STUDY ON ENTEROCOCCUS ISOLATES FROM DIFFERENT CLINICAL SAMPLES AND THEIR MICROBIAL SENSITIVITY TESTS IN A TERTIARY CARE HOSPITAL, NEPAL

Kumari Ragani Yadav[1], Brajesh Kumar Jha[2]*

Affiliation

1. *Lecturer, Department of Microbiology, Nobel Medical College Teaching Hospital, Biratnagar. Nepal, Nepal.*
2. *Associate Professor Department of Microbiology, College of Medical Sciences, Bharatpur. Nepal, Nepal.*

ARTICLE INFO

Received : 04 December, 2020

Accepted : 08 April, 2021

Published : 15 June, 2021

ORA 226

DOI: https://doi.org/10.3126/bjhs.v6i1.37566

**** Corresponding Author***

Dr. Kumari Ragani Yadav

Lecturer

Department of Microbiology

Nobel Medical College and Teaching Hospital

Email ID: raginiy385@gmail.com

ORCID ID: https://orcid.org/0000-0003-0038-473X

Citation

Kumari Ragani Yadav, Brajesh Kumar Jha. Study on Enterococcus Isolates from Different clinical samples and their Microbial Sensitivity Tests in a Tertiary Care Hospital, Nepal. BJHS 2021;6(1)14. 1315 - 1319.

ABSTRACT

Introduction

Enterococcus is as an important nosocomial pathogen due to its intrinsic and acquired resistance to antibiotics. The most common infection caused by *Enterococcus* is urinary tract infection followed by vaginal, abdominal infection, bacteremia, endocarditis and meningitis respectively. The treatment for enterococcal infections has become challenging due to development of resistance to most commonly used antibiotics.

Objective

The objective of present study was to isolate, identify, speciation of the different species of *Enterococcus* and determine their antimicrobial susceptibility pattern.

Methodology

This descriptive cross-sectional study was conducted in the department of Microbiology at Nobel Medical College Teaching Hospital, Biratnagar, Nepal with effect from July 2019 to July 2020. Identification and speciation of *Enterococcal* isolates were done by using standard microbiological guidelines. Antimicrobial susceptibility pattern were carried out according to Kirby-Bauer disc diffusion method as per the Clinical and Laboratory Standard Institute (CLSI) guidelines.

Results

Out of total 5199 different clinical samples, 2719(52.2%) samples showed microbial growth. *Enterococcus* was isolated from 136(2.56%) samples while rest of the samples 2583(49.6%) showed the growth of microorganism other than *Enterococcus*. Majority of *Enterococcus* species (51%) were isolated from the urine sample. The infection rate was higher in female as compared to male (3.85:1) and among the age group 20-49 years. *E. faecalis* was the most common species isolated. All the species of *Enterococcus* (*E. faecalis, E. faecium, E. durans, E. raffinosus* and *E. gallinarum*) were sensitive to linezolid, teicoplanin, vancomycin and nitrofuratoin.

Conclusion

In our study the most common species isolated from most of the clinical sample was *E. feacalis* and majority of the *Enterococcus* spp. were resistance to penicillin, norfloxacin and erythromycin. This presents a serious challenge for physicians treating the infection caused by *Enterococcus* species. Hence the rational use of antibiotic should be practiced based upon local epidemiological data on antibiotic susceptibility pattern.

KEYWORDS

Antibiogram, enterococcus, nosocomial infection

INTRODUCTION

Some species of the genus, *Enterococcus* are important pathogens of health care-associated infections(HAIs) globally causing urinary tract infection, soft tissue and device-associated infections.[1] *Enterococcus faecalis* (85-90%) and *Enterococcus faecium* (5-10%) are normal flora of the gastrointestinal tract of human and animal.[2] Now, *Enterococcus* spp. have emerged as medically important pathogens and are associated with both community-acquired and nosocomial infection. According to the CDC survey of nosocomial infection *Enterococci* account for 13.9% of hospital acquired UTI's and 2nd/3rd most common bacteria to cause nosocomial wound infection/ nosocomialbacteremia.[3] *Enterococci* are intrinsically resistant to cephalosporins and low level resistance to aminoglycosides. So, in penicillin sensitive strain, synergism occurs with combination treatment with penicillin and aminoglycosides.[3,4,5] The resistance patterns are more in *E. faecium* than *E. faecalis* posing a therapeutic challenge. Vancomycin resistant enterococcus (VRE) is mediated by *Van* gene and it has 5 genotypes: *VanA* to *VanE*. Strains with *VanA* gene show high level resistance to both glycopeptides vancomycin and teicoplanin, Strains with *VanB* gene show low level resistance to vancomycin, but sensitive to teicoplanin and *E. gallinarum* and *E. casseliflavus* possess *VanC* genes and they show intrinsic resistance to both the glycopeptides. *VanA* type takes the lead globally and imparts a high level of resistance to glycopeptide antibiotics seen particularly in vancomycin-resistant *E. faecium*. Genotype *VanA* is prevalent in *E. faecium* while *VanB* is prevalent in *E.faecalis*.[6] Recently; they are gaining high attention due to ability to withstand the effect of multiple antimicrobial agents, consequently limiting the treatment options and resulting in high morbidity and mortality. Tracking the route of enterococcal infection, antimicrobial susceptibility testing and antibiotic knowledge is important to formulate therapeutic challenge for treating the infection to prevent the spread of infection caused by multidrug resistant *Enterococcus*.[7] The aim of this study was to isolate, identify and characterize the different species of *Enterococcus* and determine their antimicrobial susceptibility pattern in Nobel Medical College Teaching Hospital, Biratnagar, Nepal.

METHODOLOGY

This descriptive cross-sectional study was conducted in the department of Microbiology at Nobel Medical College Teaching Hospital, Biratnagar, Nepal with effect from July 2019 to July2020 after approval from the Institutional Review Committee (*Ref: IRC*- NMCTH 338/2019) of the college. Convenient sampling was done and sample size (n) was calculated as:

$$n = z^2pq/e2$$

$$n = (1.96)^2 \times 0.4 \times 0.6/(0.1)^2 = 92.16$$

Where, Z = 1.96 at 95% confidence interval
n = sample size
p = prevalence[24], 40%
q = 100-p=100-40=60
e = margin error, 10%

Taking a 10% non-respondent rate, the sample size becomes 102. But the load of patients was high so total of 5199 clinical samples (urine, blood, high vaginal swab, bile, pus, tissue and wound swab) were collected from both inpatients department (IPD) and outpatients department (OPD) attending to hospital. Inclusion criteria for this study were all the age groups. The samples that were received unlabeled, cracked or broken container and samples from respiratory site were excluded. All the clinical samples collected were submitted to microbiological laboratory for culture and sensitivity. The clinical samples were subjected to gram staining first and then inoculated onto blood agar, MacConkey agar, chocolate agar and CLED agar and incubated at 37°c for 18-24 hours. The genus *Enterococcus* was identified on the basis of colony characteristic, gram staining, aesculin hydrolysis, salt tolerance (6.5%NaCl), growth at 45°c and 50°c and pH9.6.[8,9,10] They were further characterized up to species level by using standard test: bile aesculin test, hydrolysis of hippurate, Voges-Proskauer(VP) test, fermentation of mannitol, pyruvate, sorbitol, sucrose, lactose, raffinose, adonitol, L-rahamnose, tellurite, motility test and pigment production.[11-15]

ANTIBIOTIC SUSCEPTIBILITY TESTING (AST)

AST of the isolated strains were carried out according to Kirby-Bauer disc diffusion method as per the Clinical and Laboratory standard Institute(CLSI) guidelines against penicillin(10 units), vancomycin(30µg), teicoplanin(30µg), ciprofloxacin(5µg), linezolid(30µg) high-levelgentamicin (120µg), nitrofurantoin(300µg), norfloxacin(10µg), chloramphenicol(30µg) and erythromycin(15µg). With the help of straight inoculating wire 1-2 well isolated colonies were picked and inoculated into tube containing 5ml peptone water and incubated into the incubator at 37°c for 2-4hr and then compared with the turbidity of 0.5 McFarland standard solutions in adequate light against a card with a white background and contrasting black lines. After the turbidity was maintained, a sterile cotton swab was dipped into the peptone water containing bacterial solution and any excess solution was remove by pressing the swab on the inside wall of the tube. Followed by lawn culture on Mueller Hinton agar and thereafter antimicrobial disc supplied by HiMedia Laboratories, India, were placed onto the surface of inoculated agar plate and plate was incubated into the incubator at 37°c for 18-24 hr. Eventually, the result was read and interpreted. Zone of inhibition measured by using Vernier caliper and result were interpreted on the basis of zone size inhibition as sensitive and resistant.[16] *E. faecalis* ATCC 29212 and *S. aureus* ATCC25923 were used as control strain.

STATISTICAL ANALYSIS

The collected data were analyzed using statistical package for the social science for windows (SPSS) version 20. Parametric variables were assessed using chi-squared test, as appropriate. A difference was considered statistically significant if the p-value <0.05.

RESULTS

In the present study, out of total 5199 different clinical samples, 2719(52.2%) samples showed microbial growth. Among which *Enterococcus* was 136(2.56%) and other microorganisms were 2583(49.6%). The enterococcal infection caused by different species of *Enterococcus* was higher in female 108(79.4%) as compare to male 28(20.6%). Male: female ratio was 1:3.8. The present study showed that most of the patients were from in-patients department's (IPD) 92(67.64%) and 44(32.3%) were from out-patient department's (OPD). The cases of *Enterococcus* infection were higher in IPD as compare to OPD. The enterococcal infection were most commonly found in the age group between 20-49 years as compare to different other age group of patients (Table 1). Among the various clinical samples, urine culture yielded the highest number of *Enterococcus* isolates 69(51%), followed by high vaginal swab (HVS) 34(25%), pus 19(14%), wound swab/blood 5(3%) and bile/ ear discharge 1(1%). Table 2 shows out of 69 urine sample *E. faeacalis* were 57(82.60%), *E. faecium* were 12(17.39%). Whereas, other species of *Enterococcus* were not isolated from the urine sample. Out of total 34 sample of HVS 32(94.11%) were *E. faecalis* and 2(5.88%) were *E. faecium*. Out of 19 pus sample *E. faecalis* was isolated from 16(84.21%) while *E. durans*, *E. gallinarum* and *E. raffinosus* each were isolated from only one sample 1(5.26%).

Table 1: Age wise distribution of patients with enterococcal infections

Age groups (years)	No. of patient	Percentage
≤1	6	4.41
2-14	7	5.15
15-19	7	5.15
20-29	54	39.71
30-39	21	15.44
40-49	19	13.97
50-59	7	5.15
≥60	15	11.03

Table 2: Species distribution of *Enterococcus* in various clinical specimens

Samples	*E. faecalis* (115)	*E faecium* (17)	*E. durans* (2)	*E. gallinarum* (1)	*E raffinosus* (1)	Total
Urine(n= 69)	57	12	0	0	0	69
HVS (n=34)	32	2	0	0	0	34
Placental tissue(n= 2)	1	1	0	0	0	2
Pus (n=19)	16	0	1	1	1	19
Wound swab (n=5)	4	0	1	0	0	5
Blood (n=50	3	2	0	0	0	5
Bile (n=1)	1	0	0	0	0	1
Ear discharge (n=1)	1	0	0	0	0	1
Total	**115 (84.5%)**	**17 (12.5%)**	**2 (1.47%)**	**1 (0.7%)**	**1(0.7%)**	**136(100%)**

Antimicrobial susceptibility of urinary isolates (*E. faecalis* and *E. faecium)*

Present analysis shows, *E. faecalis* and *E. faecium* were sensitive to linezolid, Teicoplanin, vancomycin and nitrofuratoin as compare to other antibiotics. (Table 3)

Table 3: Antibiotic susceptibility pattern of *E. faecalis* and *E. faecium*

Antibiotics	*E. feacalis* (n=57)		*E. faecium* (n=12)	
	Resistance	Sensitive	Resistance	Sensitive
Penicillin	50(87.7%)	7(12.3%)	10(83.3%)	2(16.7%)
High level gentamicin	31(54.4%)	26(45.6%)	8(66.7%)	4(33.3%)
Teicoplanin	4(7.0%)	53(93.0%)	1(8.3%)	11(91.7%)
Linezolid	4(7.0%)	53(93.2%)	0(0.0%)	12(100.0%)
Ciprofloxacin	47(82.2%)	10(17.5%)	9(75.0%)	3(25.0%)
Vancomycin	5(8.8%)	52(91.2%)	2(10.0%)	10(83.3%)
Nitrofuratoin	3(5.3%)	54(94.7%)	1(8.3%)	11(91.7%)
Norfloxacilin	41(71.9%)	16(28.1%)	5(41.7%)	7(58.3%)

Antibiotic susceptibility pattern of *E. faecalis* and *E. faecium* from other clinical samples *(HVS, placental tissue, pus, wound swab, bile, blood and ear discharge)*

As shown in table 4, *E. faecalis* and *E. faecium* showed highest number of sensitivity towards linezolid, teicoplanin, vancomycin, chloramphenicol and high-levelgentamicin.

Table 4: Antibiotic susceptibility pattern of *E. faecalis* and *E. faecium*

Antibiotic	*E. feacalis* (n=58)		*E. faecium* (n=5)	
	Resistance	Sensitive	Resistance	Sensitive
Penicillin	43(74.1%)	15(25.9%)	4(80.9%)	1(20.0%)
High level gentamicin	11(18.6%)	47(81.2%)	1(20.0%)	4(80.0%)
Teicoplanin	1(1.7%)	57(98.2%)	0(0.0%0	5(100.0%)
Chloramphenicol	7(12.1%)	51(87.9%)	1(20.05)	4(80.0%)
Vancomycin	2(3.4%)	56(96.6%)	0(0.0%)	5(100.0%)
Linezolid	0(0.0%)	58(100%)	0(0.0%)	5(100.0%)
Erythromycin	36(62.0%)	22(37.9%)	2(40.0%)	3(60.9%)

Table 5: Antibiotic susceptibility pattern of *E. gallinarum* and *E. raffinosus*

Antibiotic	*E. gallinarum*(n=1)		*E. raffinosus*(n=1)	
	Resistance	Sensitive	Resistance	Sensitive
Penicillin	1(100.0%)	0(0.0%)	1(100.0%)	0(0.0%)
High level gentamicin	0(0.0%)	1(100.0%)	0(0.0%)	1(100.0%)
Teicoplanin	0(0.0%)	1(100.0%)	0(0.0%)	1(100.0%)
Chloramphenicol	0(0.0%)	1(100.0%)	0(0.0%)	1(100.0%)
Vancomycin	1(100.0%)	0(0.0%)	0(0.0%)	1(100.0%)
Linezolid	0(0.0%)	1(100.0%)	0(0.0%)	1(100.0%)
Erythromycin	1(100.0%)	0(0.0%)	1(100.0%)	0(0.0%)

DISCUSSION

In hospital setting, *Enterococcus* has become important nosocomial pathogen and has emerged as one of the leading therapeutic challenges because of the intrinsic as well as acquired antibiotic resistance. The *Enterococcus* species are intrinsically resistance to most common antibiotic like cotrimoxazole, aminoglycosides and cephalosporins being used for treatment of bacterial infection. Whereas, due to the indiscriminate use of antibiotic *Enterococcus* are acquiring resistance towards the chloramphenicol, tetracycline, glycopeptides, quinolones and nitrofuratoin.[17] So the present study was aimed to isolate, identify and speciate of Enterococci followed by their antibiogram from different clinical samples. In our study a total 136 *Enterococcus* isolates were recovered from 5199 different clinical samples over the period of 12 months duration. In this study highest number of *Enterococcus* isolates were obtained from urine sample (51%), followed by HVS (25%),

pus (14%), wound swab/blood (3%) and bile/ear discharge (1%). This was similar to other studies conducted in India.[18,19]

In our study most of the patients were from IPD 92(67.64%) as compared to OPD 44(32.3%). This was similar to study conducted by Acharya A in Dharan Nepal.[20] Maximum numbers of isolates were recovered from the age group 20-45 years with more predominance in female as compared to male which was similar to study by Preeti et al in India.[21] A study conducted by Shanmukhappa et al in India reported that maximum numbers of isolates were from age group >60 years with male predominance, which was quite difference from our study.[18] In present study among the 136 isolated Enterococci, *E faecalis* was the predominant isolate (84.5%) followed by *E faecium*(12.5%), *E durans* (1.7%), *E. gallinarun* (0.7%) and *E. raffinosus* (0.7%). This finding correlates well with study done in Nepal and India.[20,21] Present analysis shows, *E. faecalis* and *E. faecium* were highly sensitive to linezolid, teicoplanin, vancomycin and nitrofuratoin as compare to other antibiotics. Almost all *Enterococcus* isolates were resistance to penicillin and norfloxacillin and erythromycin. This was similar to study done by Agrwal N et al in India.[22] Among the isolated *Enterococci, E. faecalis* has emerged as important pathogens during the past decade and cause of significant morbidity and mortality in hospitalized patients. Species of *Enterococcus* become resistance to most commonly used antimicrobial agents and resistance can be intrinsic or acquired.[23] Further, because of over the counter use of antibiotics without supervision of health care provider and not following instructions and completing the full regimes of antibiotics, enterococci are gaining resistance to different classes of antibiotics. So, knowledge of the antimicrobial susceptibility profile is essential to formulate treatment guidelines for infections caused by *Enterococci.*

CONCLUSIONS

In our study, teicoplanin, linezolid, vancomycin and nitrofuratoin were found to be the most effective antibiotic against *enterococci* isolated from different clinical samples. *E. feacalis* is the most commonly isolated from most of the clinical samples and almost all the species of *Enterococcus* were resistance to penicillin, norfloxacin and erythromycin causing a serious challenge for physicians treating the infection caused by *Enterococcus* species. So, the prevention and control of spread of multidrug resistance *Enterococcus* require prudent use of antimicrobials.

RECOMMENDATIONS

The infection caused by *Enterococcus* has gaining increased more in a hospital setting as NMCTH. Therefore, infection control efforts have been established to limit the spread of this pathogens and regular monitoring is essential to know the species of *Enterococcus* from different types of infection. Characterization and antibiotic susceptibility test are essential to monitor susceptibility pattern of *Enterococcus* species against the drugs.

LIMITATIONS OF THE STUDY

Although utmost sincerity and dedication was invested to carry out the study it could not go beyond some limitations like: The result could not be generalized to other area because the study was conducted in urban population and therefore, it may not apply to whole population of Morang. Molecular studies for detection of various species of *Enterococcus* were not done in this study.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to all the faculties, laboratory staff and post graduates of the department of microbiology and Institutional Review Committee (IRC) of Nobel Medical College Teaching Hospital for helping us in carrying out the present research.

CONFLICTS OF INTERESTS

None

FINANCIAL DISCLOSURE

None

REFERENCES

1. Garcia-Solache M, Rice LB. The *Enterococcus:* a model of adaptability to its environment. Clin Microbiol Rev. 2019 Apr 32(2)1-28. DOI:10.1128/CMR.00058-18
2. Brooks GF, Carroll KC, Butel JS. JawetMelnick and Medical Microbiology, 26th ed. USA: LANGE; 2013.
3. Vandamme P, Vercauteran E, Lammens C, Pansart N, Ievan M. Survay of enterococcal susceptibility pattern in Belgium, J Clin Microbial. 1996 Oct ;34(10):2572-76. PMID: 8880522
4. Goliaet S, Nirmala AR, Asha S, Kamath B. Isolation, and speciation of *Enterococcus* from various clinical samples and their antimicrobial susceptibility pattern with special reference to high level aminoglycoside resistance. Int J Med Res Health Sci. 2014 Sep 29;3(3):526-29. DOI: 10.5958/2319-5886.2014.00390.7
5. Koneman ED, Allen SD, Janda WM, Schreckenbeger PC, Winn WC. Isolation and identification *Streptococci* like organisms "Koneman's color atlas and text book of diagnostic microbiology, 6th ed; Baltimore Lippincott William and wilkins. (2006):726-33.
6. Dineshraj R, Vasanthi R, Gowthami G, Priyadarshini S. Speciation of *Enterococcal* isolates in a tertiary care hospital and molecular characterization of vancomycin resistance *Enterococci* (VRE), Indian J Microbial Res. 2016;3(4):77-81. DOI:10.5958/2394-5478.2016. 00019.4
7. Bhatt P, Patel A, Sahani K. Emergence of multidrug resistance *Enterococci* at a tertiary care Centre. Medical J Armed Forces India. 2015 Apr;71(2):139-44. DOI: 10.1016/j.mjafi.2014.08.007
8. Murray BE. The life and times of *Enterococci.* ClinMicrobiol Rev. 1990 Aug;3(1):46-65. DOI: 10.1128/cmr.3.1.46
9. Desai PJ, Pandit D, Mathur M GogateA.Prevalence, identification and distribution of various species of *Enterococci* isolated from clinical specimens with special reference to urinary tract infection in catheterized patient. Indian J Med Microbiol. 2001 Jul-Sept;19(3):132-7. PMID: 17664815
10. Sahm DF, Frel Smith C, Eveland M, Manday LM. Rapid characterization schemes for surveillance isolate of vancomycin resistance *Enterococci*. J Clin Microbiol1997 Aug;35(8):2026-30. PMID: 9230375 PMCID: PMC229896

11. Burrow GI, Felthham RKA. Characters of gram-positive bacteria, Incowan and steal's manual for the identification of medical bacteria. 3rd ed, Camride university press Great Britain. 1993; 50-93.
12. Holts JG, Kraieg NR, Sneath PHA, Staley JT, Williams ST. Gram positive cocci. In Bregey's Manual of determinative bacteriology. 9th ed, East preston street Baltimore, Maryland 212202 USA, 1994;527-58.
13. Ellen JB, Lance RP, Sydney MF. *Streptococci* and related genera. In bailey and Scott's Diagnostic microbiology, 9th ed, Von Hoffmann Press, USA. 1994:333-52.
14. Alex C, Sonnenwrith LJ. Gram positive and gram negative cocci. In Gradwohl;s clinical laboratory methods and diagnosis. 8th ed, B.I publication limited, New Delhi, 1990:1629-65.
15. Cheesbrough M. Gram technique. In district laboratory practice in tropical countries art 2. Cambrigeuniversity press, United Kingdom, 2000:38-9.
16. Clinical and laboratory standard institute (CLSI), Performance standard for antimicrobial susceptibility testing, Wayne, Pennsylvania USA, 26th ed, 2016.
17. Ali S, Mirza IA, YaqoobS, Hussain A, Khan I, Rafiq MY. Antimicrobial susceptibility pattern of *Enterococcus* species isolated from patients with urinary tract infection. Gomal J of Medical Scie. 2014 Jan;12(1):11-14. DOI: https://doi.org/10.7439/ijbr.v8i6.4182
18. Shanmukhappa, Ajantha GS, Kumari A, Koppad M. Isolation, identification and speciation of *Enterococci* by conventional method and their antibiogram. National J of Laboratory Medicine. 2015 Apr;4(2):1-6. DOI: NJLM/2015/11684:2031
19. Hathiwala R,Bhimrao A, Poornima D, NandkishorJageshwar BN. Antibiogram study of clinical Isolates of *Enterococcus* in a tertiary care teaching hospital. National J of Laboratory Medicine. 2018 Jul;7(3):1-5. DOI: 10.7860/NJLM/2018/36174:2306
20. Acharya A, Khanal B, Kanuugo R, Mohapatra T. Characterization and susceptibility pattern of clinicall important *Enterococcus* species in eastern Nepal. Nepal Medical College J. 2007 Dec 1;9(4):250-45. PMID: 18298014
21. Venkatesan KD, Chander S, Abigail S, Victor K. Antibiotic resistance pattern of *Enterococcal* isolates from patients with urinary tract infection. Int J of Biomedical Research. 2017;8(6):357-60. DOI: https://doi.org/10.7439/ijbr
22. Agrawal N, Goyal L, Bachhiwal. Antimicrobial susceptibility pattern of *Enterococcus* species isolates from urine sample from tertiary care hospital. Int J of scientific Research. 2019;8 (10):1-3. DOI: 10.36106/ ijsr
23. Wade JJ, Uttley AHC. Resistant *Enterococci* mechanism, laboratory detection and control in hospitals. J Clin Pathol. 1996;49(7):700-70. DOI: 10.1136/jcp.49.9.700
24. Karna A, Baral R, Khanal B. Characterization of Clinical Isolates of Enterococci with Special Reference to Glycopeptide Susceptibility at a Tertiary Care Center of Eastern Nepal. Int J of Microbiology. 2019 Jul1;(2019):1-8. DOI: https://doi.org/10.1155/2019/7936156