Characterization and susceptibility patterns of clinically important *Enterococcus* species in eastern Nepal

A Acharya,[1] B Khanal[2] R Kanungo[3] and T Mohapatra[4]

[1]Assistant Professor, [2] Additional Professors, Department of Microbiology. B.P Koirala Institute of Health Sciences, Dharan, Nepal., [3] Professor and Head, Department of Clinical Microbiology, Pondicherry Institute of Medical Sciences. [4] Professor, Department of Clinical Microbiology BHU, India.

Corresponding Author: Dr. Anju Acharya, Assistant Professor, Department of Microbiology, B.P. Koirala Institute of Health Sciences, Dharan. Nepal. e-mail: anju_71np@yahoo.com

ABSTRACT

Life threatening infections caused by enterococcus species with multidrug resistance has emerged as a threat to medical care in the present era. This study was conducted to characterize enterococcus species isolated from different clinical samples and to detect the pattern of susceptibility to some of the commonly used antibiotics in B.P Koirala Institute of Health Sciences (BPKIHS), a tertiary care hospital in eastern Nepal. Clinical samples submitted to the microbiology unit of Central Laboratory Service (CLS) for culture and sensitivity during March 2002 - February 2003 was analyzed. *Enterococcus* species were identified by colony characteristics, gram staining and relevant biochemical tests. Antibiotic susceptibility test was done by the Kirby Bauer disc diffusion technique. Of 50 *Enterococcus* species isolated, *E. faecalis* was the predominant isolate (48.0%) followed by *E. faecium* (32.0%) and *E avium* (20.0%). Eighty-eight percent of *E. faecalis* showed sensitivity to cephotaxime and 87.0% to vancomycin. Multiple drug resistance was observed most commonly in *E. faecium*. Seventeen percent of *E. faecium* were resistant to vancomycin and 63.0% to ciprofloxacin and 44.0% to ampicillin. On the contrary *E. avium* rarely showed resistance to the antimicrobials tested including vancomycin. Enterococcal infections are common nowadays specially in hospitalized patients. Inappropriate use of antibiotics in clinical practice and poultry should be discouraged to prevent the emergence of multidrug resistant species.

Keywords: Enterococcus**,** antimicrobial susceptibility, Eastern Nepal.

INTRODUCTION

Enterococci are members of the normal flora of the gastrointestinal tract in humans and animals.[1]

Enterococci are considered important difficult-to-treat pathogens, due to their intrinsic resistance to several antimicrobial agents and their propensity to acquire resistance. Recent literatures have shown the increasing incidence of enterococcal infections with an increasing proportion of nosocomial enterococcal infections. Increasing incidence of multidrug resistant enterococci is very much agonizing fact in clinical practice.[2,3]

Knowledge of the extent of the enterococcal infections and their antimicrobial susceptibility plays an important role in the choice of appropriate therapy. But no such study has been done, in recent years; regarding the incidence and antibiotic susceptibility of enteroccocus in eastern Nepal.

So the purpose of this study was to characterize the different species of enterococcous and to determine their antimicrobial susceptibility pattern in BPKIHS, Dharan , a tertiary care hospital in eastern Nepal.

MATERIALS AND METHODS

The study was carried out in B.P.Koirala institute of health Sciences, Dharan. Nepal from March 2002 to February 2003.

A total of 8627 clinical specimens submitted to the microbiology unit of Central Laboratory Service (CLS) for culture and sensitivity were included in the present study. They comprised of urine, blood, tissue, high vaginal swabs, pus, catheter tip, wound swabs and body fluid. All the specimens were subjected to gram staining first. Then they were inoculated onto blood agar, MacConkey agar, chocolate agar, whenever necessary and incubated at 37 ^{0}c for 18-24 hr**.**

The strains were identified as being of the genus Enterococcus on the basis of colony caracteristics, gram staining findings, salt tolerance, manitol fermentation and aesculin hydrolysis.[1,4-6]

They were further characterized up to species level by using standard tests : bile aesculin test, Voges-proskauer test, hydrolysis of hippurate, arginine, fermentation of pyruvate, sorbitol, lactose, sucrose, raffinose, adonitol, L-rahamnose, D-xylose, tellurite, motility, yellow pigment production, growth at 45^{0}C, growth at 50^{0}C and growth at pH 9.4.[7-11]

Preparation of inoculum and Antimicrobial Susceptibility testing: Fine similar looking colonies of the test organism grown on blood agar were picked up with sterile loop. They were suspended into peptone water and incubated at 37^{0}C for 2-4 hours. The turbidity of the suspension was adjusted to MacFarland's Nephalometer standard tube no. 0.5. Then the test organism was spreaded with swabs on to the Muller Hinton blood agar media. Commercially prepared antibiotic discs

(obtained from-Hi-Media Ltd. India) of 6 mm in diameter were used to determine the susceptibility pattern of Enterococcus species in Muller Hinton blood agar media.

Kirby Bauer disk diffusion susceptibility testing (6 antimicrobiol disc/ plate) was performed to commonly used antimicrobial agents. The plates were incubated at 37^0C for over night. Zone of inhibition measured by using Vernier caliper and the results were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) criteria on the basis of zone size inhibition as sensitive, intermediate and resistant.[12]

E. faecalis ATCC 29212 and *E. faecium* ATCC 35667 were used as control strains.

RESULTS

A total fifty *Enterococcus* species were isolated from various clinical materials. *Enterococci* were frequently isolated from blood (30.0%) and pus (30.0%) followed by urine (22.0%).

Seventy two percent of enterococus was isolated from hospitalized patients and 28.0% from outdoor patients. Among the hospitalized patients 44.5% belonged to surgery ward and 30.5% to paediatric ward.

E. faecalis was the commonest species followed by *E. faecium* and *E. avium*. (Fig. 1).

Most of the *E.avium* were sensitive to all commonly used antibiotics except tetracycline, to which only 20.0% of *E. avium* were sensitive.

Eighty-eight percent of *E. faecalis* showed sensitivity to cephotaxime. Similarly 87.0% were sensitive to vancomycine, 67.0% to chloromphenicol and erythromycine and 62.0% to gentamycine. Only 37.0% of *E. faecalis* were sensitive to ciprofloxacine and 54.0% to ampicilline.

Eighty-three percent of *E. faecium* showed sensitivity to vancomycine**.** In contrast to *E. avium* and *E. faecalis , E. faecium* showed poor sensitivity to all other antibiotics. Only 37.0% of *E. faecium* were sensitive to erythomycine, 31.0% to gentamycine, and 25.0% to chloromphenicol and cefotaxim. Similarly only 19.0% and 12.0% were sensitive to Ciprofloxacine and Ampicilline respectively.

Sensitivity pattern of individual species to various antibiotics is given in Fig. 2

DISCUSSION

Enterococci are being isolated more frequently from clinical specimens and are considered the second leading cause of nosocomial infections.[13] In present study among the 50 isolated enterococci, *E faecalis* was the predominant isolate (48.0%) followed by *E faecium* (32.0%) and *E avium* (20.0%). This finding correlates with other similar studies.[4-6,14]

In our study 72.0% of enterococus was isolated from hospitalized patients and 28.0% from outdoor patients. Among the hospitalized patients 44.5% belonged to surgery ward and 30.5% to pediatric ward.

Longer hospital stay and immunocompromised conditions are known risk factors for nosocomial infections like enterococcal infections. Among the hospitalized patients the post surgical patients have longer hospital stay and have more chance of cross infection. So it may be the cause for higher incidence of enterococus isolation from surgical ward samples. Similarly, underdeveloped immunity and immunocompromised conditions in padiatric patients may cause them more vulnerable for nosocomial enterococcal infections.

In present study, cefotaxim and vancomycine were more effective for *E. faecalis*. But commonly used antibiotics like ciprofloxacin and ampicillin were less effective for this species. In this study 63.0% of *E. faecalis* were resistant to ciprofloxacin and 46.0% to ampicillin. Shamla had similar results of ampicillin sensitivity.[15]

Among the enterococci, *E. faecium* has emerged as an important nosocomial pathogen during the past decade. It is a cause of significant morbidity and mortality in hospitalized patients. Notorious for resistance to multiple antimicrobial agents, the acquisition of vancomycin resistance has made this organism a formidable pathogen.

In the present study, 83.0% of *E. faecium* were sensitive to vancomycin. Sensitivity to other antibiotics was observed in less than 40% of the isolates. The incidence of multidrug resistant *E. faecium* is increasing in world literatures. A study conducted by Marcus from 1993 to 1996 showed that the incidence of infections caused by resistant strains of *E. faecium* has been increased. *E. faecium* isolates resistant to ampicillin increased from 19% in 1993 to 67.0% in 1996. During the same period, resistance to vancomycin increased from 0% to 50.0%, and and high-level gentamicin resistance increased from 0% to 38.0%.[12] In present study 88.0% of enterococcus faecium were resistant to ampicillin and 69.0% to gentamicin. Sixty-eight percent were resistant to both ampicillin and gentamicin. In this study 17.0% of *E. faecium* were resistant to vancomycin. Our findings do not differ significantly from Marcus results.[16,17]

Resistance to commonly used antimicrobial agents is a remarkable characteristic of most of the enterococcal species. Antimicrobial resistance markers can be intrinsic or acquired. Intrinsic or inherent resistance traits are present in all or most of the strains and appear to be chromosomally coded.[18] The intrinsic resistance of enterococci to many commonly used antimicrobial agents may have allowed them a cumulative advantage for further acquisition of genes encoding high-level resistance to aminoglycosides, penicillins, tetracycline, chloramphenicol, and now vancomycin. Acquired resistance markers result from either mutation in existing DNA or acquisition of new DNA found in plasmid or transposons

The major factors for the emergence of multidrug-resistant enterococci are (i) baseline intrinsic resistance to several antimicrobial agents, (ii) acquired resistance via mobility of the resistance genes or plasmids, transposons, and chromosomal exchange, and (iii) the transferability of resistance. It is important to note that these genetic transfers often occur in the gastrointestinal tracts of humans and animals. So the colonization of the organisms is one of the important factor for the emergence of multi-drug resistant as well as vancomycin resistant enterococci. It appears that organism from patients or from Hospital staff first colonize in gastrointestinal tract before causing infections in patients. Further, there are evidences that Hospital person harboring resistant enterococci in their own gastrointestinal tract may be responsible for colonization of patients under their care.

The prevention of the dissemination of resistant bacteria is important. Practice guidelines for isolation precautions in hospital were proposed by the Centers for Disease Control and Prevention (CDC) in 1996. Hand hygiene has been demonstrated to be the most important measure to reduce the risk of transmitting organisms from one person to another. Gloves prevent the gross contamination of hands; reduce the likelihood of transmission of microorganisms during procedures. Placing patients in private rooms and limiting the movement visitors may prevent direct or indirect contact transmission. Masks, respiratory protection, eye protection, face shields and gowns must be worn in specific conditions. Specific measures must also be carried out with regard to patient care equipment, linen and laundry, dishes, glasses, cups and eating utensils.

The environmental burden of antimicrobial utilization also promotes the colonization and infection with multidrug resistant enterococci by basically two mechanisms:

First, the broad spectrum antibiotics have little or no anti enterococcal activity and administration commonly leads to over growth of resistant enterococci at sites at risk for infection.

Second, most antibiotics substantially reduce the normal resistance of the intestinal tract to colonization by exogenous organism. Antibiotic induced alteration in the protective flora of the intestine provides large footholds for colonization with exogenous pathogens such as multi drug resistant enterococci.

In Europe, colonization appears to occur frequently in community. An important factor associated with VRE in the community in the Europe has been avoparcin, a glycopeptide antimicrobial drug used for years in many European nations at sub-therapeutic doses as a growth promoter in food production animals. In Europe, evidence suggests that food borne VRE may cause human colonization.[19] In Nepal also there is increasing tendency to use antibiotics in poultry which may be an important factor for colonization of multidrug resistant pathogens specially VRE. Subsequently it may increase the incidence of multidrug resistant pathogens.

Similarly in our setup the unnecessary use of antibiotics in clinical practice can not be neglected. Generally the patients buy the drugs directly from the medical shop without prescription and they don't complete the course of the antibiotics. Frequent and non compliant medication in community may predispose for emergence of multi-drug resistant species. In clinical practice unnecessary use of antibiotics should be discouraged to prevent the emergence of multidrug resistant pathogens

The present study highlights that incidence of enterococcal infections in Eastern Nepal can not be ignored especially in hospitalized patients. E faecalis was the most common isolate in BPKIHS. Multiple drug resistance was observed most commonly in *E. faecium* followed by *E. faecalis*. On the contrary *E. avium* rarely showed drug resistance.

Prevention and control of spread of multi drug resistant *Enterococci* requires coordinated efforts from various departments and can only be achieved by education of hospital staff regarding problem of drug resistance, prudent use of antimicrobials in clinical practice and in poultry , early detection and reporting and immediate implementation of appropriate infection control measures.

REFERENCES

1. Murray BE. The life and times of enterococci. *Clin Microbiol* 1990; 3: 46-65.
2. Harakeh HS, Uwaydah M, Matar GM. Random amplified polymorphic DNA typing of enterococcus faecalis isolated from Lebanese individuals. *East J Med* 2000; 5: 18-20.
3. Koch S. Enterococcal infections: host response, therapeutic and prophylactic possibilities. *Vaccine* 2004; 22: 822-30.
4. Liassine N, Frei R, Jan I, Auckenthaler R. Characterization of glycopeptide resistant enterococci from a Swiss hospital. *J Clin Microbiol* 1998; 36: 1853-8.
5. Sahm DF, Frel Smith C, Eveland M, Manday LM. Rapid characterization schemes for surveillance isolates of vancomycin resistant enterococci. *J Clin Microbiol* 1997; 35: 2026-30.
6. Desai PJ, Pandit D, Mathur M, Gogate A. 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; 19:132-7.
7. Barrow GI, Felthham RKA. Characters of Gram positive bacteria. In Cowan and Steal's. Manual for the identification of medical bacteria. 3rd ed , Camridge University Press. Great Britain 1993;50-93.
8. Holts JG, Kraieg NR, Sneath PHA, Staley JT, Williams ST. Gram positive cocci. In Bergey's Manual of determinative bacteriology. 9th ed. East preston street Baltimore, Maryland 21202, USA. 1994; 527-58.

9. 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.
10. Alex C, Sonnennwrith 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.
11. Cheesbrough M. Gram technique. In District laboratory practice in tropical countries part 2. Cambridge Univercity press, United kingdom.2000; 38-9.
12. Villanova PA. Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards. 4th ed. 1997.
13. Culver S, Gaynes RP. Major trends in the microbial etiology in nosocomial infection. *Amer J med* 1991; 91: 79-82.
14. Nelson RRS, McGregor KF, Brown AR, Amyes GB, Young HK. Isolation and characterization of glycopeptide resistant enterococci from hospitalized patient over a 30 months period. *J Clin Microbiol* 2000; 38: 2112-6.
15. Shamala DP. Characterization, Antibiotic susceptibility pattern and detection of B-lactamases in Enterococci. *Indian J Ppathol Microbiol* 2002; 45: 79-82.
16. Marcus N. Rapid increase in the prevalence of antimicrobial drug resistance among enterococcal blood isolates in southern Israel. *Eur J Clin Microbiol Infect Dis* 1997; 16: 913-5.
17. Silver JEE. Epidemiologic evaluation of antimicrobial resistance in community acquired enterococci. *J Clin Microbiol* 1998; 36: 830-3.
18. Wade JJ, Uttley AHC. Resistant enterococci mechanism, laboratory detection and control in hospitals. *J Clin Pathol* 1996: 49: 700-70.
19. Clifford LM, Matthew JK, Fred CT, Jarvis WR. Vancomycin resistant enterococci outside the health care setting: prevalence, sources and public health implications. *Emerg Infect Dis* 1997; 3: 311-7.

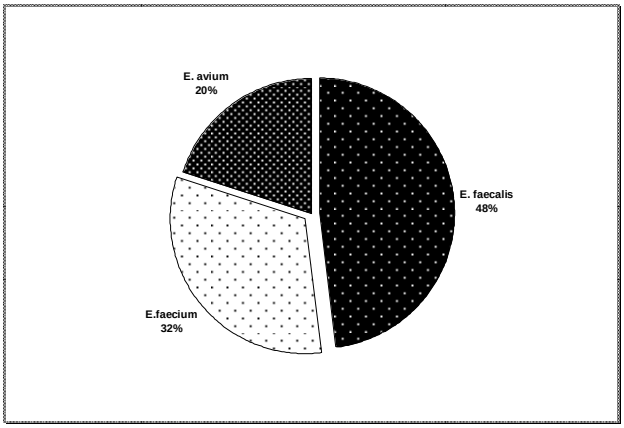

Fig. 1. Percentage of the various types of Enterococci isolated from clinical material

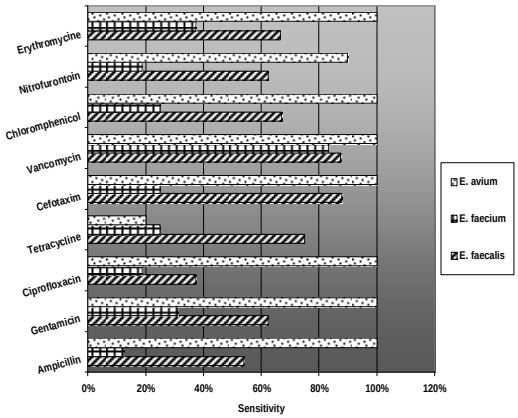

Fig. 2. Sensitivity pattern of individual species to various antibiotics