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

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**ABSTRACT**

**Introduction**

Enterococcus is 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. faecalis and majority of the Enterococcus spp. were resistance to penicillin, norfloxacine 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 2\(^{nd}\)/3\(^{rd}\) most common bacteria to cause nosocomial wound infection/nosocomial bacteremia.\(^3\) 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.\(^4\)\(^\text{-}\)\(^5\) The resistance patterns are more in E. faecium than E. faecalis posing a therapeutic challenge. Vancomycin resistant enterococccus (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 affect 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 July 2020 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:

\[
 n = \frac{z^2 \cdot pq}{e^2} \\
 n = \frac{(1.96)^2 \cdot 0.4 \cdot 0.6}{(0.1)^2} = 92.16
\]

Where, \( z \) = 1.96 at 95% confidence interval
\( n \) = sample size
\( p \) = prevalence\(^6\), 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.\(^5\)\(^\text{-}\)\(^7\) 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-rhamnose, tellurite, motility test and pigment production.\(^6\)\(^\text{-}\)\(^8\)

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-level gentamicin (120µg), nitrofurantoin(300µg), norfloxacin(10µg), chloramphenicol(30µg) and erythromycin(15µg). With the help of standard 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 inside wall of the tube. Using the standard test, 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-patient 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. faecalis 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 (5.26%).

Table 1: Age wise distribution of patients with enterococcal infections

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

Table 2: Species distribution of Enterococcus in various clinical specimens

<table>
<thead>
<tr>
<th>Samples</th>
<th>E. faecalis (133)</th>
<th>E. faecium (17)</th>
<th>E. durans (2)</th>
<th>E. gallinarum (1)</th>
<th>E. raffinosus (1)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine (69)</td>
<td>62</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>HVS (5=34)</td>
<td>32</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Placental tissue (n=2)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pat (n=19)</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Wound swab (n=3)</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Blood (n=10)</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Bile (n=1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ear discharge (n=1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>166 (89.3%)</td>
<td>18 (10.7%)</td>
<td>2 (1.2%)</td>
<td>1 (0.6%)</td>
<td>5 (3.0%)</td>
<td>198</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. faecalis (n=57)</th>
<th>E. faecium (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Penicillin</td>
<td>50 (87.7%)</td>
<td>7 (12.3%)</td>
</tr>
<tr>
<td>High level gentamicin</td>
<td>31 (54.4%)</td>
<td>26 (45.6%)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>4 (7.0%)</td>
<td>53 (93.0%)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>4 (7.0%)</td>
<td>53 (93.2%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>47 (82.2%)</td>
<td>10 (17.5%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>5 (8.8%)</td>
<td>52 (91.2%)</td>
</tr>
<tr>
<td>Nitrofuratoin</td>
<td>3 (5.3%)</td>
<td>54 (94.7%)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>41 (71.9%)</td>
<td>16 (28.1%)</td>
</tr>
</tbody>
</table>

Table 4: 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.

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 resistant 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. 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%),
Enterococcus were found to be the most effective antimicrobials.

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. 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. A study conducted by Shamukhappa et al in India reported that maximum numbers of isolates were from age group >60 years with male predominance, which was quite different from our study. 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 gallinarum (0.7%) and E raffinosus (0.7%). This finding correlates well with study done in Nepal and India. 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 norfloxacin and erythromycin. This was similar to study done by Agrwal N et al in India. 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. 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 faecalis 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 requires 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

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CONFLICTS OF INTERESTS

None

FINANCIAL DISCLOSURE

None

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