Original Article

Antibiotic resistance pattern of *Enterococci* isolates from nosocomial infections in a tertiary care hospital in Eastern India

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Abstract

Background: Resistance to commonly used antibiotics by *Enterococci* causing nosocomial infections is of concern, which necessitates judicious, responsible and evidence-based use of antibiotics. The present study was conducted to review the prevalence and identify therapeutic options for nosocomial Enterococcal infections in our tertiary care hospital. **Materials and Methods:** Isolates identified by morphological and biochemical characteristics were tested for antibiotic susceptibility using Kirby-Bauer method. **Result:** 153 of 2096 culture positive clinical samples comprised of 101 urine, 30 wound swab/pus, 13 blood and 09 high vaginal swab isolates were identified as *Enterococcus faecalis* (90.85%), *Enterococcus faecium* (8.50%) and *Enterococcus gallinarum* (0.65%). *Enterococci* accounted for 8.45%, 4.53%, 4.23%, 4.43% of urinary, wound swab or pus, blood, high vaginal swab isolates respectively, causing 7.3% of all nosocomial infections. Significant number of *Enterococci* isolated from nosocomial urinary tract infection (66.01%) and wound infections (19.6%) were multidrug resistant (MDR). Although all isolates were sensitive to vancomycin and linezolid, resistance to erythromycin (71.24%) and ciprofloxacin (49.67%) was frequently observed. High-level gentamicin resistance was observed in 43.88%, and 61.53% of *E. faecalis* and *E. faecium* isolates respectively. Minimal inhibitory concentration of vancomycin of all the isolates were ≤1 μg/ml. 7% of the Enterococcal isolates were MDR strains and vancomycin or linezolid were the only effective antibiotics. **Conclusion:** A combination of vancomycin and/or linezolid were effective against *Enterococci* causing nosocomial infections in our tertiary care facility, nevertheless continuous and frequent surveillance for resistance patterns are necessary for judicious and evidence based use of antibiotics.

Key words: Antibiotic resistance, *Enterococcus*, nosocomial infection

INTRODUCTION

The accelerated emergence of antibiotic resistance among the prevalent pathogens is of global health concern. *Enterococcus*, is one such pathogen which is the leading cause of nosocomial bacteremia, urinary tract infections (UTI), and surgical site infections.[1,2] *Enterococcus* resistance to antimicrobial agents to which the genus *Streptococcus* are generally susceptible and its ability to transfer the drug resistance genes from vancomycin-resistant strains to *Staphylococcus aureus* is of concern.[3] The therapeutic challenge of multiple-drug resistant (MDR) *Enterococci*, identifies them as important nosocomial pathogens.

Enterococci infections have traditionally been treated with cell wall inhibitor agents in combination with an aminoglycoside. Reduced susceptibility to β-lactam antibiotics and vancomycin; in combination with a high level aminoglycoside resistance (HLAR) interferes with the penetration of the aminoglycoside into the bacterial cytoplasm, thus making the antibiotic synergism ineffective.[4] Hence, this study was designed to identify the magnitude of Enterococcal infections and their antibiotic

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susceptibility pattern in a tertiary care hospital in the eastern region of India.

MATERIALS AND METHODS

Following approval from our institutional ethics committee, clinical samples were collected over a period of 1-year (February 2011 to January 2012) at a tertiary care hospital in Kolkata, India. The study population included patients of all age groups and both sexes with suspected nosocomial infections (Infection developed after 48 h of hospital stay). Patients with infections at the time of admission, within 48 h of hospital stay or 30 days after discharge were excluded.

The clinical samples of urine, wound swab/pus, high vaginal swab and blood were inoculated on blood agar and MacConkey agar. The causative bacteria were identified on the basis of colony characteristics, Gram stain morphology, motility and biochemical tests (catalase test, growth on bile aesculin agar, tolerance to 6.5% NaCl, arginine dihydrolase test, and fermentation of arabinose, mannitol, raffinose, and sorbitol).

Antibiotic susceptibility of *enterococcus* species

The isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method, as per CLSI recommendations, using commercially available 6 mm disks (HIMEDIA, Mumbai, India) on Mueller-Hinton agar. The disks used were vancomycin (30 μg), Ampicillin (10 μg), erythromycin (15 μg), ciprofloxacin (5 μg), linezolid (30 μg). For high-level gentamicin resistance (HLGR) detection, gentamicin (120 μg) disc was used. The inoculated plates were incubated for 18 h at 35°C. The diameter of the zone of inhibition of each antibiotic was measured and interpreted as sensitive, intermediate sensitive or resistant according to CLSI criteria. For HLGR, resistance was indicated by no zone, and susceptibility, by a zone of diameter ≥10 mm. *Enterococcus faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used as the susceptible and resistant quality control strains.[5]

Determination of minimum inhibitory concentrations

Minimum inhibitory concentrations (MIC) of vancomycin were determined by agar dilution method. Brain-heart infusion agar (Hi Media, Mumbai) was supplemented with different concentrations of vancomycin. The test organism was grown in broth and the turbidity matched with McFarland 0.5 standard (approximately 1.5×10^8 cfu/mL). Spot inoculation of the agar medium was done using 10 μl of bacterial culture. The plates were incubated at 37°C for 24 h and examined. The minimum concentration of vancomycin that inhibited bacterial growth was considered MIC.

RESULTS

A total of 153 *Enterococci* were isolated from 2096 culture positive clinical samples. 101 of 1194 urine isolates, 30 of 662 wound swab/pus isolates, 13 of 307 blood isolates and 09 of 203 high vaginal swab isolates were identified as *Enterococci* [Table 1]. All isolates were further speciated as *E. faecalis* (90.85%), *Enterococcus faecium* (8.50%) and *Enterococcus gallinarum* (0.65%). Most urinary isolates were from maternity ward ($n = 25$), followed by gynecology ($n = 23$), and pediatric ward ($n = 10$) respectively. Enterococcal wound infections were also most commonly reported from gynecology ($n = 7$) followed by surgical wards ($n = 5$). Nosocomial bacteremia was mostly reported from Cardiology and intensive therapy unit (ITU). *Enterococci* were the causative pathogen in high vaginal swab in patients from maternity, gynaecology and ITU. MIC of vancomycin observed in all Enterococcal isolates was <1 μg/ml which corroborates the disc diffusion test result.

DISCUSSION

The spectrum of disease produced by *Enterococci* varied from UTI, wound infection, soft tissue infection to bacteremia. Urinary tract instrumentation or catheterization, genitourinary pathology, prior use of antibiotics, prolonged hospitalization were some of the predisposing factors for Enterococcal infections. [1,2]

Urinary tract was the most common site of Enterococcal infection (66.01%) in this study which often occurred in catheterized patients. The next common infection was wound infection (19.60%) followed by bacteremia (8.50%) which corroborates with the studies from different regions of India [6-8] [Figure 1]. In India, the occurrence of Enterococcal infection varied from 1% to 36% in different institutions.[9] *E. faecalis* is the predominant Enterococcal species, which accounts for 80-90% of all clinical isolates, which is followed by *E. faecium* (5-15%).[10,11] However, a progressive increase in *E. faecium* infections has been reported and is found to be more resistant to penicillin and aminoglycosides, which is attributed to production of the

Table 1: Incidence and distribution of Enterococcal isolates in different clinical samples

Samples	Total number of isolates	Total number of Enterococcal isolates (%)	Distribution of Enterococcal isolates ($n = 153$) (%)
Urine	1194	101 (8.45)	66.01
Wound swab	662	30 (4.53)	19.60
Blood	307	13 (4.23)	8.50
Vaginal swab	203	9 (4.43)	5.89
Total	2096	153 (7.30)	100

enzyme, 6-acetyl transferase and more penicillin-binding proteins.[12] In our study, *E. faecalis* was the commonly isolated species followed by *E. faecium* and *E.gallinarum*, the incidence rates were comparable with previous reports.[13]

All *Enterococci* isolates were found to be sensitive to vancomycin and linezolid which was consistent with other studies from India,[14,15] However the prevalence of vancomycin resistant *Enterococci* (VRE) in India is reported to be between 0% and 30%.[7,16,17]

Highest prevalence of resistance was observed against erythromycin (71.24%). Almost half of the isolates were resistant to ciprofloxacin (49.67%) and Gentamicin (45.75%) [Figure 2]. In contrast, Ampicillin showed a high level of sensitivity (77.12%) among the nosocomial isolates [Table 2]. 53.33% wound and 78.22% urine isolates were resistant to erythromycin. Resistance to ciprofloxacin was also less prevalent among the wound isolates in comparison with the urine isolates. Ampicillin and gentamicin showed a higher level of resistance among the wound isolates compared with urine isolates. Isolates from nosocomial bacteremia showed a higher level of sensitivity to each of the drug tested [Table 3].

The HLAR to gentamicin was observed in 45.75% isolates with 43.88% in *E. faecalis* and 69.23% in *E. faecium* [Table 4]. HLAR to gentamicin is universally reported to be in the range of 1-48% (mean, 22.6 ± 12.3),[18] although with an increasing trend recently.[9,15,17,19,20] HLAR to gentamicin nullifies the efficacy of combination therapy, which is used to treat serious Enterococcal infections. Nevertheless, empirically chosen combination therapy with ampicillin and gentamicin would be effective in 54.25% of nosocomial infections [Figure 3]. Hence, it is necessary to distinguish the HLAR strains from simply resistant strains. [18]

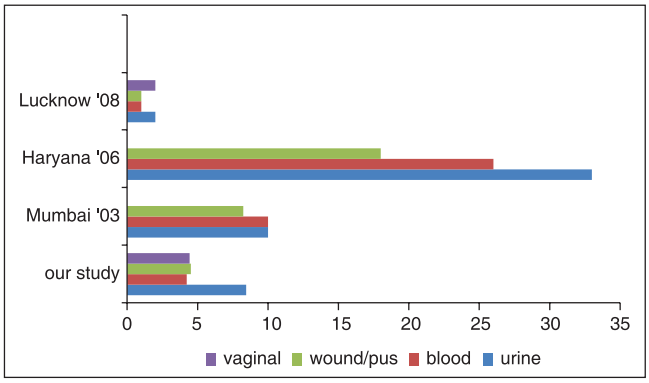

Figure 1: Comparison between isolation rates of *Enterococcus* in different clinical samples from different regions of India[6-8]

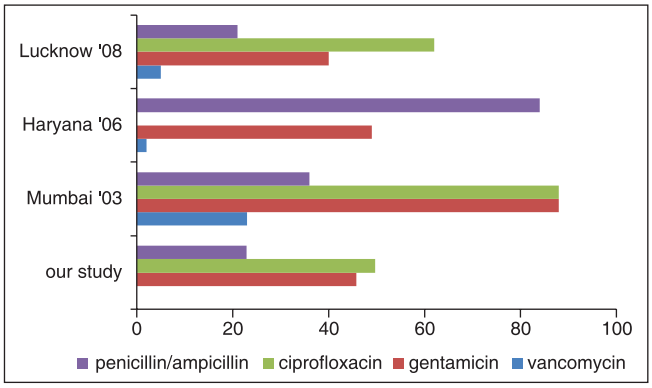

Figure 2: Antibiotic resistance pattern of *Enterococcus* as evident in different studies[6-8]

Table 2: Antimicrobial sensitivity pattern of the isolates in nosocomial infections

Antibiotic	Number of sensitive isolate	Percentage of sensitive isolate	Number of resistant isolate	Percentage of resistant isolate
Vancomycin (30 μg)	153	100	0	0
Linezolid (30 μg)	153	100	0	0
Ampicillin (10 μg)	118	77.12	35	22.88
Erythromycin (15 μg)	44	28.76	109	71.24
Ciprofloxacin (5 μg)	77	50.33	76	49.67
Gentamicin (120 μg)	83	54.25	70	45.75

Table 3: Comparison of percentage prevalence of antibiotic resistance among isolates from different sources

Name of the antibiotic	Percentage resistance among urine isolates	Percentage resistance among wound swab/ pus isolates	Percentage resistance among blood isolates	Percentage resistance among high vaginal swab isolates	Percentage resistance among all the nosocomial isolates
Vancomycin	0	0	0	0	0
Linezolid	0	0	0	0	0
Ampicillin	21.78	30.00	07.69	33.33	22.88
Erythromycin	78.22	53.33	61.54	66.67	71.24
Ciprofloxacin	53.47	43.33	46.15	33.33	49.67
Gentamicin	42.57	50.00	61.54	44.44	45.75

Table 4: HLGR in *Enterococci*

Species of *Enterococcus*	Number of isolates	Number and percentage of resistant isolates (%)
E. fecalis	139	61 (43.88)
E. fecium	13	9 (69.23)

HLGR: High-level gentamicin resistance, *E. fecalis*: *Enterococcus faecalis*, *E. fecium*: *Enterococcus fecium*

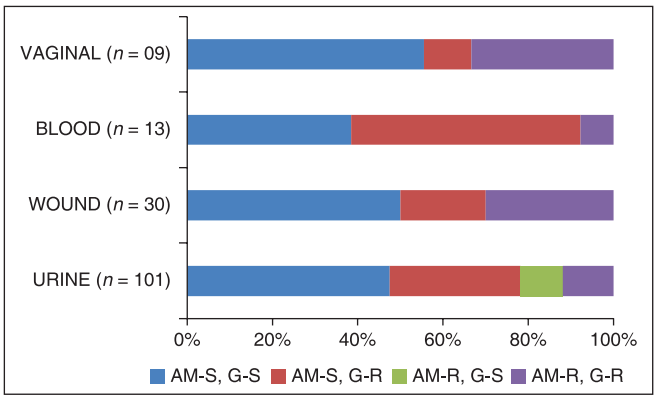

Figure 3: Comparison between β-lactam (Ampicillin) and aminoglycoside (gentamicin) susceptibility patterns of *Enterococci* isolates from different sources (n = 153) (AM: Ampicillin, G: Gentamicin, S: Sensitive, R: Resistant)

Interestingly 7% of the isolates (n = 11) in our study were MDR and vancomycin or linezolid were the only available option for treating these patients. MDR isolates constituted 9% of urinary and 7% of wound infections. None of the blood and vaginal isolates was MDR strains. The MDR strains were mostly reported from gynecology (n = 3) and maternity Wards (n = 2).

CONCLUSION

Our study reveals the prevalence of high degree of resistance to macrolide and fluoroquinolone among the nosocomial Enterococcal isolates, thereby limiting the use of these drugs for therapeutic purposes. The resistogram of the Enterococcal isolates varied among specimens from different wards, but the pattern was constant among isolates within a particular ward. Hence, the nosocomial outbreak in our hospital had not been disseminated from a single strain though isolates from a particular ward might be epidemiologically linked. The present study also revealed that despite recent trends of increasing resistance to Aminoglycosides, a combination therapy of β-lactam and Aminoglycoside as first-line drugs would be currently the best choice. Vancomycin or linezolid therapy should be restricted for use in patients infected with MDR strains only. Judicious use of vancomycin and linezolid in serious infections and appropriate infection control measures would probably recede the possible emergence of VRE outbreaks in our geographical area.

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