ORIGINAL RESEARCH PAPER

Volume-8 | Issue-10 | October - 2019 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *ENTEROCOCCUS* SPECIES ISOLATED FROM URINE SAMPLES FROM A TERTIARY CARE HOSPITAL

Microbiology

Agrawal Neha JS Microbiology DH Chittorgarh

Goyal Lokendra* JS Otolaryngology DH Chittorgarh *Corresponding Author

Bachhiwal Rekha Professor Microbiology SMS Medical College, Jaipur

ABSTRACT

Enterococcus species have emerged as very important pathogens causing urinary tract infections and multi drug resistance among *Enterococci* further add to the problem. A total of 105 *Enterococci* were isolated from urine samples. Speciation was done according to Facklams and Collins conventional method. Antimicrobial susceptibility pattern was determined by Kirby Bauer disc diffusion method. Among 105 Enterococcal isolates, *E. faecium* was predominant species 71(67.62%) over *E. faecalis* 34(32.38%). There was alarmingly high resistance to commonly used antimicrobial agents including quinolones (88-93%), ampicillin(71.4%) and high level gentamicin(62.8%). *E. faecium* was found to be more resistant than *E. faecalis* ($p < 0.05$).Considering increasing isolation of multi drug resistant *Enterococci* from urine samples there is a need to carry-out regular surveillance of antimicrobial resistance of *Enterococci* to recommend appropriate therapy.

KEYWORDS

Enterococci, speciation, vancomycin resistant enterococci(VRE)

INTRODUCTION

Enter ococci are facultative anaerobic Gram-positive cocci, which are part of the resident flora of the gastrointestinal tract of humans and animals. In spite of their weak virulence, these microorganisms may be responsible for a variety of community and hospital acquired infections such as urinary tract infections(UTI), endocarditis, bacteraemia, meningitis and are associated with intra-abdominal infections.[1]

Progress in medical technology and intensive use of broad spectrum antibiotics in the hospitals has been responsible for emergence of these organisms as important nosocomial pathogens.The risk factors for enterococcal UTI include old age, diminished host immunity, the use of broad-spectrum antibiotics and indwelling catheters.[2]

The species of the greatest clinical importance are *Enterococcus faecalis* and *Enterococcus faecium*.[3]*Enterococcus faecalis* and *Enterococcus faecium* are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. Other enterococcal species known to cause human infection include *Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus, and Enterococcus mundtii.*[4]

Enterococcus faecalis is a frequent cause of hospital acquired urinary tract infection and is being increasingly recognized as a cause of community acquired urinary tract infection. Undiagnosed and untreated enterococcal UTI is a well-known source of fatal enterococcal bacteraemia & endocarditis especially in nosocomial set up.[5]

Enterococci are considered important difficult- to- treat pathogens, due to their intrinsic resistance to several antimicrobial agents that most commonly include β lactams and aminoglycosides and also their propensity to acquire resistance.[6]

Enterococci often acquire antibiotic resistance through exchange of resistance-encoding genes carried on conjugative transposons, pheromone-responsive plasmids, and other broad-host-range plasmids. The past two decades have witnessed the rapid emergence of MDR enterococci.[7]

The largest threats are strains resistant to glycopeptides (vancomycin-resistant Enterococcus, VRE) and high-level aminoglycoside resistance (HLAR). UTIs caused by *Enterococcus* species has become challenging given the presence of underlying comorbidities in these patients and the limited therapeutic options available to treat multidrug-resistant (MDR) *Enterococcus*.[8]

MATERIALAND METHOD

Present study was carried out on 105 *Enterococcus spp* isolated from urine samples submitted to bacteriology section of Microbiology department of SMS Medical College, Jaipur (Rajasthan) from October 2017 to September 2018.

After gross and microscopic examination of urine samples were cultured by semi quantitative method with an inoculating loop (standard loop) delivering 0.001ml of urine. Culture media used were Blood agar and MacConkey agar as per the standard laboratory protocol. The inoculated media were incubated aerobically at 37°C for 18-24 hours.[9,10] Isolates with growth of $\geq 10^5$ CFU/ml were included in the study.

Isolates were identified by using standard tests like checking the colony morphology, gram staining, the catalase test, the bile esculin hydrolysis test, the salt tolerance test and the α- pyrrolidonyl β-naphthylamide test (PYR test) [9,10,11]. Their speciation was done on the basis of the sugar fermentation test (Facklam and Collin) [12], their ability to ferment pyruvate , their arginine hydrolyzing property and their motility.

All the isolates were subjected to Antimicrobial susceptibility testing by Kirby Bauer disc diffusion test .[13] The isolates were tested for antimicrobial resistance against following antimicrobials using the Disc Diffusion Method as per the recommendations of CLSI (2018).[13]Ampicillin(10µg),Ciprofloxacin(5µg), Doxycycline(30µg), High Level Gentamicin(120µg), Linezolid(30µg), Nitrofurantoin(300µg), Teicoplanin(30µg) Vancomycin(30µg), Norfloxacin(10µg), Levofloxacin(5µg), Fosfomycin(200µg). Zone diameters were interpreted according to the standard guidelines. All VRE strains were further tested by Vancomycin screen agar.[9]

OBSERVATION AND RESULTS

The present study consisted of 105 enterococcal isolates recovered from urine samples.

Enterococcal isolates were more from in-patients (76%) than out-patients (24%). Among in-patients (ward/ICU) isolates, 37(46.24%) isolates were from catheterized patients and patients underwent urological manipulation. The *Enterococci* were recovered most commonly from samples obtained from age group 21-40 years (30.47%).Among the enterococcal isolates 54(51.42%) were from females and 51(48.57%) were from males.

E. faecium was predominant species 71(67.62%) over *E. faecalis* 34(32.38%).

Table no. 1 Species wise distribution of Enterococcal isolates

Species	Number	Percentage
E. faecalis	34	32.38%
E. faecium	71	67.62%
Total	105	100%

Figure no. 1

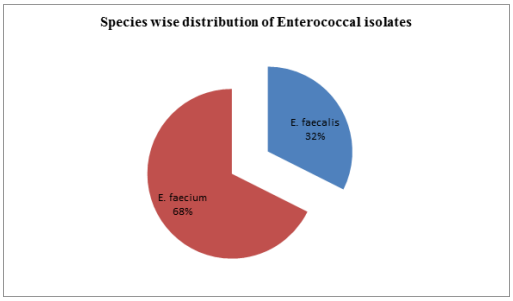


Table no. 2 Antimicrobial Sensitivity Pattern of *Enterococci* isolated from urine

Antimicrobial	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
Ampicillin	30	28.57%	75	71.42%
Norfloxacin	7	6.66%	98	93.33%
Levofloxacin	12	11.42%	93	88.57%
Ciprofloxacin	8	7.61%	97	92.38%
High level Gentamicin	39	37.14%	66	62.85%
Nitrofurantoin	77	73.33%	28	26.66%
Teicoplanin	76	72.38%	29	27.61%
Vancomycin	88	83.80%	17	16.19%
Doxycyclin	51	48.57%	54	51.42%
Linezolid	98	93.33%	7	6.66%
Fosfomycin	51	48.57%	54	51.42%

Chi-square = 407.444 with 10 degrees of freedom; P <0.001S

Table no.2 elicits that *Enterococci* isolated from urine samples showed highest resistance to fluoroquinolones (88% to 94%) and least resistance to linezolid (6.66%). Resistance for linezolid was significantly lower as compared to rest all antimicrobials tested (17% to 88%) (P<0.001). Vancomycin resistant enterococci (VRE) strains were found in 16.19% of cases while 62.85% enterococcal isolates were high level gentamicin resistant (HLGR).

Figure no. 2

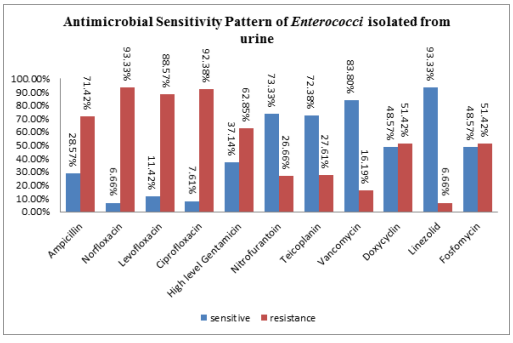
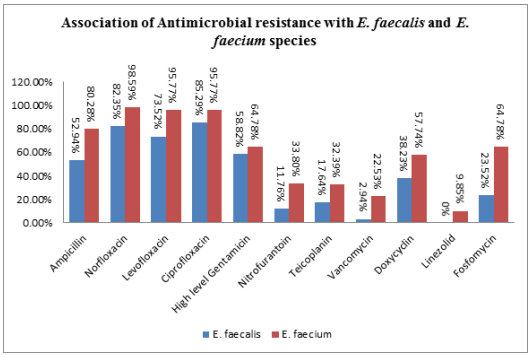


Table no. 3 Association of antimicrobial resistance with *E. faecalis* and *E. faecium* species

Antimicrobial	*E. faecalis* (n=34) resistance pattern		*E. faecium* (n=71) Resistance pattern		P value LS
	number	Percentage	number	percentage	
Ampicillin	18	52.94%	57	80.28%	0.008S
Norfloxacin	28	82.35%	70	98.59%	0.007S
Levofloxacin	25	73.52%	68	95.77%	0.002S
Ciprofloxacin	29	85.29%	68	95.77%	0.133NS
High level Gentamicin	20	58.82%	46	64.78%	0.707NS
Nitrofurantoin	4	11.76%	24	33.80%	0.031S
Teicoplanin	6	17.64%	23	32.39%	0.178NS
Vancomycin	1	2.94%	16	22.53%	0.0233S
Doxycyclin	13	38.23%	41	57.74%	0.096NS
Linezolid	0	0%	7	9.85%	0.140NS
Fosfomycin	8	23.52%	46	64.78%	<0.001S

This table shows the association of antimicrobial resistance with bacterial species. Resistance to vancomycin (VRE) was found to be statistically significant in *E. faecium* than *E. faecalis* (p<0.05). Apart from vancomycin, ampicillin, nitrofurantoin, levofloxacin, fosfomycin and norfloxacin also showed significantly more resistance in *E. faecium* as compared to *E. faecalis* (P<0.05).

Figure no. 3



DISCUSSION

Enterococci are primarily opportunistic pathogens. Progresses in medical interventions and intensive use of broad spectrum antimicrobials in hospitals have been responsible for emergence of *Enterococci* as important nosocomial pathogens particularly as urinary pathogens. So it is important to know the changing trends of *Enterococcus* infections and their antimicrobial susceptibility pattern. In our study, we found a higher rate of isolation of *Enterococci* from in-patients 80 (76.19%) than out-patients 25 (23.80%). Similar data has been obtained by various other researchers.[14,16,17]

In our study most common species recovered was *E. faecium* 71(67.62%) followed by *E. faecalis* 34(32.38%). Our study is consistent with the Karmarkar MG et al[21], who also reported *E. faecium* as predominant species from urine samples.

There was alarmingly high resistance for *Enterococci* isolated from urine samples to commonly used antimicrobial agents including quinolones (88% -93%) and penicillin (71.4%), followed by resistance to doxycyclin & fosfomycin (51.42% each), teicoplanin (27.61%) and nitrofurantoin (26.66%). Least resistance was seen with linezolid (6.66%).Resistance to HLG and vancomycin was found to be (62.85%) and (16.19%) respectively. Results were found to be similar in various other studies.[14-19]

VRE strains were more common in *E. faecium* species as compared to *E. faecalis* and this difference was found to be statistically significant (p value= 0.0233). Apart from vancomycin other antimicrobial agents including ampicillin, nitrofurantoin, levofloxacin, fosfomycin and norfloxacin were also found to be more resistant in *E. faecium* than *E. faecalis* and these findings were also statistically significant (p value <0.05) Studies from various part of India also reported *E. faecium* as more resistant than *E. faecalis*.[16,18-20]

CONCLUSION

It is evident from the present study that enterococci particularly *E. faecium* species is emerging as a common isolates from urine among hospitalized patients. Further the emergence of vancomycin resistance, in addition to the increasing incidence of high-level resistance to aminoglycosides and penicillin presents a serious challenge for physicians treating patients with infection due to these microorganisms. This call for prudent use of vancomycin and early detection of patients colonized or infected with VRE.

There is an urgent need to develop and strengthen antimicrobial policy, standard treatment guidelines to prevent the spread of these organisms and to prevent potential risk of therapeutic failure.

REFERENCES

1. Bourdon N1, Fines-Guyon M, Thiolet JM, Maugat S, Coignard B, Leclercq R, Cattoir V. Changing trends in vancomycin-resistant enterococci in French hospitals. 2011;66(4):713-21
2. Ali S, Mirza IA, Yaqoob S, Hussain A, Khan I, Rafiq MY. Antimicrobial susceptibility pattern of Enterococcus species isolated from patients with urinary tract infection. Gomal J Med Sci 2014;12:11-4.
3. Kristich C, Wells C and Dunny G.A eukaryotic-type Ser/Thr kinase inEnterococcus faecalismediates antimicrobial resistance and intestinal persistence. Pnas. 2007;104(9):3508-3513
4. Fraser L, Brusch L. 17/03/2016. Drugs & Diseases-Infectious Diseases Enterococcal Infections.

5. Bose M, Chatterjee S S, Mukherjee K, Das S, Ghosh C, Chakraborty B, ChaterjeeM,et al.Enterococcal urinary tract infection. an emerging threat. Journal of Evolution of Medical and Dental Sciences. 2015;4(17):2898-2904. DOI: 10.14260/jemds/2015/419
6. Saraswathy MP. Multidrug resistant Enterococci isolated from urine samples at a tertiary care hospital.Indian J Microbiol Res 2015;2(4):214-219
7. Mark M. Huycke, Daniel F. Sahm, and Michael S. Gilmore.Synopsis Multiple-Drug Resistant Enterococci: The Nature of the Problem and an Agenda for the Future.1998;4(2)
8. Treatment of Resistant Enterococcal Urinary Tract Infections. Current Infectious Disease Reports 2010;12(6):455–464.
9. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. The gram positive cocci. Part II: Streptococci, Enterococci and the Streptococcus-like bacteria. In Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 5th Ed.
10. Ross PW. Streptococci and Enterococci. Mackie and Mccartney's Practical Medical Microbiology, 14th edition. Elsievier, 2013;268-69.
11. Forbes BA,Sahm DF, Weissfeld AS. Overview of Bacterial Identification Methods and Strategies. Bailey and Scott 's Diagnostic Microbiology,12th edition. Mosby, 2007;216-47.
12. Facklam RR. Recognition of the Group D Streptococcal species of human origin by doing biochemical and physiological tests. J Appl Microbiol 1972;23(6):1131-39.
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
14. Ali S, Mirza IA, Yaqoob S, Hussain A, Khan I, Rafiq MY. Antimicrobial susceptibility pattern of Enterococcus species isolated from patients with urinary tract infection. Gomal J Med Sci 2014;12:11-4.
15. Saraswathy MP. Multidrug resistant Enterococci isolated from urine samples at a tertiary care hospital. Indian J Microbiol Res 2015;2(4):214-219
16. Parameswarappa J, Basavaraj VP, Basavaraj CM. Isolation, identification, and antibiogram of Enterococci isolated from patients with urinary tract infection. Ann Afr Med.2013;12:176-81.
17. Naik BT, Lavanya J, Mane V, Upadhya KA.Enterococci speciation and antibiogram: an assessment of data at a tertiary care hospital in Karnataka. Indian J Microbiol Res 2016;3(2):130-135
18. Akther J, Ahmed S, Anwar A. Antimicrobial Susceptibility Patterns of Enterococcus species Isolated from Urinary Specimens. Bangladesh Journal of Medical Microbiology 2014; 08(01):16-20.
19. Kanthishree B. Haritsa, Shashikala N, Sunil Kumar D. Chavan, Sangeetha S. "Isolation, Identification and Speciation of Enterococci and their Antimicrobial Susceptibility in a Tertiary Care Hospital". Journal of Evolution of Medical and Dental Sciences 2014;3(63):13893-13899 DOI: 10.14260/jemds/2014/3857
20. Goel V, Kumar D, Kumar R, Mathur P, Singh S. Community acquired enterococcal urinary tract infections and antibiotic resistance profile in North India. J Lab Physicians 2016;8:50-4.
21. Karmarkar MG, Gershom ES and Mehta PR. Enterococcal infections with special reference to phenotypic characterization & drug resistance. Indian J Med Res. 2004;119:22-25