ORIGINAL ARTICLE

Antimicrobial Resistance among Faecal Enterococci from the Healthy Individuals in Pokhara, Nepal

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ABSTRACT

Background: Enterococci, normal flora in the gastrointestinal tract of humans and animals. Over the last decade, multi-drug resistant (MDR) Enterococci were subject of matter of interest as it is one of the most common causes of nosocomial as well as community-acquired infection around the world. Thus, this study aimed to determine antimicrobial resistance among Fecal Enterococci from healthy individuals in Pokhara, Nepal.

Methods: A total of 100 fecal samples were collected from a community of different parts of Pokhara and processed. *Enterococcus* isolates were identified phenotypically based on colony morphology, gram staining reaction, biochemical tests and growth on 40% bile-esculin agar. Antimicrobial susceptibility test (AST) was performed as CLSI guidelines and screening for VRE was carried out by vancomycin disc diffusion method.

Results: The distribution of Enterococci was found to be highest in the age group 50-60 years 8 (61.53%) followed by the adult age group 20-30 years 3 (20%) and lowest in the 40-50 age groups (5.13%). Antibiotic susceptibility test revealed that most of the isolates were sensitive to chloramphenicol (53.84%) and resistant to linezolid and erythromycin (79.48%). Out of 39 Enterococci isolates, 71.79% isolates were found to be multi-drug resistance. Among 17 isolates of Enterococci, 7 (17.9%) isolates were vancomycin resistant.

Conclusions: Significant prevalence of Vancomycin resistant *Enterococcus* in healthy individuals indicates the risk of transmitting VRE to the environmental non-pathogenic bacteria through fecal routes which might pose serious public health threats leading to an epidemic and endemic shortly. With regard to the importance of VRE strains in the clinical and environmental settings, it seems necessary to address the issue. Therefore, regularly surveillance and effective control program must be implemented in order to prevent its dissemination. Otherwise serious community acquired infections will appear in near future.

Keywords: MDR, *Enterococcus faecalis*, *Enterococcus faecium*, Antibiotic, Nosocomial

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INTRODUCTION

Enterococci are commensals, found in the gastrointestinal tract of warm-blooded animals, stool, urine, fermented food, dairy products as well an environment that can tolerate a broad range of pH, temperature, and salt concentration.[1] Over the last few decades, Enterococci, known as opportunistic and nosocomial pathogens,[2] has become one of the most common causes of community-acquired infections and hospital-acquired infections with a high mortality rate of up to 61%.[3] *Enterococcus faecalis* contribute 80-90% of enterococcal infection whereas *Enterococcus faecium* 10-20%, but recent studies enlighten that the proportion of infections caused by *Enterococcus faecium* is increasing.[4] Due to the extended and unwise use of antibiotics in poultry farming, animal farming, and agricultural purposes, multi-drug resistance Enterococci has been emerged rapidly developing intrinsic resistance to the many antibiotics.[5] These resistance enterococci can be transmitted to healthy humans through contaminated food, water, and vegetables.[2] Although, vancomycin has been the choice of treatment due to the emergence of resistance to anti-enterococcal antibiotics and other β-lactam antibiotics;[6] a recent study showed the presence of vancomycin-intermediate Enterococci in the hospital setting.[7] All these findings are limited to hospitalized patients, therefore, this study was conducted to understand the antimicrobial resistance pattern of fecal Enterococci isolated from healthy individuals. It is believed that the study will be useful to assist the health sectors (policymakers, doctors, paramedics, academicians) for better understandings, effective control measures, and effective treatment.

MATERIALS AND METHODS

Study design , study site and sample size

This was a descriptive laboratory based cross sectional study conducted between January to March 2021, among 100 healthy people of ≥5 to ≤80 age group in different community of Pokhara valley, Nepal with no history of antibiotic therapy.

The general formula has been used to calculate the sample size. The prevalence of *Enterococcus* species is 6.2%.[2]

The formula for determining sample size will be: n= z^2 p (1-p)/e^2

n= (1.6)^2*0.062*0.938/0.05^2

Sample size (n) =59.~60 (p = prevalence; e = allowable error)

Inclusion and exclusion criteria

Only healthy participant, with no history of antibiotic therapy and provided consent, were enrolled in this study. After routine culture testing, only Gram-negative isolates had been used for further analysis. Contaminated, unlabeled, and mislabeled samples and Individuals who had recent history of antibiotic treatment were excluded.

Sampling technique

Random sampling technique was used during the study.

Sample collection and processing

From January to March 2021, a total of 100 individual sample of fecal was collected randomly from healthy people of ≥5 to ≤80 age group with no history of antibiotic therapy for at least 1 month in the community of Pokhara, Nepal. After taking the written consent from each individual, the stool samples were collected in a sterilized bottle with proper labeling. Then, the collected samples were transported as soon as possible and analyzed at the Microbiology laboratory of Prithivi Narayan Campus, Pokhara accordingly. During the collection, individuals were requested to collect the stool sample in the sterilized bottle provided by the sampler.

Bacterial isolation and identification

As reported previously, each stool sample was inoculated on Mac-Conkey agar and Bile esculin agar with sterile swabs for 24-48 hours at 37 °C under aerobic conditions.[8] After 24 hours of incubation, the agar plate with minute pink colonies of bacterial growth was selected and further isolation was done by sub-culturing on selective media and nutrient agar to obtain a pure culture.

All bacterial isolates were phenotypically identified to the genus level using conventional method as colony morphology, gram staining, growth in 6.5% sodium

chloride broth, biochemical tests, growth on 40% bile esculin agar.

Antimicrobial susceptibility test of isolated organisms

Susceptibility of isolates to the following antibiotics was examined using the Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines: Chloramphenicol (30 µg), Erythromycin (15 µg), Ciprofloxacin (5 µg), Ampicillin (10 µg), Linezolid (30 µg), Vancomycin (30 µg) and Nitrofurantoin (300 µg). The inoculum was prepared by inoculating the organisms into 2 mL of sterile saline (0.9% w/v NaCl) and the turbidity of the suspension was standardized to 0.5 McFarland standards. Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture method. Based on the susceptibility pattern of the isolates, bacterial isolates resistant to >3 classes of antibiotics were considered as multi-drug resistant.[9]

Statistical Analysis

The data were entered in Microsoft excel sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 21.0 software.

RESULTS

Among the total 100 stool samples subjected to laboratory analysis, all samples were found to be heavily contaminated by microbial flora. Discrete pink pinpoint colonies were isolated and identified as *Enterococcus* species characterized by gram-positive, catalase-negative, and growth in 6.5% sodium chloride broth. Out of 100 samples, 39% were found to be growth positive with *Enterococcus* whereas 61 (61%) were growth negative as shown in figure 1.

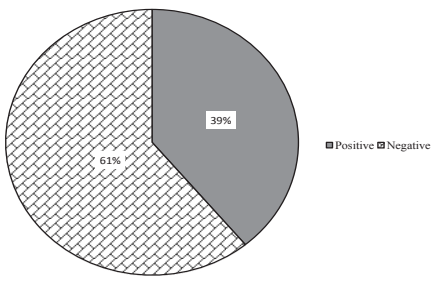

Figure 1: Growth rate of *Enterococcus* isolates in feacal samples

Distribution of *Enterococcus* isolates

Among the total samples processed, more than half of the isolates were isolated from the female population (54%). Likewise, the majority of samples collected fell upon the age group of ≥60 (28%) whereas least in the age group of 40-50 (6%). Out of the total 39 *Enterococcus* isolates, the bacterial isolate was in high percentage in the age group 50-60 years 8 (61.53%) followed by the adult age group 30-40 years 7(58.33%) as shown in table 1.

Table 1: Distribution of *Enterococcus* isolates according to age and gender

Character	No. of the total sample	Culture positive	
		Number	%
Gender			
Male	46	17	43.58
Female	54	22	56.41
Age group in years			
<20	26	7	26.92
20-30	15	3	20
30-40	12	7	58.33
40-50	6	2	33.33
50-60	13	8	61.53
≥60	28	12	42.85
Total	100		

Antibiotic susceptibility pattern of *Enterococcus* species

Antibiotic susceptibility of *Enterococcus* species revealed a higher degree of susceptibility towards the antibiotics chloramphenicol 21 (53.84%) followed by nitrofurantoin 20 (51.28%). Meanwhile, 31 (79.48%) were resistant to linezolid and erythromycin. Similarly, out of a total of 17 isolates, 7 (17.9%) were resistant to vancomycin antibiotics as shown in table 2.

Table 2: Antibiotic susceptibility pattern of *Enterococcus* isolates

Antibiotic used	*Enterococcus*	
	Sensitive, N (%)	Resistant, N (%)
Ampicillin	17 (43.58)	22 (56.41)
Ciprofloxacin	17 (43.58)	16 (41.02)
Chloramphenicol	21 (53.84)	15 (38.46)
Erythromycin	2 (5.12)	31 (79.48)
Linezolid	7 (17.94)	31 (79.48)
Nitrofurantoin	20 (51.28)	11 (28.20)
Vancomycin	10 (74.35)	7 (17.9)

Distribution of MDR (Multidrug Resistance) Enterococci

Among the total 39 *Enterococcus* isolates, 28 (71.79%) bacterial isolates were found to be multidrug-resistant out of which 12 (30.76%) isolates were resistant to 3 antibiotics, 6 (15.38%) isolates to four antibiotics and 10 (25.64%) isolates to 5 antibiotics as shown in table 3.

Table 3: Distribution of MDR *Enterococcus* isolates

Organism	Total isolates	No. of isolates exhibiting MDR pattern			Total MDR
		R3	R4	R5	
Enterococcus	39	12 (30.76%)	6 (15.38%)	10 (25.64%)	28 (71.79%)

*R3 = resistance to 3 antibiotics; R4 = resistance to 4 antibiotics; R5 = resistance to 5 antibiotics.

DISCUSSION

This study is the first to describe the prevalence of *Enterococci* in fecal samples from healthy people of a certain community of Nepal. It was observed that the prevalence of *Enterococci* in the community sample was found to be 39%. which was incompatible with the findings of other researchers, reporting the prevalence rate in Belgium (100%),[9] in Portugal (73%),[10] and Iran (70.5%).[2] On the other hand, the prevalence of Enterococci isolates, in the present study, was found to be higher than reported from previous studies such as 1.61%[11] and 0.57%[12] conducted in clinical samples in the context of Nepal. The difference in prevalence rate may be due to different clinical specimens, health status, geographical locations, hygiene status, and during the processing of specimens.

From our study frequency of Enterococci was highest in the above age group ≥60 whereas a contrasting result was reported by Karna,[12] and Dupont[13] in a clinical sample from the hospital. In elderly patients, high risk of a life-threatening disease like diabetes, depression, high blood pressure, cholesterol, thyroid, peptic diseases, urinary tract infection, organ failure, and suffering from immuno-compromised diseases, such conditions favors the Enterococcal infections and as a result, the possibility of the highest number of enterococci isolation from fecal sample will be high.

In the current study, the highest number of Enterococci was isolated from female (56.41%) participants as compared to males (43.58%). This study goes along with the study by.[14,15] The unequal ratio of sample size, poor health, and females suffering from chronic diseases and frequently visiting the hospital for treatment might be the reason for the highest isolated Enterococci from female participants as compared to males.

This study showed chloramphenicol (53.8%) to be most effective against Enterococci isolates. This finding is similar to various studies conducted in Switzerland,[2] New Zealand[16] and Iran.[17,18] Similarly, nitrofurantoin (51.28%) also showed good activity which is in accordance with the conducted in Australia.[19] The low incidence of chloramphenicol resistance is most likely due to the less prescription of it than other antibiotics in Nepal.[20]

The study demonstrated 79.48% of *Enterocoocus* isolates are resistant to Linezolid and erythromycin which is in contract with the findings reported in previous studies.[2,21,22] High-level resistance in these drugs might be due to gene transmission from animal sources, might also be from hospital settings.[23] Different research suggested that the resistance in this drug may lead to antibiotic resistance in Enterococci.

The spread of VRE has been a global health problem to public health, the prevalence of vancomycin resistance

enterococci (VRE) in this study was 17.9%. This finding was significant to countries like Greece (18.8%),[24] Brazil (13.6%),[25] and France (11.8%)[26] including community and hospitalized patients. From the above studies, it can be confirmed the presence of VRE in the fecal samples suggesting that VRE are part of the gut microbial flora and can be acquired in the community.[2]

MDR Enterococci is the leading cause of enterococcal infection in the hospital and rearing animals. The prevalence of MDR Enterococci was found to be 71.79% in healthy people which is similar to the finding by,[18, 27–29] in hospital and healthy individuals. The current practice of Antibiotic Therapy in different parts of Nepal, especially in eastern Nepal and across the border is seemingly ruining many antibiotic treatment regimens. As a result, healthy carriers are prior exposed to the irrational use of antibiotics without medical prescription.[12]

This finding is new in the case of healthy individuals in the community and probably the first in the context of Nepal. The less percentage of isolated Enterococci might be because of various reasons which include not having enough knowledge about the antibiotics, availability of substandard and counterfeit drugs, lack of proper advice on scientific husbandry practices with poor hygiene and sanitation, overuse of antimicrobials, lack of awareness and observance of drug withdrawal periods. These are also some of the most important factors responsible for the growing AMR (Antimicrobial resistance) burden in Nepal. Self-prescription, over-prescription, under-prescription, and irrational prescription of powerful antibiotics intended for rapid cure have all contributed to antibiotic resistance.[30]

CONCLUSION

According to our results, 17.9% of fecal samples were carried vancomycin-resistant Enterococci. This study figures out MDR enterococci and vancomycin resistance enterococci from the gut of healthy carriers which indicates the risk of transmitting VRE to the environmental non-pathogenic bacteria through fecal routes. Enterococcal infections possess serious problem in hospital and clinical sample whereas it has very few options of treatment globally. Vancomycin, linezolid, Nitrofurantoin are the drug of choice in developed and undeveloped countries as very few antibiotics can treat enterococccal infections if these drug showed resistance towards enterococci no other option can treat enterococcal infection and large number of people affected by this diseases. Irrational and irregular use of antibiotics may not treat disease caused by enterococcal infections in the near future which automatically enhance the morbidity and mortality of people. Due to increasing resistance rate of Enterococci to vancomycin and most common antibiotics, appropriate surveillance and control measures are essential to reduce the amount of antimicrobial agents and a load of resistant bacteria in community as well as clinical sectors.

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ETHICAL APPROVAL

Ethical approval was taken from the research committee of the Prithivi Narayan campus**.**

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CONFLICTS OF INTEREST

There is no conflicts of interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors RRM, CT, ST, PP, NB and PS designed the study, performed the statistical analysis and wrote the protocol. Authors PS and RRM wrote the first draft of the manuscript and managed the analyses of the study. Author RRM managed the literature searches. Author KG supervised the study. All authors read and approved the final manuscript.

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