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RESEARCH ARTICLE

Increasing Ciprofloxacin Resistance Among Prevalent Urinary Tract Bacterial Isolates in Gaza Strip, Palestine

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This article presents the incidence of ciprofloxacin resistance among 480 clinical isolates obtained from patients with urinary tract infection (UTI) during January to June 2004 in Gaza Strip, Palestine. The resistance rates observed were 15.0% to ciprofloxacin, 82.5% to amoxycillin, 64.4% to cotrimoxazole, 63.1% to doxycycline, 32.5% to cephalexin, 31.9% to nalidixic acid, and 10.0% to amikacin. High resistance to ciprofloxacin was detected among *Acinetobacter haemolyticus* (28.6%), *Staphylococcus saprophyticus* (25.0%), *Pseudomonas aeruginosa* (20.0%), *Klebsiella pneumonia* (17.6%), and *Escherichia coli* (12.0%). Minimal inhibitory concentration (MIC) of ciprofloxacin evenly ranged from 4 to 32 μg/mL with a mean of 25.0 μg/mL. This study indicates emerging ciprofloxacin resistance among urinary tract infection isolates. Increasing resistance against ciprofloxacin demands coordinated monitoring of its activity and rational use of the antibiotics.

INTRODUCTION

Urinary tract infections (UTIs) are the second most common infections in community practice. Worldwide, about 150 million people are diagnosed with UTI each year costing the global economy in excess of 6 billion US dollars [1].

All over the world, *Escherichia coli* accounts for 75% to 90% of UTI isolates and *Staphylococcus saprophyticus* accounts for 5% to 15% of cases of uncomplicated cystitis [2].

Antibiotics used in the therapy of UTI are usually able to reach high urinary concentrations, which are likely to be clinically effective. Fluoroquinolones are preferred as initial agents for empiric therapy of UTI in areas where resistance is likely to be of concern [3, 4]. This is because they have a high bacteriologic and clinical cure rates, as well as low rates of resistance, among most common uropathogens [4].

Ciprofloxacin is the most frequently prescribed fluoroquinolone for UTIs because of its availability in oral and intravenous formulations. Ciprofloxacin has shown an excellent activity against pathogens commonly encountered in complicated UTIs. It is well absorbed from oral doses and is rapidly excreted from the body under normal conditions [3, 5, 6].

Resistance to fluoroquinolones has increased markedly since their introduction for UTI treatment. Many studies worldwide reported a clear increase in ciprofloxacin resistance. For instance, in China, from 1998 to 2002 the incidence of ciprofloxacin resistance increased steadily from 46.6% to 59.4% [7]. In Spain, it was 14.7% [8], and in Bangladesh, it was 26.0% [9]. However, in previous studies in Gaza Strip, the resistance to ciprofloxacin among all isolates in 2000 was 4.1% and among *E coli* was only 2.9% [10] whereas, it increased to 11.3% in 2002 [11].

Evolving changes in drug resistance in various communities have forced the importance to a reassessment of local empiric choices for managing UTI [8, 12]. The present study describes the most common organisms causing UTI in Gaza Strip and evaluates the antibacterial activity of ciprofloxacin against recently isolated UTI pathogens.

METHODOLOGY

Sample collection and processing

A total of 1278 clean voided midstream urine samples were collected from the main three Gaza Strip governmental hospitals (Al Shefaa, Khan Younis, and the

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Gaza European hospital) from UTI adult outpatients (the physician suspected infection) aged 18–60 years during January to June 2004. The study was carried out at Khan Younis Hospital Laboratory, Palestine.

One sample per patient was collected consecutively from each of the 1278 UTI suspected cases (831 female and 447 male) to avoid strain duplication. Samples were stored at $2 - -4^\circ$C until being processed on the same day. Positive culture was defined as the culture of a single microorganism at a concentration of $\geq 10^5$ CFU/mL [13].

The nature of the work followed in the present study was fully explained to all subjects, and the study was conducted with their informed consent.

Each specimen was inoculated on both blood agar (with 5% defibrinated sheep blood) and MacConkey agar plates using a 0.01 mL standard loop (for semiquantitative counts) and incubated aerobically at 37°C for 24–48 hours and the number of colonies was counted. Significant growth was identified biochemically and serologically in a systematic way according to standard methods [14]. All Gram-negative rods were identified by using API 20E strips. Staphylococci were identified by catalase, coagulase, novobiocin, DNase, and Staphylococcus latex tests. The initial characterization of enterococci was based on catalase reaction, hemolysis, and colony morphology. Further identification of enterococci was accomplished by the use of bile esculin test. Enterococci were also confirmed by a serologic procedure "strep-check test" (Lorne Laboratories Ltd, Twyford, UK).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the bacterial isolates was performed by the disk diffusion method [15] in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) [16].

Quality controls employed standard strains of *E coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* 29212. Interpretive criteria for susceptibility or resistance followed NCCLS guidelines [16]. For this report, we present susceptibility data for amoxycillin, 25 μg; cephalexin, 30 μg; cefuroxime, 30 μg; ceftazidime, 30 μg; cotrimoxazole, 1.25–23.75 μg; nalidixic acid, 30 μg; ciprofloxacin, 5 μg; doxycycline, 30 IU; nitrofurantoin, 300 μg; gentamycin, 10 μg; and amikacin, 30 μg.

The ciprofloxacin minimum inhibitory concentration (MIC) was confirmed by E-test strips (AB Biodisk, Solna, Sweden). The ciprofloxacin MIC (μg/mL) used to define resistant isolates was ≥ 4, as outlined by the National Committee for Clinical Laboratory Standards [16].

Statistical analysis

Statistical analysis was performed by the chi-square test and P values of ≤ 0.05 were considered significant.

RESULTS

Of the 1278 urine samples processed, 492 (38.5%) showed positive monomicrobial cultures. Gram-negative bacteria represented 437 (91.0%) of the positive bacterial cultures (480), whereas Gram-positive were 43 (9.0%).

Table 1. Frequency of microorganisms isolated from 480 outpatients positive urine cultures. Figures reflect the number of the isolates.

Microorganisms	Frequency	%
Escherichia coli	252	52.5
Proteus mirabilis	47	9.8
Klebsiella pneumonia	44	9.2
Pseudomonas aeruginosa	43	9.0
Enterobacter cloacae	33	6.9
Acinetobacter haemolyticus	18	3.8
Enterococcus faecalis	25	5.2
Staphylococcus saprophyticus	18	3.8
Total	480	100.0

Twelve isolates (2.5% of 492) of yeast were encountered during the screening of UTI specimens. The yeast isolates were not included in this analysis because our study is concerned only with bacterial uropathogens and their antimicrobial susceptibility.

The overall sex distribution of the subjects was 831 (65.0%) females and 447 (35.0%) males and the sex distribution for the 480 positive cultures was 360 (75.0%) females and 120 (25.0%) males with a statistical significance ($P = .03$) predominance of females with UTI. The patients mean age was 31.6 ± 10.3 years.

A summary of the different microorganisms isolated during the study period is shown in Table 1. It is clear that *E coli* was the predominant uropathogen (52.5%) causing UTI followed by *Proteus mirabilis* (9.8%) and *Klebsiella pneumonia* (9.2%) whereas *E faecalis* was the most common uropathogen (5.2%) isolated among the Gram-positive bacteria.

High rates of resistance were found to amoxycillin (82.5%), followed by cotrimoxazole (64.4%) and doxycycline (63.1%) while the lowest resistance was to amikacin and ceftazidime (10.0%). The resistance rate to ciprofloxacin was 15.0%. The MICs for ciprofloxacin resistant isolates evenly ranged from 4 to 32 μg/mL with a mean of 25.0 μg/mL.

The isolated bacteria showed wide differences in their susceptibility to the tested antimicrobial drugs. A high resistance rate to ciprofloxacin was observed among the *Acinetobacter haemolyticus* (28.6%), *S saprophyticus* (25.0%), and *P aeruginosa* (20.0%) whereas *E faecalis* was the lowest resistant one (9.1%). The resistance to nitrofurantoin was only 2.7% among *E coli* and 28.6% among *A haemolyticus*. On the other hand, the resistance to nalidixic acid was 16.0% among *E coli* and 57.1% among *A haemolyticus*.

DISCUSSION

The importance of this study lies in describing the most common bacteria causing UTI among outpatients

Table 2. Antimicrobial resistance percentage of 480 clinical bacterial strains isolated from urinary tract infections. AMX, amoxycillin; CF, cephalexin; CTX, cefuroxime; CTZ, ceftazidime; GM, gentamycin; AN, amikacin; SXT, cotrimoxazole; DOX, doxycycline; NA, nalidixic acid; CIP, ciprofloxacin; and NF, nitrofurantoin.

Isolates	Antimicrobial agent										
	AMX %	GM %	AN %	SXT %	DOX %	CF %	CTX %	CTZ %	NA %	CIP %	NF %
Escherichia coli	78.7	14.7	2.7	58.7	58.7	18.7	10.7	4.0	16.0	12.0	2.7
Proteus mirabilis	84.2	36.8	10.5	68.4	63.2	47.4	21.1	15.8	31.6	15.8	89.5
Klebsiella pneumonia	88.2	11.8	11.8	76.5	76.5	23.5	11.8	11.8	23.5	17.6	5.9
Pseudomonas aeruginosa	93.3	33.3	13.3	66.7	73.3	60.0	53.3	13.3	56.7	20.0	100.0
Enterobacter cloacae	83.3	41.7	8.3	66.7	66.7	50.0	25.0	16.7	25.0	16.7	8.3
Acinetobacter haemolyticus	85.7	57.1	14.3	71.4	71.4	71.4	42.9	14.3	57.1	28.6	28.6
Enterococcus faecalis	81.8	45.5	36.4	63.6	45.5	NT	NT	NT	100.0	9.1	27.3
Staphylococcus saprophyticus	75.0	50.0	50.0	75.0	75.0	25.0	25.0	25.0	100.0	25.0	25.0
Resistance mean	82.5	25.6	10.0	64.4	63.1	32.5	19.4	10.0	31.9	15.0	26.3
P value	0.917	0.018	0.005	0.911	0.708	0.004	0.011	0.482	0.000	0.921	0.000

in Gaza Strip and their resistance to 11 selected antimicrobial agents.

The sex distribution of patients in the present study is consistent with that of other studies [17, 18]. The significant differences in UTI rates between females and males are thought to be due to anatomical differences between the sexes. Among other factors, the length of the urethra, a drier environment surrounding the meatus, and antibacterial properties of prostatic fluid contribute to a lower rate of infection in males [19].

In this study, the predominance of *E coli* among Gram-negative bacteria followed by *P mirabilis*, *K pneumonia*, and, among Gram-positive bacteria, *E faecalis* (Table 1), was similar to many authors results all over the world [20, 21, 22, 23].

The prevalence of *E coli* may be due to its existence as a normal flora in the large intestine and female vagina. The possible source of *E faecalis* infection could be due to a previous catheterization and those patients may be considered "complicated UTI" cases.

Notably, comparison among different studies concerning resistance of uropathogens to different antimicrobial agents should take into account the different periods in which such studies were carried out as well as various socioeconomical, socioepidemiological, and clinical parameters of the target population. Moreover, the comparison must consider the limitation of resistance to antimicrobials, which can vary from country to another.

The resistance of antimicrobial agents tested showed high resistance rates to amoxycillin, cotrimoxazole, and doxycycline while the lowest resistance was to amikacin and ceftazidime. The resistance rate of ciprofloxacin was 15.0% whereas, in a previous study (2000) carried out in Gaza Strip, lower resistance to ciprofloxacin (4.1%) was reported [10]. The widespread and more often the misuse of antimicrobial drugs in Gaza Strip have led to a general rise in the emergence of resistant bacteria, particularly to ciprofloxacin.

Higher resistance was reported in the USA to ampicillin and cotrimoxazole [24] whereas, for ciprofloxacin resistance, lower rates were found in other countries [25, 26].

Among Gram-negative bacteria, *E coli*, *K pneumonia*, and *Enterobacter cloacae* were more susceptible to nitrofurantoin (Table 2). These data suggest that nitrofurantoin may still be useful for the treatment of UTIs, especially for the mentioned organisms.

When comparing the high resistance rates in this study to ciprofloxacin against *Acinetobacter haemolyticus*, *S saprophyticus*, *P aeruginosa*, *E coli*, and *E faecalis* with other authors, higher resistance rates were reported [7, 8].

There are many reasons for this alarming phenomenon, including inappropriate prescribing of antibiotics and poor infection control strategies [27]. The situation in Gaza Strip, in terms of antimicrobial drug use, is not so different from that of many developing countries, where people usually take antimicrobial drugs without prescription or without performing the necessary culture testing.

The considerably high MIC values for ciprofloxacin reflects the extent of treatment problem for resistant isolates.

Overall susceptibility testing of this study demonstrates increased resistance to many commonly used agents especially to ciprofloxacin and illustrates the need for a continuous evaluation for the common antibiotics used in the therapy of uropathogens.

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