Original Research Article

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

Rasha H. El-Mahdy¹, Mohammed A. Saleh²* and Aalaa Aboelnour³

¹Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Egypt
²Department of Medical Microbiology and Immunology, Faculty of Medicine, Al-Azhar University, New Damietta, Egypt
³Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt

*Corresponding author

**A B S T R A C T**

*Escherichia coli* (*E. coli*) is one of the 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 HAUTI 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 determined, 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%) 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 mutations at positions 87 were 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.

**Keywords** Ciprofloxacin Resistant, *E. coli*, and gyrA Mutations.

**Accepted:** 20 January 2017

**Available Online:** 10 February 2017

**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 fragment length polymorphism (RFLP) analysis of PCR products. The PCR products were digested with HinfI (Fermentas, 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 49 years±13.79 (range, 21 to 70 years) with 46 samples (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, while 9 (26.5%) had a single mutation at Asp-87. No single mutation at Ser-83 was detected. MIC of ciprofloxacin 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)                  |
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 ingyrA. 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 ingyrA represents a significant mechanism in resistance to ciprofloxacin.

References

Arslan, H., Azap, O. K., Ergonul, O., and Timurkaynak, F. (2005). Risk factors for ciprofloxacin resistance among Escherichia coli strains isolated from community-acquired urinary tract infections in Turkey. J Antimicrob...
Bean, D. C., Krahe, D., and Wareham, D. W. (2008). Antimicrobial resistance in community and nosocomial Escherichia coli urinary tract isolates, London 2005-2006. *Ann Clin Microbiol Antimicrob*, 7, 13. doi: 10.1186/1476-0711-7-13

Cavaco, L. M., Frimodt-Moller, N., Hasman, H., Guardabassi, L., Nielsen, L., and Aarestrup, F. M. (2008). Prevalence of quinolone resistance mechanisms and associations to minimum inhibitory concentrations in quinolone-resistant Escherichia coli isolated from humans and swine in Denmark. *Microb Drug Resist*, 14(2), 163-169. doi: 10.1089/mdr.2008.0821

Chenia, H. Y., Pillay, B., and Pillay, D. (2006). Analysis of the mechanisms of fluoroquinolone resistance in urinary tract pathogens. *J Antimicrob Chemother*, 58(6), 1274-1278. doi: 10.1093/jac/dkl404

Connie, R Mahon, Mahon, D, Lehman, C, and Manuselis, G. (2000). Textbook of diagnostic microbiology. *WB Saunder, UK*.

Cullen, I. M., Manecksha, R. P., McCullagh, E., Ahmad, S., O’Kelly, F., Flynn, R. J., Thornhill, J. A. (2012). The changing pattern of antimicrobial resistance within 42,033 Escherichia coli isolates from nosocomial, community and urology patient-specific urinary tract infections, Dublin, 1999-2009. *BJU Int*, 109(8), 1198-1206. doi: 10.1111/j.1464-410X.2011.10528.x

Ena, J., Lopez-Perezagua, M. M., Martinez-Peinado, C., Cia-Barrio, M. A., and Ruiz-Lopez, I. (1998). Emergence of ciprofloxacin resistance in Escherichia coli isolates after widespread use of fluoroquinolones. *Diagn Microbiol Infect Dis*, 30(2), 103-107.

Fasugba, O., Gardner, A., Mitchell, B. G., and Mnatzagianian, G. (2015). Ciprofloxacin resistance in community- and hospital-acquired Escherichia coli urinary tract infections: a systematic review and meta-analysis of observational studies. *BMC Infect Dis*, 15, 545. doi: 10.1186/s12879-015-1282-4

Jadhav, S., Hussain, A., Devi, S., Kumar, A., Parveen, S., Gandham, N., Ahmed, N. (2011). Virulence characteristics and genetic affinities of multiple drug resistant uropathogenic Escherichia coli from a semi urban locality in India. *PLoS One*, 6(3), e18063. doi: 10.1371/journal.pone.0018063

Karlowsky, J. A., Kelly, L. J., Thornsberry, C., Jones, M. E., and Sahm, D. F. (2002). Trends in antimicrobial resistance among urinary tract infection isolates of Escherichia coli from female outpatients in the United States. *Antimicrob Agents Chemother*, 46(8), 2540-2545.

Khorvash, F., Mostafavizadeh, K., Mobasherizadeh, S., and Behjati, M. (2009). Susceptibility pattern of *E. coli*-associated urinary tract infection (UTI): a comparison of spinal cord injury-related and nosocomial UTI. *Med Sci Monit*, 15(11), Cr579-582.

Kresken, M., and Wiedemann, B. (1988). Development of resistance to nalidixic acid and the fluoroquinolones after the introduction of norfloxacin and ofloxacin. *Antimicrob Agents Chemother*, 32(8), 1285-1288.

Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., and Fridkin, S. K. (2014). Multistate point-prevalence survey of health care-associated infections. *N Engl J Med*, 370(13), 1198-1208. doi: 10.1056/NEJMoa1306801

Minarini, L. A., and Darini, A. L. (2012). Mutations in the quinolone resistance-determining regions of gyrA and parC in *Enterobacteriaceae* isolates from Brazil. *Braz J Microbiol*, 43(4), 1309-1314. doi: 10.1590/s1517-83822012004000010

Moon, D. C., Seol, S. Y., Gurung, M., Jin, J. S., Choi, C. H., Kim, J., Lee, J. C. (2010). Emergence of a new mutation and its accumulation in the topoisomerase IV
gene confers high levels of resistance to fluoroquinolones in Escherichia coli isolates. Int J Antimicrob Agents, 35(1), 76-79. 

Sotto, A., De Boever, C. M., Fabbro-Peray, P., Gouby, A., Sirot, D., and Jourdan, J. (2001). Risk factors for antibiotic-resistant Escherichia coli isolated from hospitalized patients with urinary tract infections: a prospective study. J Clin Microbiol, 39(2), 438-444. doi: 10.1128/jcm.39.2.438-444.2001

Ruiz, J. (2003). Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J Antimicrob Chemother, 51(5), 1109-1117. doi: 10.1093/jac/dkg222

Zhanel, G. G., Karlowsky, J. A., Harding, G. K., Carrie, A., Mazzulli, T., Low, D. E., and Hoban, D. J. (2000). A Canadian national surveillance study of urinary tract isolates from outpatients: comparison of the activities of trimethoprim-sulfamethoxazole, ampicillin, mecillinam, nitrofurantoin, and ciprofloxacin. The Canadian Urinary Isolate Study Group. Antimicrob Agents Chemother, 44(4), 1089-1092.

How to cite this article:

Rasha H. El-Mahdy, Mohammed A. Saleh and Aalaa Aboelnour. 2017. GyrA Mutations in Nosocomial Ciprofloxacin-Resistant Escherichia coli Isolates Associated with Urinary Tract Infections. Int.J.Curr.Microbiol.App.Sci. 6(2): 1902-1907. doi: http://dx.doi.org/10.20546/ijemas.2017.602.215