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GyrA Mutations in Nosocomial Ciprofloxacin-Resistant *Escherichia coli* Isolates Associated with Urinary Tract Infections

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A B S T R A C T

Escherichia coli (*E. coli*) is one of most common organisms responsible for health care-associated urinary tract infection (HAUTI). The aim of this study was to determine antibiotic susceptibility pattern of *E. coli* isolated from HAUT I and to study ciprofloxacin resistance caused by *gyrA* gene mutations among these isolates. Urine Samples were collected from patients with suspected HAUTI. *E. coli* identification and antimicrobial susceptibility testing, minimum inhibitory concentration (MIC) of ciprofloxacin were done followed by detection of mutations in the gyrA gene by *PCR*-RFLP. Eighty seven isolates *E. coli* were isolated from HAUTI. Thirty-six isolates (41.3%) isolates were ciprofloxacin resistant. Double mutations at *gyrA* were detected at positions 83 and 87 of the quinolone resistance determining regions (QRDRs) in 25 (69.4%) ciprofloxacin resistant isolates, while single mutation at positions 87 revealed in 9 (25%) isolates. The pressure of the abuse of ciprofloxacin shares significantly to their resistance among *E. coli* isolated from urinary tract with double mutations at gyrA at positions 83 and 87 of the QRDRs represents an important factor for resistance.

Introduction

Urinary tract infections (UTIs) are the fourth health care-associated infection (Magill *et al*., 2014). *E. coli* is a major pathogen in healthcare-associated urinary tract infection (HAUTI) (Cullen *et al*., 2012).Antimicrobial resistance in *E. coli* causing UTIs is rising in several countries (Niranjan and Malini, 2014), (Karlowsky *et al*., 2002).

In recent decades, fluoroquinolones have been broadly used to treat health care-associated Gram-negative bacterial infections.

Ciprofloxacin is the most commonly used fluoroquinolone for treatment of UTIs as it exists in oral and intravenous preparations (Schaeffer, 2002). However, resistance to fluoroquinolones has become prevalent due to this widespread use (Ena *et al*.,1998). Mutation in DNA gyrase and DNA topoisomerase IV are the most important mechanisms of resistance to fluoroquinolones (Minarini and Darini, 2012; Moon *et al*., 2010).Other resistance mechanisms, including, alteration in the outer membrane

proteins, efflux pump, target mutation and drug enzymatic modification are found (Cavaco *et al*., 2008). The aim of this study was to assess antibiotic susceptibility pattern of *E. coli* isolated from HAUTI, in addition, determine ciprofloxacin resistance and the role of mutations in the *gyrA* gene in ciprofloxacin-resistance.

Materials and Methods

Urine samples (mid-stream, catheter aspirated) were collected from January to August 2016 from the patients suspected to have UTIs in Mansoura University Hospitals referred to Microbiology Department in Faculty of Medicine, Mansoura University. They were processed by the semi-quantitative culture technique on the cystine lactose electrolyte deficient (CLED). Colonies identified by colonial morphology, Gram-stained films and conventional biochemical tests including, oxidase test, Kligler- iron agar test, IMVC tests (Mahon *et al*., 2000).

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was done by disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines (Wayne, 2007). The group of intermediate susceptibility was considered together with the resistant strains. Minimum inhibitory concentration was done for ciprofloxacin using E-test strips (bioMérieux Inc., MO, USA) following the manufacturer's instructions.

Detection of *GyrA* mutations

Genomic DNA extraction from the isolates was done through a boiling technique. PCR was performed and primers and thermocycling conditions used were designed as described previously by Ozeki *et al.*, (1997). *gyrA* PCR product is about 164 bp.

Point mutation at positions Ser-83 and Asp-87 of *gyrA* was tested by restriction fragmet length polymorphism (RFLP) analysis of PCR products. The PCR products were digested with *HinfI* (Ferment as, Thermo Fisher Scientific Inc) to detect mutations at positions Ser-83 and Asp- 87 (Ozeki *et al*., 1997). Digestion products according to mutation site are listed in table 1. Products were determined by electrophoresis in 3% (w/v) agarose gel then visualized under UV light using 50 bp ladder as DNA size marker

Results and Discussion

During the study period, a total of 87 *E. coli* isolates were collected from patients with HAUTIs. Sixty women (79 %) and 27 men (31%), mean age was 49years+13.79 (range, 21 to 70 years) with 46samples (52.8%) were collected from patients in intensive care units (ICUs).

The highest rates of resistance were found for cefotaxime(78.2%), aztreonam (65.5%), sulphametoxazole-trimethoprim (65.5%). Thirty-six isolates (41.3%) were ciprofloxacin resistant. Antibiotic resistance pattern of *E. coli* among ciprofloxacin resistant and ciprofloxacin sensitive are summarized in table 2.

Ciprofloxacin resistance in *E. coli* was significantly higher in old age, prior urinary catheterization and prior quinolone use (Table 3).

No mutation was detected in ciprofloxacin sensitive isolates. Most of ciprofloxacin resistant isolates 34 (94.4%) had mutation in *gyrA* with 25 isolates (73.5%) had mutations at both Ser-83 and Asp-87, while9 (26.5%) had a single mutation at Asp-87. No single mutation at Ser-83 was detected. MIC of cirprofloxacin in *gyrA* mutations are listed in table 4. Treatment of UTIs becomes more

difficult because of emergence of antibiotic-resistant bacteria (Arslan *et al*., 2005). In this study, high rate of antibiotics resistance was found in *E. coli*. Similarly, high rate of resistance was previously reported in several studies (Niranjan and Malini, 2014), (Jadhav *et al*., 2011), (Khorvash *et al*., 2009). On other hand, lower resistance rate was observed by Sotto *et al.*, (2001). This variation in resistance may be due to difference in local antibiotic prescription policy (Sotto *et al*., 2001).

The most common antibiotics used in treatment of UTIs are trimethoprim-sulfamethoxazole, quinolones, cephalosporins and semisynthetic penicillins with or without beta-lactamase inhibitors (Arslan *et al*., 2005).

Table.1 HinfIPCR-RFLP patterns of gyrA

Mutation site	Restriction fragment length
Mutations at both Ser-83 and Asp-87	164bp
No mutations at either Ser-83 or Asp-87	109 bp and 40 bp
Single mutation at Ser-83	124 bp and 40 bp
Single mutation at Asp-87	109 bp and 55 bp

Table.2 Antibiotic resistance profiles of ciprofloxacin resistant and ciprofloxacin sensitive *E. coli* isolates

Antibiotics	Ciprofloxacin resistant (36)	Ciprofloxacin sensitive (51)	Total Resistance No. (%)	P value
Amoxicillin-clavulanic acid	19	36	55(63.2)	.09
Cefotaxime	31	37	68(78.2)	.13
Gentamicin	18	30	48 (55.2)	.41
Imipenem	14	13	27 (31)	.18
Amikacin	11	21	32 (36.8)	.31
Nitrofurantoin	15	20	35 (40.2)	.81
Sulphamethoxazole-Trimethoprim	29	28	57(65.5)	.013*
Aztreonam	21	36	57 (65.5)	.23
Tazocin	20	18	38(43.7)	.06

Table.3 Risk factors for ciprofloxacin resistance among urinary *E. coli*

Risk factors	Ciprofloxacin resistant	ciprofloxacin sensitive	P value	Odds Ratio (Confidence Interval)
Age > 60 years	16	10	.01*	.30 (.117-.791)
Urinary catheter	26	25	.03*	2.7 (1.08-6.7)
ICU	22	24	.19	1.7 (.7-4.2)
Prior ciprofloxacin use (6 months)	16	12	.04*	2.6 (1.03-6.5)

Table.4 MIC of Ciprofloxacin and gyrA gene mutations in ciprofloxacin resistant *E. coli*

gyrA	**Total Number (36)**	**No. of *E. coli* isolates corresponding Ciprofloxacin MIC (µg/ml)**					
		4	**8**	**16**	**32**	**64**	**>64**
Wild type	2 (5.5%)	2	0	0	0	0	0
Ser83 and Asp87	25 (69.4%)	0	1	15	1	5	3
Asp87	9 (25.1%)	6	3	0	0	0	0

In this study, high resistance was reported to cefotaxime, trimethoprim and lower resistance for imipenem and nitrofurantoin. In Consistent with our results, high trimethoprim resistance and low nitrofurantoin resistance was also observed in previous reports (Bean *et al*., 2008; Cullen *et al*., 2012; Schito *et al*., 2009).

At first, the incidence of fluoroquinolone resistance was very low (Kresken and Wiedemann, 1988). In the last decade, widespread use of fluoroquinolones has leaded to increase resistance among urinary *E. coli* (Fasugba *et al*., 2015). In this study, it was found that ciprofloxacin resistance rate was 41.3% and this is in consistent with previous study of Tandogdu *et al.,* (2014) in which Ciprofloxacin resistance among urinary *E. coli* in different geographic areas varies from 35-57%. On other contrary, lower prevalence 5.3% was reported by other researcher (Sotto *et al*., 2001). In our work, ciprofloxacin resistance isolates were significantly associated with sulphamethoxazole-trimethoprim resistance. Previous works observed concomitant trimethoprim and ciprofloxacin resistance in urinary *E. coli* (Karlowsky *et al*., 2002), (Zhanel *et al*., 2000). Prior quinolone use, old age and prior urinary catheterization were significantly associated with ciprofloxacin resistance. The same factors were previously recognized (Sotto *et al*., 2001). Moreover, Ena *et al.,* (1998) showed increase in fluoroquinolone resistance in urinary *E coli* from 3 to 20% that associated with usage of ciprofloxacin. Mutation in *gyrA* is the most frequent mechanism of fluoroquinolone resistance in clinical isolates (Ruiz, 2003).The majority of ciprofloxacin resistant isolates in this study showed double mutations in*gyrA.* The same was previously reported by several publications (Minarini and Darini, 2012; Moon *et al*., 2010; Chenia *et al*., 2006). Intermediate to high-level resistance was associated with these strains with double mutations comparable with previous studies who stated that low-level fluoroquinolone resistance in *E. coli* is related to a single mutation in the *gyrA* whereas high-level resistance associated with multiple mutations (Chenia *et al*., 2006; Minarini and Darini, 2012)

From the previous results, it was concluded that the uncontrolled use of certain antibiotics such as quinolones that should be reserved for resistant isolates strongly lead to increase the frequency of their resistance. Moreover, double mutations in*gyrA* represents a significant mechanism in resistance to ciprofloxacin.

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