Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in the Gaza Strip

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ABSTRACT

Introduction: This study aims to assess common organisms causing urinary tract infection (UTI) in the Gaza Strip, Palestinian Authority and to examine the incidence of ciprofloxacin resistance in the strains of bacteria isolated from patients suspected with UTI over a six-month period.

Methods: Ciprofloxacin was evaluated along with other commonly-used antibiotics against a total of 480 clinical isolates obtained from urine samples. The samples were collected from community patients from different parts of the Gaza Strip. Susceptibility tests were done by the Kerby Bauer method.

Results: Among the tested drugs, the percent resistance rate to ciprofloxacin was 15.0 percent. However, high resistance to ciprofloxacin was detected among *Acinetobacter haemolyticus* (28.6 percent), *Staphylococcus saprophyticus* (25.0 percent), *Pseudomonas aeruginosa* (20.0 percent), *Klebsiella pneumonia* (17.6 percent) and *Escherichia coli* (12.0 percent). Minimal inhibitory concentration of ciprofloxacin was measured for all resistant UTI isolates.

Conclusion: This study indicates emerging ciprofloxacin resistance among most UTI bacterial pathogens. Increasing resistance against ciprofloxacin demands coordinated monitoring of its activity and rational use of the antibiotics.

Keywords: antibiotic resistance, ciprofloxacin, urinary tract infection

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INTRODUCTION

Urinary tract infection (UTI) is the second most common infectious presentation 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 (E. 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 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 high bacteriological 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]. In previous studies in the Gaza Strip, the resistance to ciprofloxacin among community-acquired UTIs isolates in 2000 was 4.1% and among *E. coli*, it was only 2.9%[10]. However, the resistance among *E. coli* increased to 11.3% in 2002[11].

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

METHODS

A total of 1,278 clean voided midstream urine samples were collected from adult patients (aged 18-60 years) having community-acquired UTI and who were referred to the main three Gaza Strip governmental

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hospital laboratories (Al Shefaa, Khan Younis and the Gaza European hospitals with 1,083 beds) during the period, January to June 2004. The study was carried out at Khan Younis Hospital Laboratory. Susceptibilities of the common isolated bacteria *(E. coli, Klebsiella pneumonia, Proteus mirabilis, Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter haemolyticus, Staphylococcus saprophyticus and Enterococcus faecalis)* to certain antimicrobial agents causing UTI were examined. The incidence of ciprofloxacin resistance was compared with that of previous local studies. The patient population and bacteriological methods used (culture and antimicrobial susceptibility testing) were not changed during the three studies.

One sample per patient was collected consecutively from each of 1,278 UTI suspected cases (831 females and 447 males) to avoid strain duplication. Samples were stored at 2-4ºC until they were 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 semi-quantitative counts), and incubated aerobically at 37ºC 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, novabiocin, D'Nase and Staph latex tests. The initial characterisation of enterococci was based on catalase reaction, haemolysis and colony morphology. Further identification of enterococci was accomplished by the use of the bile esculin test. Enterococci were also confirmed by a serological procedure, "Strep-Check test" (Lorne Laboratories Ltd). Antimicrobial sensitivity testing of all isolates was performed on diagnostic sensitivity test plates by the Kerby Bauer method[15] in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) assessment criteria[16].

Bacterial inoculum were prepared by suspending the freshly-grown bacteria in 4-5 ml sterile normal saline and adjusted to a 0.5 McFarland standard. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates in three directions (except for the *Streptococcus* species, where blood agar plates were used). Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (Sanofi Diagnotic Pasteur) were used. The antimicrobial agents tested were 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; nitrofurantoin, 300 μg; gentamycin, 10 μg and amikacin, 30 μg.

The resistance to ciprofloxacin was confirmed by breakpoint minimum inhibitory concentration (MIC) [in microgrammes per millilitre] by using E-test strips (AB Biodisk, Solna, Sweden). The resistance breakpoint for ciprofloxacin was >4 μg/ml according to those defined by NCCLS M100-S11, where the higher value was used as the MIC[16]. Quality controls were carried out once weekly with reference strains of *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Statistical analysis was performed by the chi-square test and p-values of <0.05 were considered significant.

RESULTS

Of 1,278 urine samples processed, 492 (38.5%) showed positive mono-microbial cultures. Gram-negative bacteria represented 437 (91.0%) of the positive bacterial cultures (480), whereas gram-positive was 43 (9.0%). 12 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 was 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. 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 I. 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%). *Enterococcus faecalis* was the most common uropathogen (5.2%) isolated among the gram-positive bacteria. The mean percentages of resistance of all isolates to the ten antimicrobial agents were: 82.5% to amoxycillin, 64.4% to cotrimoxazole, 32.5% to cephalexin, 31.9% to nalidixic acid, 26.3% to nitrofurantoin, 25.6% to gentamycin, 19.4% to cefuroxime, 15.0% to ciprofloxacin, and 10.0% to ceftazidime and amikacin. 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%),

Staphylococcus saprophyticus (25.0%) and *Pseudomonas aeruginosa* (20.0%). *Enterococcus faecalis* had the lowest resistance (9.1%). The resistance to nitrofurantoin was only 2.7% among *E. coli* and 28.6% among *Acinetobacter haemolyticus*. On the other hand, the resistance to naldixic acid was 16.0% among *E. coli* and 57.1% among *Acinetobacter haemolyticus.*

Table I. Distribution of the isolates according to sex.

Isolates	Sex				Among all subjects	
	Female		Male			
	No.	%	No.	%	No.	%
E. coli	212	58.9	40	33.3	252	52.5
Proteus mirabilis	31	8.6	16	13.3	47	9.8
Klebsiella pneumonia	33	9.2	11	9.2	44	9.2
Pseudomonas aeruginosa	22	6.1	21	17.5	43	9.0
Enterobacter cloacae	21	5.8	12	10.0	33	6.9
Acinetobacter haemolyticus	11	3.1	7	5.8	18	3.8
Enterococcus faecalis	13	3.6	12	10.0	25	5.2
Staphylococcus saprophyticus	17	4.7	1	0.8	18	3.8
Total	360	100.0	120	100.0	480	100.0

No. reflects the number of the isolates.

p-value = 0.03

DISCUSSION

The importance of this study lies in describing the most common bacteria causing UTI among outpatients in the Gaza Strip and their resistance to ten selected antimicrobial agents. In this study, the predominance of *E. coli* among gram-negative bacteria followed by *Proteus mirabilis, Klebsiella pneumonia* and among gram-positive bacteria, *Enterococcus faecalis* (Table I) was similar to many study results all over the world[17-20]. The possible source of *Enterococcus faecalis* infection could be due to a previous catheterisation 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 one country to another. The resistance of antimicrobial agents tested showed high resistance rates to amoxycillin and cotrimoxazole, while the lowest resistance was to amikacin and ceftazidime. Of significance is the mean resistance rate of ciprofloxacin in this study (15.0%), compared to a lower resistance to ciprofloxacin (4.1%) reported in a previous study carried out on the same population and with the same bacteriological methods in 2000[10].

The widespread use, and more often the misuse, of antimicrobial drugs in the Gaza Strip, has 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[21]. For ciprofloxacin resistance, lower rates were found in other countries[22,23]. Among gram-negative bacteria, *E. coli, Klebsiella pneumonia* and *Enterobacter cloacae* were more susceptible to nitrofurantoin (Table II). This data suggest that nitrofurantoin may

Table II. Percentage of resistance to the selected antimicrobial agents among different isolates.

Isolates	Antimicrobial agent									
	AMX %	GM %	AN %	CO %	CF %	CTX %	CTZ %	NA %	CIP %	NTFN %
E. coli	78.7	14.7	2.7	58.7	18.7	10.7	4.0	16.0	12.0	2.7
Proteus mirabilis	84.2	36.8	10.5	68.4	47.4	21.1	15.8	31.6	15.8	89.5
Klebsiella pneumonia	NT	11.8	11.8	76.5	23.5	11.8	11.8	23.5	17.6	5.9
Pseudomonas aeruginosa	NT	33.3	13.3	66.7	60.0	NT	13.3	56.7	20.0	100.0
Enterobacter cloacae	83.3	41.7	8.3	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	42.9	14.3	57.1	28.6	28.6
Enterococcus faecalis	81.8	45.5	36.4	63.6	NT	NT	NT	100.0	9.1	27.3
Staphylococcus saprophyticus	75.0	50.0	50.0	75.0	25.0	25.0	25.0	100.0	25.0	25.0
p-value	0.917	0.018	0.005	0.911	0.004	0.011	0.482	0.000	0.921	0.000

NT = not tested.

Significant at p-value of <0.05

AMX = amoxycillin; CF = cephalexin; CTX = cefuroxime; CTZ = ceftazidime; GM = gentamycin; AN = amikacin; CO = cotrimoxazole; NA = nalidixic acid; CIP = ciprofloxacin; NTFN = nitrofurantoin.

still be useful for the treatment of simple cystitis and uncomplicated UTIs, especially for the above-mentioned organisms. When comparing the high resistance rates in this study to ciprofloxacin against *Acinetobacter haemolyticus, Staphylococcus saprophyticus, Pseudomonas aeruginosa, E. coli* and *Enterococcus faecalis* (Table II) with other studies, 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[24]. The situation in the 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 of the common antibiotics used in the therapy of uropathogens.

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