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VANCOMYCIN RESISTANT ENTEROCOCCI AT A TERTIARY CARE HOSPITAL IN NORTHERN INDIA

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ABSTRACT

Resistant enterococci is a real threat and continuous surveillance is necessary to monitor its magnitude**.** Risk factors and phenotypic characteristics of VRE isolated in Gandhi memorial and associated hospital, K.G's .Medical University Lucknow a tertiary care setup. Method: Enterococci were isolated from various clinical specimens and identified phenotypically, vancomycin resistance pattern using both disc diffusion and minimum inhibitory concentration methods. High level resistance (HLR) to streptomycin, kanamycin, and gentamicin was determined and beta lactamase production was detected using three methods: Acidometric, iodometric and chromogenic beta lactams. Results: Amongst 86 enterococci isolated 4 isolates were resistant to vancomycin but sensitive to teicoplanin, 34 were found to have high level resistance (HLR) to some aminoglycosides. HLR was more to kanamycin than streptomycin and gentamicin. Only 1 isolate produced beta lactamase. Conclusion: Frequency of isolation of VRE was not very high in our setting and prolonged hospital stay was the common risk factor . Frequent occurrence of HLR to kanamycin makes amikacin a poor choice to achieve synergistic therapy with cell wall active agent.

KEYWORDS:*Enterococci species, Vancomycin resistance, High-level aminoglycosideresistance, beta lactamase.*

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INTRODUCTION

Enterococci have evolved over the past century from being an intestinal commensal organism of little clinical significance to becoming the second most common nosocomial pathogen associated with significant morbidity and mortality[1, 2] In recent years, there has been a rapid increase in the incidence of infection and colonization of patients with vancomycin-resistant enterococci (VRE). The prevalence of VRE has dramatically increased worldwide. The resistance may be intrinsic or acquired via gene transfer1. This genus is intrinsically resistant to a number of antimicrobial agents including low concentration of aminoglycosides mortality[3]. None of the currently available cephalosporins have clinically useful activity against them. In addition *enterococcus* is rapidly acquiring resistance to many commonly used antibiotics in hospitals. Of concern is the rapid dissemination of high level aminoglycosides resistant enterococci. The synergistic effect obtained by combination of aminoglycosides with cell wall active agents disappear in strains that show High level resistance(HLR) to former mortality[3]. The National Nosocomial Infection Surveillance (NNIS) system in the USA has revealed a significant increase in the percentage of invasive nosocomial *Enterococcus* strains displaying high level vancomycin resistance mortality[4]. *Enterococcus faecalis* was the most common species associated with nosocomial infections, followed by mortality *Enterococcus faecium,* and both species are responsible for about 95% of infections caused by Enterococci mortality[4] . Infections caused by VRE were found to be associated with adverse outcome such as extended length of hospital stay, increased cost and increased mortality mortality[2 ,4]. The glycopeptides vancomycin is the first choice alternative to penicillin-amino glycoside combination for treatment of systemic Enterococci infections mortality [5]. Different types of vancomycin resistance genes have been reported in enterococci. Glycopeptide-resistace genotypein enterococci include Van (high-level resistance), which is detected in a wide variety of enterococcal species , Van B, Van B2 and Van D with moderate to high-level resistance and Van C(C1, C2, C3) causing intrinsic low-level resistance mortality [6] . Vancomycin resistance is most commonly found in *E. faecium* and is encoded by the Van A gene cluster carried on the mobile genetic element Tn1546 [7]. Enterococci as reservoirs of antibiotic resistance genes, tend to transfer their resistance genes to the other bacteria, including methicillin resistant *Staphylococcus aureus*[8] . Monitoring the antibiotic resistance of enterococci isolated from clinical specimen is a useful tool to get information about the prevalence of VRE and will be essential for the controlling the spread of bacterial resistance. So, the present study was planned with aim to isolate and characterize *enterococcus* species from clinical specimens and to study prevalence of drug resistance among these isolates, especially with regard to high level aminoglycoside resistance (HLAR).

MATERIALS AND METHODS

Specimens were collected for this study over the period from May 2004 to April 2005, and included 5,890 different clinical samples referred for bacteriological culture (5,516 in patients department (IPD) and 374 outpatient's department (OPD) samples. There were 3,277 urine, 1,666 blood, 344 pus, and 183 cerebrospinal fluid (CSF) samples, 175 vaginal swabs, 70 throat swabs, and 175 samples of other body fluids such as ascitic fluid, pleural fluid, tissue aspirate, tissue pieces, and catheter tips. A detailed history of patients including drug intake, hospital stay duration during current and previous admission, underlying disease, invasive devices, ventilator use etc. The isolated Enterococci were identified to the species level using phenotypic methods 3 antibiotic sensitivity test was performed by Kirby-Bauer's method on Muller Hinton sheep blood agar. The following discs were used (Hi-

Media pvt. Ltd., Mumbai, India): ampicillin, 10µg/disc; ampicillin/sulbactam, 10/10 µg/disc; chloramphenicol, 30 µg/disc; ciprofloxacin, 5µg/disc ; erythromycin, 15 µg/disc; teicoplanin, 30 µg/disc; tetracycline, 30 µg/disc; vancomycin, 30 µg/disc. Aminoglycosides discs were used in high level concentration, diffusion in solid medium using discs impregnated with 120 µg of gentamicin (which also predicts susceptibility to tobramycin and netilmycin) and kanamycin (also predicts response to amikacin), and 300 µg of streptomycin was performed [3, 9] . Before use, each lot of discs was checked with standard strains of *E.faecalis* ATCC 29212. Inhibition zones were interpreted according to CLSI guidelines. Beta lactamase production was detected by three methods: Iodometric, Acidometric and Chromogenic beta lactam[10].

RESULTS

From 5,890 clinical specimens, a total of 86 enterococci were isolated. Isolation rate of enterococci was 1.46%. Spectrum of infection associated with enterococci was diverse and majority (75/86, 87%) was caused by *E. faecalis* and *E. faecium*. 77% (66/86) enterococci were sensitive to ampicillin (Table 1). Resistance to ampicillin was found significantly higher amongst *E. faecium* as compared to *E. faecalis* (11/33 vs. 3/42 respectively). 66 isolates (66/86) were sensitive to combination of ampicillin and sulbactam. For the tetracycline, erythromycin, ciprofloxacin, and chloramphenicol sensitivity were 63 (63/86), 25 (25/86), 28 (28/86), and 27 (27/86) respectively. For all other groups of antibiotics except ampicillin there was no significant difference between resistance pattern of *E. faecalis* and *E. faecium*. All enterococci were sensitive to teicoplanin whereas 4 were found resistant to vancomycin by disc diffusion method. The minimum inhibitory concentration (MIC) for vancomycin amongst those which were resistant by disc diffusion method ranged from 32-256 µg/ml by both agar and broth dilution methods, and these VRE were *E.faecium* isolated from urine samples. All VRE isolates were from patients having history of more than 4 days stay in hospital and taking broad spectrum antibiotics. Over all 40% (34/86) enterococci were found to have HLR to some aminoglycosides. HLR was more to kanamycin than streptomycin and gentamicin (48.83%, 20.93%, and 8.30% respectively). Species wise there was no significant difference for the presence of HLAR. Seven patterns of HLR were found; commonest was HLR to kanamycin alone in 26 isolates, followed by HLR to kanamycin and streptomycin together in 5 isolates. HLR to gentamicin alone was not found in any enterococci, it was always associated with resistance to kanamycin or streptomycin or both. All isolated VRE were sensitive to high level aminoglycosides and teicoplanin. Of the 86 isolates, only one produced beta-lactamase (positive by all three methods) which was *E. faecalis* isolated from blood and by routine disc diffusion it was sensitive to ampicillin and this isolate was also sensitive to vancomycin.

Table 1

Antibiotic Sensitivity pattern of enterococci by disc diffusion method (n-86)

Sensitivity pattern	A/S	T	Cp	E	C	Va	Te
Sensitive	66	15	28	25	27	82	86
Moderately sensitive	2	8	5	6	7	-	-
Resistant	18	63	53	55	52	4	-

A/S-ampicillin/sulbactam, T-tetracyclin, Cp-Ciprofloxacin, E-erythromycin, C-Chloramphenicol, Te-teicoplanin, Va-vancomycin.

DISCUSSION

During the study period, we examined different clinical samples from 5,890 patients for the presence of VRE at King George's medical college (Gandhi memorial and associated hospital) Lucknow. In our study, isolation rate of enterococci was 1.46 (86/5890), Parvathi et al. (2003). from Taminadu India reported rate of enterococci 3.38 %, although that study includes samples from fistulae and stool. Stool was a significant source of enterococci in their study. The vast majority of the isolates in this study were either *E.faecalis* which caused 48.83% infection or *E.faecium* which was responsible for 38.37 % of infection, while *E. avium, E. durans, E. dispar, E. casseliflavus, E. cecorum* and *E. hirae* accounted for only 12.79%. From urine 54 (1.64) isolates were obtained from 3277 samples which was consistent with finding of other studies which reported 1.49 % isolated from urine within our country (Taneja et al., 2004) [11]. Of the total enterococci isolated in present study the highest isolation rate was from urine 62.79 % (54/86), blood culture 24.41 % (21/86) and the least incidence was noted in pus 4.65 % (4/86), vaginal swab 3.49 % (3/86), catheter tip 3.49 % (3/86) and tissue 1.16 % (1/86). Higher incidence than Parvathi et al. (2003) in urine, blood and pus in our study may be due to higher number of samples processed by us. Finding in vaginal swab (3.49 %) was approximately same as the studies by Udo et al. (2003), 3.0 % of the total enterococci isolated obtained by them were from vaginal swab. Enterococci are intrinsically resistant to several antimicrobials and can develop resistance to many others, which complicates treatments of their infections. All isolates were susceptible to teicoplanin, four (4.65 %) were resistant to vancomycin. Out of the total 86 enterococci resistant was found in tetracycline 63 (73.25 %), ciprofloxacin 53 (61.62 %), erythromycin 55(63.54%), chloramphenical 52 (60.46%), ampicillin/sulbactam18 (20.93%). Ampicillin seemed to work better for *E. faecalis* as compared to *E.faecium* as reported earlier. Resistance tetracycline was the highest noted in present study followed by resistance to erythromycin and ciprofloxacin. Based on the result of the MIC studies and their susceptibility to teicoplanin, isolated VRE appear to be Van B phenotype. The high-level resistance to gentamicin, streptomycin and kanamycin were 8.30%, 20.93%, and 48.83% respectively. Vandamane et al. (1996) [12]. reported high level gentamicin resistance among enterococci isolate to be 8.7 which is similar to our findings. A low prevalence of HLR to gentamicin among enterococci has been reported earlier which is similar to our observation, suggesting that gentamicin should maintain a synergistic effect when combined with cell-wall active agent such as vancomycin and ampicillin in the treatment of serious enterococcal infection. Udo et, al.(2003) reported similar findings for streptomycin resistance, although resistance to kanamycin was higher in our set up in comparison to study done by Edet E.Udo et al.(2003). Frequent occurrence of HLR to kanamycin confirms that amikacin is a poor choice when attempting to achieve synergistic therapy as observed by others also. 20% HLR for streptomycin has been reported in a study though HLR to kanamycin was higher in our set up in comparison to their study. High-level doses of aminoglycosides in combination of beta lactamase appear to be helpful in the treatment of VRE. According to Udo et al. (2003) of 415 isolates of enterococci, resistance to erythromycin was 63.3% and to tetracycline 60.5 %. In our study resistance to erythromycin was similar. Tetracyclin was resistant to 73.25% (63/86) isolates, which were in higher percentage than in the above study. It was the highest resistance noted in present study. Resistance to ciprofloxacin was similar to a study done by Calderon et al. (2003) [13] ,who found resistance to ciprofloxacin (60%). Of the 86 isolates, 60.46% were resistant to chloramphenical which were higher than the level of resistance reported for this antibiotic among enterococci in the U.K. (Woodford et al.,

1993)[14] and Germany (Reinert et al., 1999) . Beta-lactamase production amongst enterococci is not very common. But what is important is that this is often missed on routine disc susceptibility testing due to inoculums effect also observed by us.

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