Original Article
E-ISSN: 2538-4449

Frequency Distribution of Fluoroquinolones-Resistant *Enterococcus faecalis* Isolates from Patients with Prostatitis in Golestan Province, Iran

Hamideh Askari (MSc)
Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran

Leila Fozouni (PhD)
Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran

Hamid Reza Pordeli (PhD)
Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran

Corresponding author: Leila Fozouni **(PhD)**

Email: lili_kia@yahoo.com

Tel: +98-9111518674

Address: Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran

Received: 06 Aug 2018
Revised: 01 Nov 2018
Accepted: 05 Nov 2018

ABSTRACT

Background and Objectives: *Enterococcus faecalis* is a major cause of bacterial prostatitis, which can increase the risk of developing prostate cancer if mistreated or left untreated. The aim of this study was to evaluate resistance of *E. faecalis* strains isolated from patients with prostatitis to three fluoroquinolones.

Methods: In this cross-sectional study, we collected urine specimen from 164 patients hospitalized in six hospitals in the Golestan Province, Iran. Biochemical and bacteriological tests were carried out to identify *E. faecalis* strains. Pattern of resistance to ciprofloxacin, levofloxacin and norfloxacin was studied using the agar disk diffusion method (Kirby-Bauer method). The broth microdilution test was performed to determine minimum inhibitory concentrations (MICs) of fluoroquinolones according to the CLSI M100-S25 (2015) criteria.

Results: Of 164 isolates, 39 (23.8%) were identified as *E. faecalis*. Frequency of resistance to ciprofloxacin, norfloxacin and levofloxacin was 12.8%, 12.8% and 2.6%, respectively. The MIC_{90} of ciprofloxacin against the isolates was 4 µg/ml, which was 4-fold lower than that of norfloxacin (MIC_{90}=16µg/ml) and 2-fold lower than that of levofloxacin (MIC_{90}=8µg/ml). We found no significant difference between the isolates in terms of resistant to the fluoroquinolones ($P>0.01$).

Conclusion: Our results show that *E. faecalis* is one of the most common causes of bacterial prostatitis, and fluoroquinolones are still effective for treating the infection despite the reports of fluoroquinolones resistance in Iran. Moreover, levofloxacin may be a more suitable and effective antibiotic than ciprofloxacin and norfloxacin for treatment of this infection.

Keywords: *Enterococcus faecalis*, Prostatitis, Drug Resistance, Iran.

This paper should be cited as: Askari H, Fozouni L, Pordeli HR [Frequency Distribution of Fluoroquinolones-Resistant *Enterococcus faecalis* Isolates from Patients with Prostatitis in Golestan Province, Iran]. mljgoums. 2019; 13(4):29-33

INTRODUCTION

Enterococci are important causes of nosocomial and community-acquired infections (1). Hospital-acquired infections prolong hospitalization period, which significantly increases health care costs (2). *Enterococcus faecalis* and *Enterococcus faecium* are the most common *Enterococci* that cause human infections, such as urinary tract infections (UTIs), ulcers and endocarditis (3, 4). *E. faecalis* is an anaerobic, gram-positive and catalase-negative bacterium that can ferment glucose without gas production. The bacterium often resides in the urinary tract and is regarded as the second most common nosocomial pathogen (5). The intrinsic resistance of *Enterococci* to antibiotics and their ability to acquire resistance contribute to the survival of these organisms in hospital settings (6).

Bacterial prostatitis (BP) is caused by localization of UTI-causing bacteria (e.g. *E. faecalis*) (7). According to the World Health Organization, the increased prevalence of drug-resistant pathogens is an important global health problem. Fluoroquinolones such as ciprofloxacin and norfloxacin have been effectively used for the treatment of various bacterial infections including BP. Levofloxacin is a new-generation fluoroquinolone that is widely applied for treatment of infections caused by gram-positive, gram-negative, aerobic and anaerobic microorganisms. All three antibacterial agents are recommended as first-line treatments for BP (8). In this study, we investigate frequency of resistance to fluoroquinolones among *E. faecalis* isolates from patients with BP.

MATERIALS AND METHODS

In this cross-sectional study, urine specimens were taken from 164 patients (average age: 57 ± 9 years) with symptoms of prostatitis who were admitted to six hospitals in the Golestan Province, north of Iran, from September 2015 to October 2017. The symptoms included perineal pain, pelvic pain, testicular pain and bladder symptoms such as dysuria and frequent or urgent urination. Before sampling, we made sure that patients had not taken any antibiotics. About 5-10 ml of midstream specimens of urine was collected. The specimens were cultured on blood agar (Merck, Germany) for colony counting, and were then cultured in sodium azide broth and sodium azide agar (Sigma Aldrich, USA). After incubation, the bacteria were subjected to gram staining and catalase test. Gram-positive, catalase-negative *Enterococci* were further studied using standard biochemical tests including L-Pyrrolidonyl-beta-naphthylamide hydrolysis test, culture in tryptic soy broth (TSB) containing 6.5% salt, and Esculin hydrolysis test in the presence of bile and telluride 0.04%.

Antibiotic susceptibility testing was performed by the agar disk diffusion (Kirby-Bauer**)** method using ciprofloxacin (5 µg), norfloxacin (10 µg) and levofloxacin (5 µg) disks purchased from Mast Co., UK. In this test, a bacterial suspension equal to 0.5 McFarland standard was prepared and cultured in Müller-Hinton agar (Merck, Germany). After placing the antibiotic disks, plates were incubated for 16-18 h at 37 °C. Diameter of inhibition zone around the disks was measured and recorded in millimeters. The results were interpreted as resistant, susceptible and intermediate based on the CLSI M100-S25 (2015) criteria (9).

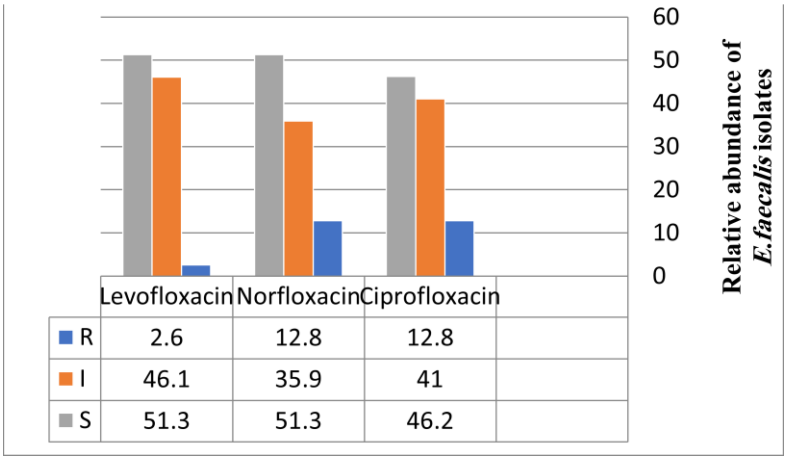
In order to determine minimum inhibitory concentration (MIC) of the antibiotics, all resistant strains were further investigated using the broth microdilution test. Stock solutions of ciprofloxacin (Sigma Aldrich, USA) in water and norfloxacin and levofloxacin (Sigma Aldrich, USA) in water with 0.1 mol/L NaOH were prepared. Next, serial dilutions were made and added to a 96-well microplate containing Müller-Hinton broth (Merck, Germany). Bacterial suspensions with turbidity of 0.5 McFarland standard were inoculated in the wells. The plate was incubated at 37 °C for 16 to 20 h. Wells containing the drug and broth were considered as the negative control, while those containing the broth and bacterial suspension were considered as the positive control. Minimum concentration of the antibiotic that inhibited growth of 90% of isolates was regarded as the MIC_{90}. The plates were placed in an ELISA reader to confirm results of the optical turbidity measurement. According to the CLSI-M100-S25 standard (9), the strains with MIC≤1 µg/ml were identified as susceptible, the ones with MIC=2 µg/ml as intermediate, and the ones with MIC≥4 µg/ml were identified as resistant to ciprofloxacin; the strains with MIC≤2 µg/ml were identified as susceptible, the ones with MIC=4 µg/ml as

intermediate, and the ones with MIC≥8 µg/ml were identified as resistant to levofloxacin; the strains with MIC≤4 µg/ml were identified as susceptible the ones with MIC=8 µg/ml as intermediate, and the ones with MIC≥16 µg/ml were regarded as resistant to norfloxacin (9). To determine the minimum bactericidal concentration (MBC), 100 µl of the wells' content with a concentration higher than the MIC value and 100 µl of the positive control were separately transferred onto Müller-Hinton agar and incubated at 37 °C for 24 h. *E. faecalis* ATCC29212 was used as the control strain. Data were analyzed using the chi-square test and one-way ANOVA in SPSS software (version 18). A p-value of less than 0.01 was considered as statistically significant.

RESULTS

Of 164 isolates from patients with symptoms of prostatitis, 39 (23.8%) were identified as *E. faecalis*. According to the results of the disk diffusion method, frequency of resistance to ciprofloxacin, norfloxacin and levofloxacin was 12.8%, 12.8% and 2.6%, respectively (Figure 1).

Figure 1– Frequency distribution of drug resistance among *E. faecalis* isolates. R: resistant; I: intermediate; S: susceptible.



The MIC_{90} of ciprofloxacin against the isolates was 4 µg/ml, which was 4-fold lower than that of norfloxacin (MIC_{90}=16µg/ml) and 2-fold l ower than that of levofloxacin (MIC_{90}=8µg/ml). Concentration of 8 µg/ml inhibited the growth of all isolates except Those resistant to norfloxacin (32 µg/ml) and levofloxacin (16 µg/ml) (Table 1). As shown in table 2, *E. faecalis* isolates did not differ significantly in terms of resistance to the fluoroquinolones.

Table 1- MIC and MBC values of fluoroquinolones against *E. faecalis* isolates

Strains	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	MBC (µg/ml)	Concentrations range (µg/ml)	Antibiotics
E. faecalis	2	4	8	16-0.03	Ciprofloxacin
E. faecalis	4	16	32	64-0.125	Norfloxacin
E. faecalis	4	8	16	32-0.06	Levofloxacin

Table 2- Comparison of growth of *E. faecalis* strains under equal concentrations of fluoroquinolones

Concentration (8 µg/ml)	Strains (1.5×10^8 cfu/ml)	No Growth		Growth		Comparison
		Number	Percent	Number	Percent	
Ciprofloxacin	*E. faecalis*	39	100	0	0	X^2=0 NS
Norfloxacin	*E. faecalis*	35	89.7	4	10.3	X^2=0 NS
Levofloxacin	*E. faecalis*	36	92.3	3	7.7	X^2=0 NS

NS: No significant difference in terms of drug resistance based on one-way ANOVA

DISCUSSION

E. faecalis is a gram-positive, commensal bacterium in the gastrointestinal tract of humans that can cause UTI and BP in hospitalized patients (7). Nosocomial infections caused by *E. faecalis* and regular use of antibiotics for preventing recurrence have resulted in the emergence of resistant strains, which complicates the treatment process (10). A 4 to 12 weeks treatment with fluoroquinolones including ciprofloxacin, levofloxacin and norfloxacin has been recommended for eradication of bacteria and treatment of BP (11, 12).

In present study, the frequency of *E. faecalis* strains was 23.8% in patients with symptoms of BP. In a previous study in Iran, of 339 *Enterococcus* strains isolated from patients in the Labbafinejad and Shahid Chamran hospitals, 273 (77.5%) were identified as *E. faecalis* (13). In Hamedan (Iran), the frequency of *E. faecalis* was 62.5% (14). In another survey, 92.3% of *Enterococcus* strains were identified as *E. faecalis* (15). Researchers detected *E. faecalis* in 79% of the samples taken from patients, of which 29% were ciprofloxacin resistant (16). The disparity between the results could be attributed to geographical factors and difference in health standards and conditions of the patients. In the present study, of 39 *E. faecalis* isolates, 12.8% showed resistance to ciprofloxacin, which is higher than the rates observed in previous studied (7, 13). Norfloxacin and levofloxacin are effective for treatment of BP caused by *E. faecalis, Escherichia coli* and *Proteus mirabilis* (12,17,18).

In a study in western Iran, the frequency of resistance to ciprofloxacin and levofloxacin was reported to be 68% in gram-negative isolates (19), which is higher than the rate observed in our study. Moreover, the frequency of resistance of gram-negative isolates to these two antibiotics in Canada and Syria was 79% and 68%, respectively (20, 21). This disparity between the findings could be related to the difference in bacteria strains, sources of antibiotic resistance and geographical factors.

In this study, the frequency of ciprofloxacin- and norfloxacin-resistant *E. faecalis* isolates was identical and similar to results of Yumi and Gilho (7).

In our study, 56.4% of the strains were susceptible to levofloxacin, and only one strain (2.6%) was levofloxacin resistant. This finding suggests that levofloxacin may be a more effective agent for treatment of BP caused by *E. faecalis*.

CONCLUSION

Our results reveal that *E. faecalis* is one of the most common causes of BP, and fluoroquinolones are still effective for treating the infection despite the reports of fluoroquinolones resistance in Iran. Results of the antibiotic susceptibility testing indicate that levofloxacin may be a more suitable and effective antibiotic than ciprofloxacin and norfloxacin for treatment of BP.

It is recommended to conduct annual screening programs for men, especially those over the age of 50 years and with symptoms of prostatitis, to help reduce prevalence of BP and UTI.

ACKNOWLEDGMENTS

This study has been supported by the Research Council of the Islamic Azad University of Gorgan, Iran. We wish to thank the staff of hospitals in the Golestan Province for cooperating in the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this study.

REFERENCES

1. Goossens H, Jabes D, Rossi R, Lammens C, Privitera G, Courvalin P . *European survey of vancomycin-resistant enterococci in at-risk hospital wards and in vitro susceptibility testing of ramoplanin against these isolates*. J Antimicrob Chemother. 2003; 51(3): 5-12.

2. Askarian M, Gooran NR. *National nosocomial infection surveillance system-based study in iran: Additional hospital stay attributable to nosocomial infections*. Am J Infect control .2003; 31(8): 465-8.

3. Maria DA, Citron DM, K wok R. *Evaluation of the velogene genomic assay for detection of vanA and vanB gene in vancomycin resistant Enterococcus Species*. J Clin Microbial. 2004; 42(4): 1751-52. doi: 10.1128/JCM.42.4.1751-1752.2004.

4. Murray BE. *Vancomycin resistant Enterococcal infections*. New Eng J Med. 2000; 342(10): 710-21.

5. Jawetz E, et al. *Medical microbiology*. 26 th ed. New York; Toronto; MCGraw Hill Medical. 2013; 209-40.

6. Walker TS. *Microbiology*. philadelphia. W.B. 2003; Saunders Company.

7. Yumi Seo, Gilho Lee. *Antimicrobiol Resistance Pattern in Enterococcus Faecalis Strains Isolated From Expressed Prostatic Secretions of Patients with chronic Bacterial Prostatitis*. Korean J Urol. 2013; 54(7): 477-81.

8. Dennis Kasper, Anthony Fauci, Stephen Hauser. *Harrisons Principles Of Internal Medicine.* 19th ed. 2015; 340-43.

9. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial 324 susceptibility testing; Twenty-Fifth Informational Supplement M100-S25. 325. 2015:Clinical and Laboratory Standards Institute. Wayne, PA.

10. Lee G .*Ciprofloxacin Resistance in Enterococcus faecalis Strains Isolated From Male Patients With Complicated Urinary Tract Infection*. Korean J Urol. 2013; 54(6): 388-93. doi: 10.4111/kju.2013.54.6.388.

11. Dan M, Golomb J, Gorea A, Braf Z, Berger SA. *Concentration of ciprofloxacin in human prostatic tissue after oral administration.* Antimicrob Agents Chemother. 1986; 30(1): 88-89.

12. Jang WH, Yoo DH, Park SW. *Prevalence of and risk factors for levofloxacin-resistant E. coli isolated from outpatients with urinary tract infection*. Korean J Urol. 2011; 52(8): 554-9.

13. FeizAbadi, M.,Asadi S . *Study of Antibiotic Resistance Pattern of Enterococcus faecalis and Enterococcus faecium Strains in Labafinezhad and Shahidchamran Hospitals*.Pajoohandeh J. 2004; 6:333-39.

14. Najafi Mosleh M, Nasaj M, Rahimi F, Arabestani MR. *Distribution Rates and Antibiotic Resistance Pattern of Enterococcus Spp. Isolated from Clinical Specimens of Hospitals in Hamedan*. J Mazandaran Univ Med Sci. 2014; 24(117): 92-102.

15. Firoozeh F, Akha M, Oskoii, Antibiotic M. *Resistance in Enterococcus Strains Isolated from Clinical Specimens*.Tabriz University J .2010;1:61-7.

16. Hällgren A, Abednazari H, Ekdahl C, Hanberger H, Nilsson M, Samuelsson A, et al. *Antimicrobial susceptibility patterns of Enterococci in intensive care units in Sweden evaluated by different MIC breakpoint systems*. Journal of Antimicrobial Chemotherapy. 2001; 48(1): 53-62.

17. Naber KG, Busch W, Focht J. *Ciprofloxacin in the treatment of chronic bacterial prostatitis: a prospective, non-comparative multicentre clinical trial with long-term follow-up. The German Prostatitis Study Group*. Int J Antimicrob Agents. 2000; 14(2): 143-9.

18. Panagopoulos P, Antoniadou A, Kanellakopoulou K, Tsiodras S, Katsarolis I, Papadopoulos A, et al. *Fluoroquinolone treatment of chronic bacterial prostatitis: a prospective cohort study*. J Chemother. 2009; 21(3): 317-21.

19. Lorestani R, Akya A, Elahi A. *The Mutations of Topoisomerase Genes and Their Effect on Resistance to Fluoroquinolones in Extended-Spectrumβ-Lactamase-Producing Escherichia coli*. Jundishapur J Nat Pharm Prod. 2018; 13(1): 1-6.

20. Lagace-Wiens PR, Nichol KA, Nicolle LE, Decorby MR, McCracken M, Alfa MJ, et al. *ESBL genotypes in fluoroquinolone-resistant and fluoroquinolone-susceptible ESBL-producing Escherichia coli urinary isolates in Manitoba*.Can J Infect Dis Med Microbiol. 2007; 18(2): 133-7.

21. Alheib O, Al Kayali R, Abajy MY. *Prevalence of plasmid-mediated quinolone resistance (PMQR) determinants among extended spectrum beta-lactamases (ESBL)-producing isolates of escherichia coli and klebsiella pneumoniae in Aleppo, Syria*. Arch Clin Infect Dis. 2015;10(3): e20631.