up. Many of these strains apparently are pre-existing and are selected during prophylaxis, but we also provide evidence for in vivo mutation to a resistant phenotype of previously susceptible strains. Recent reviews and meta-analysis studies suggest that prophylactic treatment results in a reduction in death from all causes[26-31]. However, these conclusions are based on original studies performed before 2005 and therefore do not take sufficiently into account the increased prevalence of fluoroquinolone-resistant Gram-negative strains observed in recent years, which may lead to a reduced efficacy of fluoroquinolone prophylaxis. Our study underlines the necessity for an ongoing discussion on this topic and the need for alternative strategies for patients in whom fluoroquinolone-resistant Gram-negative strains have been detected.

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Chapter 7

Summarizing Discussion

Samenvatting in het Nederlands

Dankwoord

Curriculum Vitae

Bibliography

The aim of the studies described in this thesis was to analyze some aspects of ciprofloxacin use and clinical and (molecular) epidemiology of ciprofloxacin resistance in different settings, both within hospitals (**chapter 3,4 and 6**), community and nursing homes (**chapter 2 and 5**). With its broad spectrum against gram negative organisms and favorable pharmacokinetics, ciprofloxacin use has increased over the last two decades, as did resistance against ciprofloxacin.

Community

Use of ciprofloxacin in primary care in the Netherlands more than doubled from 1997 till 2010.[1] The Practice Guidelines developed by the Dutch College of General Practitioners (NHG) however, emphasize that the use of fluoroquinolones should be preserved for serious infections in hospitals and should not be prescribed in primary care in for example respiratory tract infections, urinary tract infections and sexually transmitted diseases. Ciprofloxacin is considered a reserve asset, only used exceptionally and based upon cultures and susceptibility tests. However, it may not only be ciprofloxacin use in human medicine that is responsible for the emergence of resistance. The extent to which antibiotics are used for veterinary and aquaculture (salmon) purposes in food producing animals can pose a risk to public health as it is an important determinant for the development of antibiotic resistance. Antibiotic usage expressed in terms of grams per kg live weight had doubled in 2007 compared to 1999.[2] Fluoroquinolones (e.g. enrofloxacin) were introduced in veterinary medicine in the late 1980s and early 1990s, especially in poultry. The increase of ciprofloxacin resistance in *Campylobacter* species in the Netherlands and other countries in Europe has been related to this introduction. [3-6]

Campylobacter is a leading cause of acute diarrhea worldwide. [7-9] *Campylobacter* enteritis is a food borne disease, although infection can also be acquired through direct contact with animals or their products. *Campylobacters* inhabit the intestinal tracts of a wide range of animal hosts, notably poultry, and it is from these sources that they enter the food chain. Although less dangerous than salmonellosis, *Campylobacter* enteritis causes considerable morbidity and high economic costs. **Chapter 2** describes a nation-wide epidemiological analysis of culture-proven *Campylobacter* infections in the Netherlands over the years 2000-2004 and the effect of region, degree of urbanization and season on the incidence of *campylobacter*iosis and development of resistance. High stable rates of resistance to fluoroquinolones (35%) were observed and resistance was higher in travel-related infections (54%) than in endemic infections (33%). The high resistance rates to fluoroquinolones warrants reconsideration of its use as drug of first choice in the empiric treatment of gastrointestinal infections in the Netherlands.

Hospital

Hospital use of fluoroquinolones in the Netherlands increased from 7.6 DDD per 100 patient-days in 1999 to 9.6 DDD per 100 patient-days in 2008. This increase was exclusively due to the steep increase in use of ciprofloxacin from 5.3 to 8.6 DDD per 100 patient-days.[1]

In **chapter 3** the effects of intervention to reduce and improve ciprofloxacin use in a hospital have been described. Despite relatively low baseline ciprofloxacin consumption, intervention led to 3-4 fold sustained reduction in the use of ciprofloxacin and significant improvement in quality of ciprofloxacin prescription. Close collaboration within a hospital between medical microbiologists and clinicians is an important condition to reduce liberal and inappropriate use of antibiotics. Routine (prophylactic) use of ciprofloxacin should be discouraged, to prevent unnecessary use. An example is given in **chapter 4**. A 3-day course of ciprofloxacin was common practice in our hospital when removing a urinary catheter. Previously, Harding et al. demonstrated a single-dose of antibiotics for patients with asymptomatic bacteriuria to be as effective in preventing urinary tract infection as a 10 days course.[10] In our randomized double-blind placebo-controlled trial ciprofloxacin use appeared not beneficial at all in reducing occurrence of significant bacteriuria and urinary tract infection after catheter removal. Another example is the use of ciprofloxacin for prophylaxis in haematology patients. Due to the use of fluoroquinolones for prophylaxis, fluoroquinolone resistance is emerging as a major type of antibacterial resistance in *E. coli* with reported resistance rates exceeding 50%. [11] In **chapter 6** we investigated the evolution of ciprofloxacin resistance and molecular epidemiology of clinical *E. coli* isolates in haematology patients receiving ciprofloxacin prophylaxis on the population and individual patient level. We provide evidence for cross-transmission of ciprofloxacin resistant *E. coli* and in vivo mutation to a resistant phenotype of previously sensitive *E. coli* strains (in 7% of patients).

In our study we observe that in 27% of patients ciprofloxacin-resistant *E. coli* strains can be demonstrated either from the onset or during follow-up. Recent reviews and meta-analysis studies suggest that prophylactic treatment results in a reduction in death from all causes.[12-17] However, these conclusions are based on original studies published before 2005 and therefore do not take sufficiently in account the increased prevalence of fluoroquinolone-resistant Gram-negative strains observed in recent years, which may lead to a reduced efficacy of fluoroquinolone prophylaxis. Nowadays standard use of ciprofloxacin for prophylactic purpose in neutropenic patients should be reconsidered. Alternative strategies for patients in whom fluoroquinolone-resistant Gram-negative strains have been detected should be adopted.

Nursing Homes

Relatively little is known about the role of nursing homes in the epidemiology of resistant bacteria. Nursing homes have been considered important reservoirs of antibiotic resistant bacteria [18-20], as their residents frequently harbour risk factors for colonization and infection by multiresistant bacteria, such as indwelling bladder catheters, malnutrition, immunocompromised status, skin and soft-tissue breakdowns, recurrent urinary tract infections [20-24] and episodes of treatment with broad-spectrum antibiotics. [22,25] Transfer of colonized patients between health-care settings facilitates the spread of resistant bacteria. We determined the prevalence and molecular epidemiology of ESBL-producing and of ciprofloxacin-resistant *Enterobacteriaceae* in a teaching hospital and nursing homes in its immediate catchment area in a point-prevalence and retrospective analysis in **chapter 5**. Our findings demonstrate a persistently high prevalence of ciprofloxacin-resistant (26%) and ESBL-producing (7%) *Enterobacteriaceae* in nursing home residents over the period 2005-2010. In the point-prevalence survey 38% and 7% of 513 patients carried ciprofloxacin resistant and ESBL-producing *Enterobacteriaceae*, respectively. Highest resistance rates were found in the nursing home with the highest patient transfer rate between nursing home and hospital, which may partly be explained by the cycle of institutionalization and hospitalization. Regardless of the causes of the high prevalence of resistant strains in nursing homes our findings have several implications. Our study suggests an important role of patient admission (and readmissions) from nursing homes in the epidemiology of antibiotic-resistant Gram-negative bacteria. From one of these nursing homes an ESBL-carrier is admitted to the hospital every 19 days on average. This admission risk would further increase if ESBL-carriage is associated with a higher likelihood of hospital admission. Vice versa, hospitalization may also increase the risk of acquisition of ESBL-carriage and subsequent introduction in a nursing home. The effect of screening nursing home residents for resistant microorganisms at the time of admission in the hospital is now being assessed in an ongoing prospective trial.

Regular education of nursing and physician personnel on infection-control measures and interventions to reduce the amount of antibiotics prescribed, should be considered. [26,27] The unnecessary use of broadspectrum antibiotics should be strongly discouraged.

Emergence of fluoroquinolone resistance

Resistance to antibiotics is becoming an increasingly important worldwide problem. Inappropriate use of antibiotics is considered to be the most important reason for development of antibiotic resistance.[28-30] The use of fluoroquinolones is associated with a more rapid development of resistance than the use of beta-lactam antibiotics. [31] Considering the large scale use of ciprofloxacin the development of resistance and cross-

resistance has raised global concern.[28] Reducing the selective pressures of antibiotic usage will prevent or delay the emergence of resistant strains. [32,33] In comparison to infections caused by susceptible bacteria, infections caused by resistant bacteria are associated with higher incidences of mortality and morbidity, prolonged hospital stay and increased financial burden.[34]

Maintaining fluoroquinolone class efficacy

The rise in Gram-positive pathogen resistance in recent years has prompted the pharmaceutical industry to develop fluoroquinolones with greater activity against Gram-positive microorganisms. The introduction of new agents such as levofloxacin, gatifloxacin and moxifloxacin, expanded the traditional gram-negative coverage of fluoroquinolones with improved activity to Gram-positive organisms. In particular they manifest greater activity against *Streptococcus pneumoniae*. Clinical applications include upper and lower respiratory infections, gastrointestinal infections, gynecologic infections, sexually transmitted diseases, and skin and soft tissue infections. Although the newer fluoroquinolones have shown promising in vitro activity against Gram-positive bacteria, caution must be exercised to avoid the potential for selection of widespread resistance, which may occur if they would be used indiscriminately.[35,36] Levofloxacin clinical failures have already been reported in the management of patients with pneumococcal community-acquired pneumonia.[36]

Overuse of a single agent will ultimately result in resistance to the entire class. It has been suggested that ciprofloxacin-resistant, levofloxacin-susceptible *S. pneumoniae* may already possess first-step mutations. [37,38]

Given the fact that penicillin resistance among pneumococci hardly occurs in The Netherlands and the risk of the development of resistance in both Gram-positive and Gram-negative microorganisms when the new fluoroquinolones are used, they should not be used as first-line agents and their area of indication should be limited.[39]

Appropriate antibiotic stewardship is important in the fight against antimicrobial resistance. Although fluoroquinolones are clinically effective against a broad range of infectious agents, emergence of resistance and associated clinical failures have prompted re-consideration of their empiric use. Two key objectives are concerned in appropriate use: 1. only prescribing antimicrobial therapy when it is beneficial and 2. using the agents with optimal activity against the expected pathogens. Emphasizing “correct-spectrum” coverage may reduce development of antimicrobial resistance and maintain class efficacy. Evidence is rising that suggests a link between inappropriate fluoroquinolone use, development of antimicrobial resistance against the entire fluoroquinolone class, and clinical failure.[40] Antibiotic pressure in the community, hospitals and nursing homes, should be reduced by a shift to treatment based on results of microbiologic investigation

rather than empiric treatment. Also ciprofloxacin should not be regarded as the magic bullet, but used with restraint.

Given large quantities of antibiotics currently use in veterinary medicine, judicious and targeted use of antibiotics in clinical medicine alone will not be enough to prevent ever increasing levels of resistance. The use of veterinary antimicrobial drugs associated with induction of resistance to antibiotics in humans should be limited, preferably by government regulations. New antimicrobial drugs should be explicitly approved for use in animals, permission only being granted for drugs that are not of vital importance for the treatment of infectious disease in humans, and do not contribute to development of resistance to antimicrobial analogues used in human medicine. Stringent antibiotic policies in human health and development of new antibiotics alone will not be effective enough.

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Nederlandse Samenvatting en Discussie

Het doel van de studies beschreven in dit proefschrift was het analyseren van een aantal aspecten van ciprofloxacine gebruik en klinische en (moleculaire) epidemiologie van ciprofloxacine resistentie in verschillende settings. Zowel in ziekenhuizen (**hoofdstuk 3,4 en 6**), als in de bevolking en verpleeghuizen (**hoofdstuk 2** en **5**). Gezien het brede spectrum tegen gramnegatieve micro-organismen en de gunstige farmacokinetische eigenschappen, is het gebruik van ciprofloxacine de afgelopen 2 decennia toegenomen, als ook de resistentie tegen ciprofloxacine.

In de bevolking

Het gebruik van ciprofloxacine in Nederland in de eerste lijn is meer dan verdubbeld van 1997 tot 2010.[1] De NHG-standaarden, ontwikkeld door het Nederlands Huisartsen Genootschap (NHG), benadrukken echter dat het gebruik van fluorochinolonen behouden moet blijven voor ernstige infecties in ziekenhuizen en niet voorgeschreven zou moeten worden in de eerstelijns zorg in bijvoorbeeld luchtweginfecties, urineweginfecties en seksueel overdraagbare aandoeningen.
Ciprofloxacine wordt beschouwd als een reserve middel en alleen te gebruiken bij uitzondering en op basis van kweken en gevoeligheidsbepalingen.
Echter, niet alleen het gebruik van ciprofloxacine in de humane geneeskunde is verantwoordelijk voor het optreden van resistentie. De hoeveelheid antibiotica die gebruikt worden voor veterinaire en viskweek (zalm) doeleinden in voedsel producerende dieren kunnen een risico vormen voor de volksgezondheid omdat het een belangrijke factor is in de ontwikkeling van antibiotica resistentie. Antibiotica gebruik, uitgedrukt in grammen per kilogram levend gewicht, is verdubbeld in 2007, vergeleken met 1999. [2] Fluorochinolonen (bijv. enrofloxacine) werden in de late jaren ’80, begin jaren ’90 geïntroduceerd in de veterinaire geneeskunde, met name in de pluimveesector. De toename van ciprofloxacine resistentie in *Campylobacter* species in Nederland en andere landen in Europa is gerelateerd aan deze introductie.[3-6]

Campylobacter is de meest voorkomende oorzaak van acute diarree wereldwijd. [7-9] *Campylobacter* enteritis is een door voedsel overdragen ziekte, hoewel een infectie ook opgelopen kan worden door direct contact met dieren of hun producten. *Campylobacters* zijn bewoners van het darmkanaal van tal van dierlijke gastheren, met name pluimvee, en vanuit deze bronnen komen zij de voedselketen binnen. Hoewel minder gevaarlijk dan salmonellose , veroorzaakt *Campylobacter* enteritis aanzienlijke morbiditeit en hoge economische kosten.

Hoofdstuk 2 beschrijft een landelijke epidemiologische analyse van kweek-bewezen *Campylobacter*-infecties in Nederland in de jaren 2000-2004 en het effect van de regio, de mate van verstedelijking en het seizoen op het optreden van campylobacteriose en de ontwikkeling van resistentie. Er werden hoge resistentie percentages tegen fluorochinolonen waargenomen (35%) en de resistentie was hoger in reisgerelateerde infecties (54%) dan in endemische infecties (33%). De hoge resistentie percentages tegen fluorochinolonen maakt heroverweging van het gebruik ervan als middel van eerste keuze in de empirische behandeling van gastro-intestinale infecties in Nederland nodig.

Ziekenhuis

Het gebruik van fluorochinolonen in het ziekenhuis in Nederland is toegenomen van 7,6 DDD (Defined Daily Dose) per 100 patiëntdagen in 1999 tot 9,6 DDD per 100 patiëntdagen in 2008. Deze stijging was uitsluitend te danken aan de sterke stijging in het gebruik van ciprofloxacine, van 5,3 tot 8,6 DDD per 100 patiëntdagen. [1]

In **hoofdstuk 3** worden de effecten van interventie met als doel het gebruik van ciprofloxacine in een ziekenhuis te verlagen en te verbeteren, beschreven. Ondanks de al relatief lage basis consumptie van ciprofloxacine, leidde interventie tot een blijvende 3-4-voudige reductie in het gebruik van ciprofloxacine en een significante verbetering van de kwaliteit van de voorschriften van ciprofloxacine. Nauwe samenwerking tussen artsen-microbioloog en clinici is een belangrijke voorwaarde om vrij en ongeoorloofd gebruik van antibiotica te verminderen. Routinematig (profylactisch) gebruik van ciprofloxacine moet worden ontmoedigd, om onnodige gebruik te voorkomen. In **hoofdstuk 4** wordt een voorbeeld gegeven. Een 3-daagse kuur ciprofloxacine was standaard procedure in ons ziekenhuis wanneer een urinekatheter werd verwijderd. Eerder toonde Harding et al. aan dat een eenmalige gift antibiotica even effectief is in het voorkomen van een urineweginfectie als een 10 daagse kuur, in patiënten met asymptomatische bacteriurie.[10] In ons gerandomiseerde, dubbelblinde placebogecontroleerde onderzoek bleek het gebruik van ciprofloxacine geen gunstig effect te hebben op het verminderen van het optreden van significante bacteriurie en urineweginfectie na urinekatheter verwijdering.
Een ander voorbeeld is het profylactisch gebruik van ciprofloxacine in hematologie patiënten. Door het profylactisch gebruik van fluorochinolonen is fluorochinolon resistentie in opkomst als een belangrijke vorm van antibiotica resistentie in *E. coli* met gerapporteerde resistentie percentages boven de 50%.[11] In **hoofdstuk 6** is de evolutie van ciprofloxacine resistentie en de moleculaire epidemiologie van klinische *E. coli* isolaten in haematologie patiënten die ciprofloxacine profylaxe kregen, onderzocht, op populatie niveau en op het niveau van de individuele patiënt. We leveren bewijs

voor kruistransmissie van ciprofloxacine resistente *E. coli* en in vivo mutatie naar een resistent fenotype in voorheen gevoelige *E. coli* stammen (in 7% van de patiënten).

In onze studie zien we dat in 27% van de patiënten ciprofloxacine resistente *E. coli* stammen kunnen worden aangetoond, hetzij vanaf het begin, of gedurende de follow-up. Recente reviews en meta-analyse studies suggereren dat profylactische behandeling resulteert in minder sterfte door alle oorzaken.[12-17] Echter, deze conclusies zijn gebaseerd op studies die gepubliceerd zijn voor 2005 en houden dus onvoldoende rekening met de in de laatste jaren toegenomen prevalentie van fluorochinolon resistente Gram-negatieve stammen, wat kan leiden tot een verminderde werkzaamheid van fluorochinolon profylaxe. Standaard gebruik van ciprofloxacine als profylaxe in neutropene patiënten moet worden heroverwogen. Alternatieve strategieën voor patiënten bij wie fluorochinolon-resistente Gram-negatieve stammen zijn ontdekt, moeten worden ontwikkeld.

Verpleeghuizen

Er is relatief weinig bekend over de rol van verpleeghuizen in de epidemiologie van resistente bacteriën. Verpleeghuizen worden beschouwd als een belangrijke bron voor antibiotica resistentie[18-20], daar de bewoners risicofactoren bij zich dragen voor kolonisatie en infectie met multiresistente bacteriën, zoals blaaskatheters, ondervoeding, immuuncompressie, huid en weke delen problemen, recidiverende urineweginfecties[20-24] en episodes van behandeling met breed-spectrum antibiotica. [22,25]

Overplaatsing van gekoloniseerde patiënten tussen gezondheidsinstellingen faciliteert de verspreiding van resistente bacteriën. In **hoofdstuk 5** bepaalden we de prevalentie en moleculaire epidemiologie van ESBL-producerende en ciprofloxacine-resistente *Enterobacteriaceae* in een opleidingsziekenhuis en verpleeghuizen in het verzorgingsgebied, middels een punt-prevalentie studie en een retrospectieve analyse. Onze bevindingen tonen een aanhoudend hoge prevalentie van ciprofloxacine-resistente (26%) en ESBL-producerende (7%) *Enterobacteriaceae* in verpleeghuis bewoners aan, over de periode 2005-2010.
In de punt-prevalentie studie droeg 38% respectievelijk 7% van de 513 patiënten ciprofloxacine resistente en ESBL-producerende *Enterobacteriaceae* bij zich. De hoogste resistentie percentage werden gevonden in het verpleeghuis met de meeste overplaatsingen tussen verpleeghuis en ziekenhuis, wat gedeeltelijk verklaard zou kunnen worden door cyclus van opname en heropname tussen verpleeghuis en ziekenhuis. Ongeacht de oorzaak van de hoge resistentie prevalentie in verpleeghuizen hebben onze bevindingen een aantal implicaties. Onze studie suggereert een belangrijke rol voor patiënt opname (en heropname) vanuit verpleeghuizen in de epidemiologie van antibiotica resistente

Gram-negatieve bacteriën. Iedere 19 dagen wordt er een ESBL drager opgenomen in het ziekenhuis vanuit 1 van deze verpleeghuizen. Dit opname risico zou nog verder kunnen stijgen als ESBL-dragerschap geassocieerd is met een hogere waarschijnlijkheid van ziekenhuis opname. Vice versa kan ziekenhuisopname ook het risico op het verkrijgen van ESBL-dragerschap vergroten en daarmee introductie in een verpleeghuis. Het effect van screenen van verpleeghuispatiënten op resistente micro-organismen bij opname in het ziekenhuis wordt onderzocht in een nog lopend prospectief onderzoek. Regelmatige nascholing van verpleegkundigen en verpleeghuisartsen op het gebied van infectiepreventie maatregelen en interventies om het antibioticum gebruik te reduceren, zouden moeten worden overwogen. [26,27] Het onnodig gebruik van breed-spectrum antibiotica moet sterk worden ontmoedigd.

Opkomst van fluorochinolon resistentie

Antibiotica resistentie begint wereldwijd een steeds belangrijker probleem te worden. Onjuist gebruik van antibiotica wordt als de meest belangrijk oorzaak van de ontwikkeling van antibiotica resistentie beschouwd. [28-30] Het gebruik van fluorochinolonen is geassocieerd met een snellere resistentie ontwikkeling dan het gebruik van beta-lactam antibiotica. [31] Het ciprofloxacine gebruik op grote schaal in aanmerking genomen, heeft de resistentie ontwikkeling en kruisresistentie wereldwijd tot grote zorgen geleid. [28] Het verminderen van de selectieve druk door antibiotica gebruik zal de opkomst van resistente stammen voorkomen of vertragen. [32,33] In vergelijking met infecties veroorzaakt door gevoelige bacteriën, zijn infecties veroorzaakt door resistente bacteriën geassocieerd met een hogere mortaliteit en morbiditeit, verlengde ziekenhuisopnameduur en hogere kosten. [34]

Behoud van werkzaamheid van fluorochinolonen

De stijging van resistentie in Gram-positieve pathogenen de afgelopen jaren heeft ertoe geleid dat de farmaceutische industrie fluorochinolonen is gaan ontwikkelen met grotere activiteit tegen Gram-positieve micro-organismen. De introductie van nieuwe middelen als levofloxacine, gatifloxacine en moxifloxacine, breidde de traditionele Gram-negatieve dekking van fluorochinolonen uit met verbeterde activiteit tegen Gram-positieve micro-organismen. Met name laten zij en betere activiteit tegen *Streptococcus pneumoniae* zien. Klinische indicaties zijn onder meer hogere en lagere luchtweginfecties, gastro-intestinale infecties, gynaecologische infecties, seksueel overdraagbare aandoeningen en huid en weke delen infecties. Hoewel de nieuwere fluorochinolonen een veel belovende in vitro activiteit tegen Gram-positieve bacteriën laten zien, is voorzichtigheid geboden om wijdverspreide resistentie te voorkomen, welke kan ontstaan als ze zonder onderscheid gebruikt worden. [35,36] Klinisch falen van levofloxacine is al gerapporteerd in de behandeling van patiënten met pneumococcen pneumonie. [36]

Overmatig gebruik van een enkel middel zal uiteindelijk resulteren in resistentie tegen de gehele klasse. Er zijn aanwijzingen dat ciprofloxacine-resistente, levofloxacine-gevoelige *S. pneumoniae* reeds de eerste mutaties bezitten. [37,38]

Gezien het feit dat penicilline resistente pneumococcen in Nederland nauwelijks voorkomen en gezien het risico op de ontwikkeling van resistentie in zowel Gram-positieve als Gram-negatieve micro-organismen bij gebruik van de nieuwere fluorochinolonen, dienen deze niet ingezet te worden als eerstelijns middelen en hun indicatie gebied zou beperkt moeten zijn. [39]

Goed antibiotica beleid is belangrijk in het gevecht tegen antimicrobiële resistentie. Hoewel fluorochinolonen een brede dekking hebben tegen vele bacteriën, heeft het optreden van resistentie en bijbehorend klinisch falen hun empirische gebruik doen heroverwegen. Twee doelstellingen zijn van belang bij gepast gebruik: 1. alleen antibiotica voorschrijven wanneer het nuttig is en 2. gebruik het antibioticum met het optimale spectrum tegen de verwachte micro-organismen. De nadruk op "correcte-spectrum" dekking kan de ontwikkeling van antibiotica resistentie verminderen en de werkzaamheid behouden.

Steeds meer bewijs suggereert dat er een verband is tussen onjuist fluorochinolon gebruik, ontwikkeling van antimicrobiële resistentie tegen de hele fluorochinolonen klasse, en klinisch falen.
Antibiotische druk in de bevolking, ziekenhuizen en verpleeghuizen zou verminderd moeten worden door een verschuiving van empirische therapie, naar therapie gebaseerd op resultaten van microbiologisch onderzoek. Ciprofloxacine moet niet gezien worden als 'magic bullet', en met terughoudendheid gebruikt worden.

Gezien de grote hoeveelheden antibiotica die momenteel gebruikt worden in de veterinaire geneeskunde, zal verstandig en gericht gebruik van antibiotica in de humane geneeskunde alleen, niet genoeg zijn om de almaar toenemende resistentie te voorkomen. Het gebruik van veterinaire antimicrobiële geneesmiddelen geassocieerd met inductie van resistentie tegen antibiotica bij mensen, moet worden beperkt, bij voorkeur door regelgeving van de overheid. Nieuwe antimicrobiële geneesmiddelen moeten expliciet worden goedgekeurd voor gebruik bij dieren, waarbij alleen toestemming moet worden verleend voor geneesmiddelen die niet van vitaal belang zijn voor de behandeling van infectieziekten bij de mens, en die niet bijdragen aan de ontwikkeling van resistentie tegen antimicrobiële analogen die worden gebruikt in de humane geneeskunde. Streng antibiotica beleid in de humane gezondheidszorg en de ontwikkeling van nieuwe antibiotica alleen zal niet effectief genoeg zijn.

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Curriculum vitae

Babette van Hees werd geboren in Nijmegen op 31 januari 1979. Tot haar 6de woonde zij in Malden, daarna in Maarn. In 1997 werd het diploma Gymnasium gehaald aan het Stedelijk Gymnasium Johan van Oldenbarnevelt, te Amersfoort. In dat jaar werd de studie geneeskunde aangevangen in Utrecht. Het arts-examen werd op 30 januari 2004 behaald. In mei 2004 startte zij de opleiding tot arts-microbioloog in het St. Antonius Ziekenhuis te Nieuwegein en het UMCU. Tijdens haar opleiding was zij actief binnen de Landelijk Vereniging van Assistent Geneeskundigen (LVAG) en vertegenwoordigde zij de arts-assistenten in de Medisch Specialisten Registratie Commissie (MSRC). Als lid van de Projectgroep modernisering van de opleiding NVMM, Commissie HOMM (Herstructurering Opleiding Medische Microbiologie) was zij betrokken bij het vormgeven van de nieuwe opleiding. De opleiding tot arts-microbioloog werd voltooid op 1 mei 2009. Vanaf deze datum is zij werkzaam als arts-microbioloog in Gelre ziekenhuizen. Sinds 1 juni 2010 is zij hoofd van de afdeling Medische Microbiologie en Infectiepreventie.

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Molecular analysis of ciprofloxacin resistance and clonal relatedness of clinical *Escherichia coli* isolates of haematologic patients reveiving ciprofloxacin prophylaxis
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