Emergence of fluoroquinolone resistance in *Escherichia coli* isolates at the Department of Clinical Hematology

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SUMMARY

The aim of this study was to evaluate the dependence of *Escherichia coli* resistance to fluoroquinolones on their use in the outpatients and inpatients in the Hradec Kralove region of the Czech Republic.

Data on inpatient fluoroquinolones use were obtained from the database of the Charles University Teaching Hospital Pharmacy and expressed as defined daily dose per 100 beds – days (DBD). Data on outpatient prescriptions were obtained from the database of the General Health Insurance Company and expressed in defined daily doses per 1000 clients per day (DID). *Escherichia coli* strains were isolated from samples of urine of both community and hospitalized patients suffering from acute bacterial urinary tract infection, examined using aerobic cultivation, and determined by standard biochemical procedures.

The utilization of fluoroquinolones in inpatients has significantly ($p<0.01$) increased from 2.73 DBD in 2001 to 4.89 DBD in 2006. In outpatients, fluoroquinolone utilization has also increased significantly from 0.29 DID to 1.15 DID ($p<0.01$). In the same period, 11 856 *Escherichia coli* strains were isolated from inpatients and outpatients with urinary tract infection and tested for the susceptibility to fluoroquinolones. Resistance increased significantly ($p<0.01$), both in the hospital (from 2 % to 10 %) and in the community (from 1% to 11%). The development of *Escherichia coli* resistance to fluoroquinolones correlates significantly with their utilization both in hospital ($r=0.996$, $p=0.005$) and in the community ($r=0.878$, $p=0.029$).

Results of this study shows the impact of fluoroquinolone utilization on *Escherichia coli* resistance, and support the need of controlled use of these effective antibiotics.

KEY WORDS: Fluoroquinolones, Drug utilization, *Escherichia coli*, Resistance, The Czech Republic

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IMPACT STATEMENT

In a recently published clinical study by Lautenbach *et al.*, describing the trends in Enterobacteriaceae resistance to fluoroquinolones during a 12-year period, *Escherichia coli* and *Proteus mirabilis* demonstrated a significant increase in resistance, with a significant difference between

inpatients and outpatients. As part of ongoing surveillance of infections and a review of antibiotic policy in the Charles University Teaching Hospital of Medical Faculty Hradec Kralove, Czech Republic, a recent increase in the rate of isolation of *E. coli* strains with high-level of resistance to fluoroquinolones prompted us to analyze the impact of fluoroquinolones selective pressure on bacterial resistance of *Escherichia coli* causing urinary tract infections in a community population (Hradec Kralove region, Czech Republic) and in the hospital facility located in the centre of the region (the Charles University Teaching Hospital of Medical Faculty Hradec Kralove).

This survey identified several areas for improvement in the area of fluoroquinolone empiric therapy and the development of bacterial resistance in inpatients and outpatients.

Ongoing co-operation between clinical teams and medical microbiologists is important to detect trends in epidemiology, which can be used to design empirical antibiotic regimens, effectively manage infections in patients and guide infection control policies in the Czech Republic.

INTRODUCTION

Fluoroquinolones, belonging to the quinolone chemotherapeutical class of agents, are antimicrobial drugs frequently used in the treatment of bacterial infections in human medicine. The first generation of quinolone involved nalidixic acid, oxolinic acid, and a novel pipemidic acid. Fluorinated quinolones (ofloxacin, ciprofloxacin, norfloxacin, perfloxacin, and others), introduced into practice in late 80s of the last century, represented a breakthrough in the development of this group of antimicrobial drugs. These agents, known as fluoroquinolones, manifest a very wide antibacterial spectrum, covering the majority of gram-negative rods including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter spp*, and other bacterial species causing human nosocomial infections. Nowadays, a serious problem is an increasing bacterial resistance to antibiotics, including fluoroquinolones (Flournoy *et al.,* 2000; Kolář *et al.,* 2001; Lesh *et al.* 2001). Fluoroquinolones possess bactericidal activity by the formation of enzyme-DNA complexes involv-

ing DNA gyrase and topoisomerase enzymes responsible for unwinding of the bacterial chromosome during replication. The primary mechanisms of resistance to fluoroquinolones are mutations that result in alteration of the target proteins DNA gyrase and topoisomerase IV, and decreased intracellular drug accumulation due to drug efflux or changes in outer membrane proteins (Drlica 2003; Ellington & Woodford 2006). The increasing bacterial resistance to fluoroquinolones is evidently connected with their overuse (Lautenbach *et al.,* 2004; Killgore *et al.,* 2004).

In the largest published clinical study, describing the trends in Enterobacteriaceae resistance to fluoroquinolones during a 12-year period, *Escherichia coli* and *Proteus mirabilis* demonstrated a significant increase in resistance, with a significant difference between inpatients and outpatients (Lautenbach *et al.,* 2004). In other epidemiological studies on inpatients and outpatients, independent risk factors for fluoroquinolone resistance of *Escherichia coli* were fluoroquinolone use, long-term care facility residence, aminoglycoside use, or underlying cancer (Lautenbach *et al.,* 2001; Lautenbach *et al.,* 2001; Killgore *et al.,* 2004).

As part of ongoing surveillance of infection and a review of antibiotic policy in the Charles University Teaching Hospital of the Medical Faculty Hradec Kralove, a recent increase in the rate of isolation of *E. coli* strains with high-level of resistance to fluoroquinolones (Zemkova *et al.,* 2005; Zemkova *et al.,* 2007) prompted us to analyze the impact of fluoroquinolones selective pressure on bacterial resistance of *Escherichia coli* causing urinary tract infections in a community population (Hradec Kralove region, the Czech Republic) and in the hospital facility located in the centre of the region (the Charles University Teaching Hospital of Medical Faculty Hradec Kralove).

MATERIALS AND METHODS

Design and setting
This study was designed as a retrospective drug utilization study conducted in the period of 2001 to 2006. Data on fluoroquinolones utilization and urinary tract infections causing *Escherichia coli* resistance to fluoroquinolones were collected both from hospitalized patients in the Charles

University Teaching Hospital of the Medical Faculty Hradec Kralove and the Hradec Kralove region community, and the hypothesis tested was dependence of *Escherichia coli* resistance on total fluoroquinolones utilization. The study was reviewed and approved by the Ethical Committee of the Charles University Teaching Hospital of Medical Faculty Hradec Kralove, Czech Republic. In 2001, the Hradec Kralove region had 232 000 inhabitants; the Teaching Hospital was the main in-patient facility of the region with 1517 acute–care beds and 436,138 bed-days per year. In 2006, there were 229,000 inhabitants in the region and 1530 acute–care beds and 431,392 bed-days per year (IHIS, Prague, the Czech Republic 2002; IHIS, Prague, the Czech Republic 2007).

Fluoroquinolones utilization data
Utilization of fluoroquinolones in the Teaching Hospital of Medical Faculty Hradec Kralove in the period followed was obtained from the computerized database of the Charles University Teaching Hospital Pharmacy. The database contains all antibiotics used. The data were processed according to the ATC/DDD system valid in 2006, and explained as the number of defined daily doses per 100 bed-days (DBD) (WHO 2006).

Data from outpatient prescriptions of antibiotics in the Hradec Kralove region were obtained from all prescriptions for patients registered with the General Health Insurance Company (GHIC) in the region, obtained from the company's database. The General Health Insurance Company is the largest state health care institution in the Czech Republic with the broadest spectrum of patients of all ages and social groups, and this cohort contained more than 55 % of the total population of the region. All drugs thus prescribed are registered on this database. In the Czech Republic antibiotic treatment is by medical prescription paid and registered by the health insurance company. The data were processed according to the ATC/DDD system, and explained as the number of defined daily doses per 1000 GHIC patients in the region per day (DID).

Escherichia coli susceptibility testing
Escherichia coli strains were isolated from the samples of urine both community and hospitalized patients suffering from acute bacterial urinary tract infection, examined using aerobic cul-

tivation, and determined by standard biochemical procedures (NCLLS 2002), using Enterotest 24 (Pliva Lachema, the Czech Republic). Only one *Escherichia coli* strain per patient was entered into the database. The repeated isolation of the same organism from clinical relevant specimens over a four-week period was considered part of the same episode.

Minimal inhibitory concentrations (MICs) of ofloxacin and ciprofloxacin tested for isolated *Escherichia coli* strains were further determined by the micro-dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCLLS) (NCLLS 2002). Concentrations of tested strains in Mueller-Hinton broth (Oxoid) were prepared to obtain 0.5 McFarland turbidity scale. MICs were read after 18 hours of incubation at 37°C. The MIC was interpreted as the lowest concentration of the antibacterial drug-agent that visibly inhibited bacterial growth. As MIC breakpoints, the following values were used: 1mg/L for ciprofloxacin, and 2 mg/L for ofloxacin. Reference strains *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853 were used for protocol quality control.

STATISTICAL ANALYSIS

Fox-Pro®, relation database software, was used for database management and internal quality and validation procedures. The statistical package SPSS 10.0 software for Windows was used to perform regression analysis (SPSS Inc., Chicago, III).

For testing the significance of resistance development and changes in fluoroquinolone utilization a linear regression analysis was used. Pearson correlation was used to determine the relationship between the use of fluoroquinolones and the susceptibility of pathogenic *Escherichia coli* strains to ofloxacin and ciprofloxacin. Statistical significance was accepted at the 5% level.

RESULTS

In spite of continuously decreasing antibiotic use in the hospital, utilization of fluoroquinolones significantly ($p < 0.01$) increased from 2.73 DBD

in 2001 to 4.89 DBD in 2006. Moreover, the proportion of fluoroquinolones out of total antibiotic utilization increased in these 5 years from 5.17% to 9.47% (Table 1).

In the regional community too, total antibiotic usage decreased, but fluoroquinolone utilization increased significantly from 0.29 DID to 1.15 DID (p<0.01). Increase in their proportion of all prescribed antibiotics is even more impressive - from 0.74% to 6.23% (Table 2).

In the same period, 17 682 samples of urine from community patients and hospitalized patients at the Charles University Teaching Hospital of Medical Faculty Hradec Kralove suffering from acute bacterial tract infection were cultivated, in which 11 856 *Escherichia coli* strains were isolated and tested for susceptibility to fluoroquinolones. Frequencies of *Escherichia coli* strain occurrence based on origin (community or hospital) and the numbers of resistant strains in each year of the followed period are described in Table 3. Resistance increased significantly (p<0.01)

TABLE 1 - *Utilization of fluoroquinolones in the Charles University Teaching Hospital of the Medical Faculty Hradec Kralove over the period 2001-2006.*

Year	2001	2002	2003	2004	2005	2006
Antibiotics [DDD/100bd]	52.81	43.60	42.92	46.10	46.08	51.64
Fluroquinolones [DDD/100bd]	2.73	2.86	2.94	3.43	3.82	4.89
Fluroquinolones [%]	5.17	6.56	6.85	7.44	8.29	9.47

TABLE 2 - *Utilization of fluoroquinolones in the Hradec Kralove region over the period 2001-2006.*

Year	2001	2002	2003	2004	2005	2006
Antibiotics [DDD/1000/d]	39.19	23.28	21.66	19.48	17.81	18.46
Fluroquinolones [DDD/1000d]	0.29	0.54	0.68	0.89	0.95	1.15
Fluroquinolones [%]	0.74	2.32	3.14	4.57	5.39	6.23

TABLE 3 - *Number of Escherichia coli strains tested and resistant to fluoroquinolones in the followed period.*

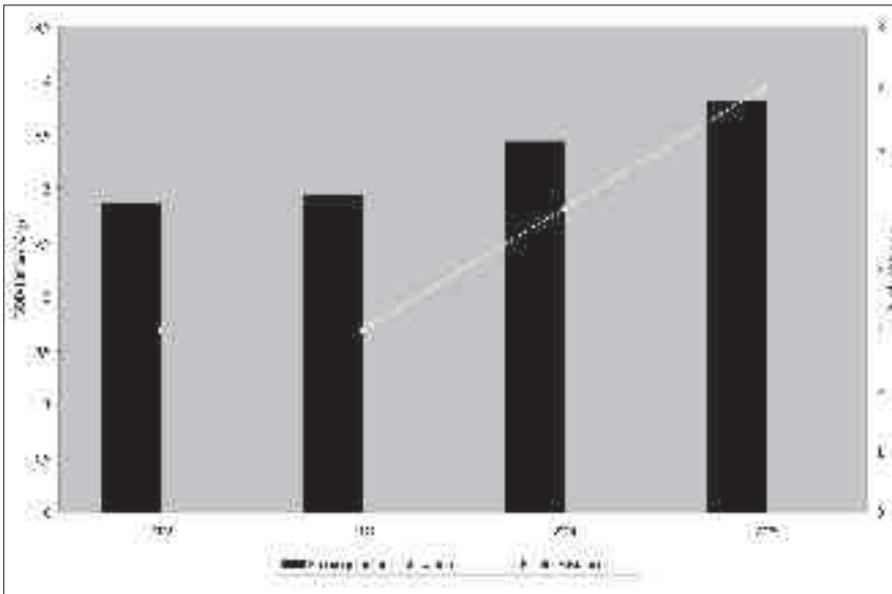| Year	Hospital			Community		
	Number of strains tested	Resistant to ofloxacin [%]	Resistant to ciprofloxacin [%]	Number of strains tested	Resistant to ofloxacin [%]	Resistant to ciprofloxacin [%]
2001	947	2	2	748	1	1
2002	1006	3	3	842	3	3
2003	1132	3	3	1074	4	4
2004	1117	4	5	745	5	6
2005	1195	6	7	963	9	9
2006	1061	10	10	1026	11	11

FIGURE 1 - *Utilization of fluoroquinolones and* Escherichia coli *resistance to fluoroquinolones in inpatients of the Charles University Teaching Hospital of the Medical Faculty Hradec Kralove.*

both in the hospital (from 2% to 10%) and in the community (from 1% to 11%).

The dependence of the development of *Escherichia coli* resistance to fluoroquinolone on their utilization in Charles University Teaching Hospital of the Medical Faculty Hradec Kralove is shown in Figure 1, the same for community utilization and resistance in Figure 2. A significant correlation was found both in the hospital (r=0.996, p=0.005) and in the community (r=0.878, p=0.029).

DISCUSSION

The Czech Republic is a country where utilization of antimicrobial agents is below the average for European Union countries (according to European Surveillance of Antimicrobial Consumption project data) (Vander Stichele *et al.,* 2004), with a relatively low consumption of fluoroquinolones. In 2001, the utilization of antibiotics there reached 18.55 DID (IHIS, Prague, the Czech Republic 2002), from which fluoroquinolones
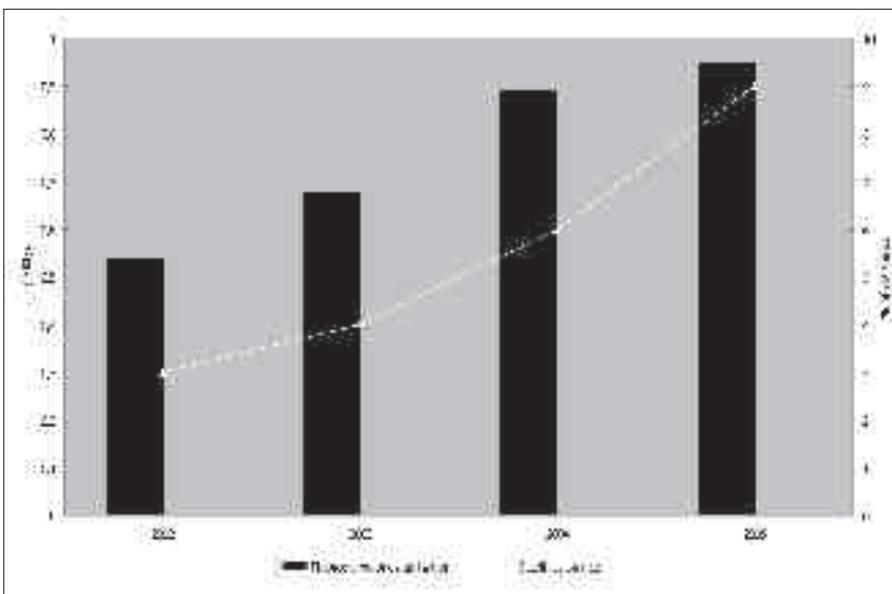

FIGURE 2 - *Utilization of fluoroquinolones and* Escherichia coli *resistance to fluoroquinolones in outpatients of the Hradec Kralove region.*

was 1.37 DID (7.4%). Until 2006, total antibiotic utilization increased only to 20.14 DID, but the fluoroquinolones proportion increased to 1.83 DID (9.1%) (IHIS, Prague, Czech Republic 2007). At the beginning of this study, utilization of fluoroquinolones was low both in hospital (2.73 DBD), and in out-patient care in the region (0.29 DID). *Escherichia coli* resistance in inpatients was at the level of 2%, respectively 1% in outpatients. In 2002, fluoroquinolone utilization in the community increased significantly and this increase remained stable until 2005, when it started to diminish. The resistance followed this trend very closely and reached 11% in 2006. Moreover, the development of *Escherichia coli* resistance in the hospital more closely followed the utilization of fluoroquinolones, which started to increase with a delay of 3 years - the greatest increase was found from 2004 to 2005, when the resistance increased from 7% to 10%.

The influence of hospital antibiotic policy is responsible for this delay, in contrast to an absence of this in out-patient care. Policies for antibiotic use in the Czech Republic were first compiled about 30 years ago, with many large hospitals having antibiotic committees. After 1989, the position of those committees was partly diminished and since 1997 antibiotic policy has been partially set up in the market economy (Kolář & Látal 1999).

The Pearson correlation for both parts of the study was significant, supporting the hypothesis of the strong general influence of antibiotic utilization on *Escherichia coli* resistance.

It should be stressed that although we have shown a correlation between increased use of fluoroquinolones and increase in bacterial resistance, this does not necessarily imply that this is the only cause, despite the fact that it would be the most plausible explanation. Other factors and reasons for the development of bacterial resistance in risk population groups have to be taken into account (Lautenbach *et al.,* 2004; Killgore *et al.,* 2004).

On the other hand, the increasing bacterial resistance to fluoroquinolones in the human population is evidently connected with their overuse (McDonald *et al.,* 2001). In addition, there is a high association with ciprofloxacin resistance in the strains producing extended-spectrum beta-lactamases in hospitals (Einhorn *et al.,* 2002; Shah *et al.,* 2004). Nevertheless the selective pressure of oxyimino-cephalosporins in the Czech communi-

ty is minimal; in the Hradec Kralove region the use of 3rd generation cephalosporins is at zero level and their use in Charles University Teaching Hospital of the Medical Faculty Hradec Kralove is very low. It can be assumed that the increasing number of fluoroquinolone-resistant *Escherichia coli* strains in our study is associated with the overuse of fluoroquinolones in the community.

This study demonstrates the importance of surveillance of infection in hospitalized patients to detect trends in infection and the emergence of multi-resistant organisms. By contrast, in some recently published papers the importance of restrictive antibiotic policy is more or less doubtful, sometimes with a reason like impossible measurement of economic impact of the resistance or impossibility of finding patient-connected outcomes (Leverstein-van Hall & Fluit 2001; Anonymous 2004).

In our opinion, the fact that infection by resistant strains cannot be treated by routinely used antibiotics has been sufficiently proven, and a rapid increase in resistance can render unusable otherwise useful and safe antibiotics.

A prospective study to evaluate the appropriateness of antimicrobial prophylaxis in terms of compliance with the guidelines and antibiotic susceptibility patterns in local hospitals is needed in the future. Adopting the national guidelines for antimicrobial prophylaxis in high - risk populations would provide a standard for improving the quality of local guidelines and a background for an evaluating guideline quality study.

We have used this information to inform the hospital antibiotic and other policies (e. g. infection control and vaccination policies) and to emphasize the importance of rational antibiotic use.

In conclusion, close co-operation between clinical teams and medical microbiologists is very important in effectively managing infections in patients, and in reviewing infection control and antibiotic policies.

KEY POINTS

- Resistance of uropathogenous *Escherichia coli* to quinolones depends on their use.
- Resistance increases more rapidly in outpatients.
- Restrictive antibiotic policy is needed.

Abbreviations
ATC/DDD - Anatomical Therapeutic Chemical in-
dex (including defined daily doses) for plain
substances
DID - defined daily doses per 1000 General Health
Insurance Company patients per day
DBD - defined daily dose per 100 beds - days
DNA - deoxyribonucleic acid
GHIC - General Health Insurance Company
IHIS - Institute of Health Informatics and Sta-
tistics
MIC - minimal inhibitory concentration
NCLLS - National Committee for Clinical Labo-
ratory Standards

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