Original Research

Assessment of infections caused by Enterococcal species and their antimicrobial susceptibility pattern with reference to High Level Gentamicin Resistance (HLGR) and Vancomycin Resistant Enterococci (VRE)

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ABSTRACT

Background: Over the past few decades, enterococci—Gram positive, facultative anaerobic cocci—have changed from being common intestinal commensals in humans and animals to serious nosocomial infections linked to considerable morbidity and mortality. The present study was conducted to assess infections caused by Enterococcal species and their antibiotic resistance pattern with respect to High Level Gentamicin Resistance (HLGR) and Vancomycin Resistant Enterococci (VRE).

Materials & Methods: 86 clinical isolates of Enterococci were selected. The isolates were identified, speciated using standard methods and antibiotic susceptibility was determined by Kirby Bauer disc diffusion method; and Vancomycin MIC was determined by E-test method.

Results: Out of 86 samples, 21 were isolated from urine, 40 from pus, 17 from blood, 6 from synovial fluid. E. faecalis was isolated in 63, E. faecium in 12, E. raffinosus in 3, E. durans in 6 and E. avium in 2 cases. Sensitive strains and resistant strains of Penicillin was 35% and 65%, Ampicillin was 62% and 38%, Vancomycin was 94% and 6%, Gentamicin (10 µg) was 75% and 25%, high level gentamicin (120 µg) was 68% and 32%, Nitrofurantoin (for 18 urinary isolates) was 84% and 16%, Ciprofloxacin was 72% and 28%, Erythromycin was 21% and 79% and Linezolid was 100% respectively. The difference was significant ($P< 0.05$).

Conclusion: More drug resistance to E. faecium isolates was found in the study, with a high rate of resistance to Aminoglycosides, Vancomycin, Penicillin, and Ciprofloxacin. This underscores the urgent need for more prudent use of antimicrobials and infection control.

Keywords: Aminoglycosides, Vancomycin, Penicillin

INTRODUCTION

Over the past few decades, enterococci—Gram positive, facultative anaerobic cocci—have changed from being common intestinal commensals in humans and animals to serious nosocomial infections linked to considerable morbidity and mortality.[1] They are responsible for a broad spectrum of illnesses, including urinary tract infections (UTI), surgical site infections (SSI), bacteremia, intra-pelvic and intra-abdominal abscesses, and on rare occasions, pneumonia and meningitis.[2]

The two most common Enterococci species that infect humans are E. faecalis (80–90%) and E. faecium (5–10%). These bacteria are becoming more and more important because they are

intrinsically resistant to a number of common antibiotics and can develop resistance to a number of additional antibiotics through mutation or the transfer of plasmids and transposons.[3] Enterococci are known for their antibiotic resistance; E. faecium is more resistant to antibiotics than E. faecalis. Despite the fact that enterococci have an innate resistance to low concentrations of aminoglycosides, these medications work in concert with beta lactams to provide bactericidal effects. Different processes cause streptomycin and gentamicin resistance in enterococci.[4] Gentamicin resistance is caused by the inactivating enzyme 20-phosphotransferase-69-acetyltransferase, which also confers resistance to amikacin, kanamycin, netilmicin, and tobramycin. With the exception of streptomycin, gentamicin is therefore thought to be resistant to all other aminoglycosides. Enterococci that generate streptomycin adenyl transferase are the primary source of streptomycin resistance. These isolates continue to be gentamicin-sensitive.[5]

AIM AND OBJECTIVES: The present study was conducted to assess infections caused by Enterococcal species and their antibiotic resistance pattern with respect to High Level Gentamicin Resistance (HLGR) and Vancomycin Resistant Enterococci (VRE).

MATERIALS & METHODS

The present cross-sectional study was conducted on 86 clinical isolates of Enterococci in the Department of Microbiology, Gouri Devi Institute of Medical Sciences & Hospital, Durgapur, West Bengal, India. All participants gave written consent after being made aware of the study. The study was approved by the Institutional Ethics Committee. The duration of the study was from January 2020 to August 2020. All were informed regarding the study and their written consent was obtained.

When found in pure culture or in significant numbers as part of mixed cultures, the isolated strains were deemed clinically significant and were included in the study; isolates from stool samples were excluded. Speciation and antibiotic susceptibility testing were conducted simultaneously during the study period, resulting in a total of 86 consecutive culture isolates of Enterococci. Clinical samples, including blood, urine, pus, and other bodily fluids like pleural fluid, peritoneal fluid, and CSF (cerebrospinal fluid), were analysed to obtain the isolates. Samples were inoculated with blood and MacConkey agar and then aerobically incubated for 24 to 48 hours at 37 °C. Blood samples in Brain Heart infusion broth were subcultured on Blood and MacConkey agar on the second and seventh days of incubation.

The isolates were identified, speciated using standard methods and antibiotic susceptibility was determined by Kirby Bauer disc diffusion method; and Vancomycin MIC was determined by E-test method.

The species was determined by:

- Sugar fermentation: 1% of glucose, sucrose, lactose, maltose, mannitol, arabinose, and raffinose.
- The Moller decarboxylase test for arginine hydrolysis.
- Tellurite reduces in Tellurite agar.

To differentiate between E. faecalis and E. faecium, use this test. Black colonies are produced by E. faecalis.

Statistical Analysis

Data thus obtained were subjected to statistical using Microsoft excel and using IBM SPSS version 16.0. P value < 0.05 was considered significant.

RESULTS

Table I: Species distribution of Enterococci in various clinical specimens

Samples	Total no. of Enterococci	E. faecalis	E. faecium	E. raffinosus	E. durans	E. avium
Urine	21	11	1	3	4	2
Pus	40	30	9	0	1	0
Blood	17	16	0	0	1	0
Synovial fluid	8	6	2	0	0	0
Total	86	63	12	3	6	2

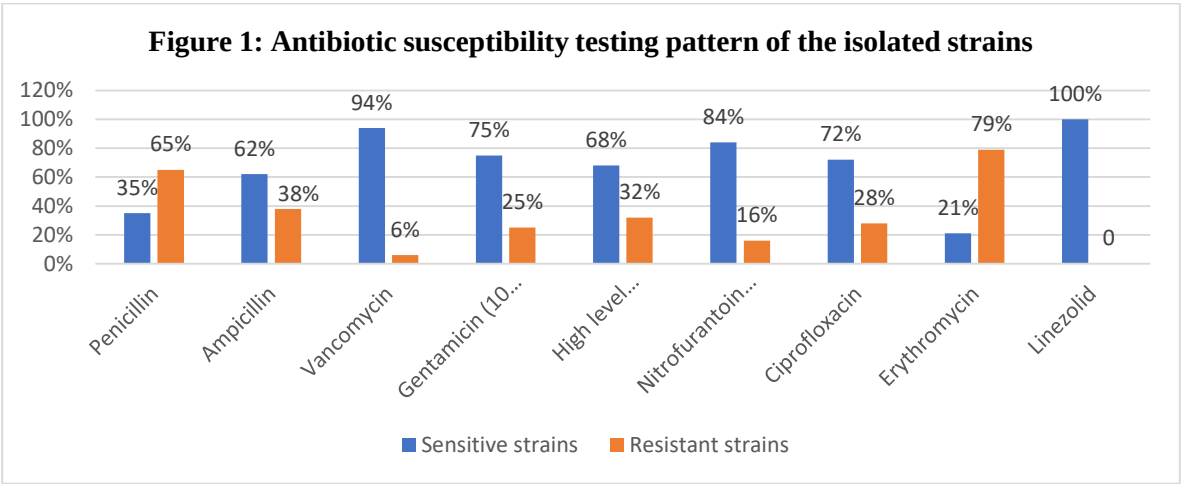
Table I shows that out of 86 samples, 21 were isolated from urine, 40 from pus, 17 from blood, 6 from synovial fluid. E. faecalis was isolated in 63, E. faecium in 12, E. raffinosus in 3, E. durans in 6 and E. avium in 2 cases.

Table II: Antibiotic susceptibility testing pattern of the isolated strains

Antibiotics	Sensitive strains	Resistant strains	P value
Penicillin	35%	65%	0.05
Ampicillin	62%	38%	
Vancomycin	94%	6%	
Gentamicin (10 µg)	75%	25%	
High level Gentamicin (120 µg)	68%	32%	
Nitrofurantoin (for 18 urinary isolates)	84%	16%	
Ciprofloxacin	72%	28%	
Erythromycin	21%	79%	
Linezolid	100%	0	

Table II and figure 1, shows that sensitive strains and resistant strains of Penicillin was 35% and 65%, Ampicillin was 62% and 38%, Vancomycin was 94% and 6%, Gentamicin (10 µg) was 75% and 25%, high level gentamicin (120 µg) was 68% and 32%, Nitrofurantoin (for 18 urinary isolates) was 84% and 16%, Ciprofloxacin was 72% and 28%, Erythromycin was 21% and 79% and Linezolid was 100% respectively. The difference was significant (P< 0.05).

Figure 1: Antibiotic susceptibility testing pattern of the isolated strains



DISCUSSION

High level aminoglycoside-resistant enterococci (Streptomycin MIC, >2,000 mg/mL; Gentamicin MIC, >500 mg/mL) do not exhibit penicillin-aminoglycoside synergy.[6] Alarming High Level Gentamicin Resistance (HLGR) rates of 60–80% have been reported in India by a number of studies.[7,8] Vancomycin Resistant Enterococci (VRE) have been identified as one of the world's primary nosocomial infection causes since 1988. This is particularly concerning because it has restricted the therapeutic choices available to doctors.[9] The present study was conducted to assess infections caused by Enterococcal species and their antibiotic resistance pattern with respect to High Level Gentamicin Resistance (HLGR) and Vancomycin Resistant Enterococci (VRE).

We found that out of 86 samples, 21 were isolated from urine, 40 from pus, 17 from blood, 6 from synovial fluid. E. faecalis was isolated in 63, E. faecium in 12, E. raffinosus in 3, E. durans in 6 and E. avium in 2 cases. Varghese V et al.[10] found that out of the 75 Enterococci strains, 50 (66.7%) were E. faecalis, 16 (21.4%) were E. faecium, 6 (8%) were E. raffinosus, 2 (2.6%) were E. durans and 1 (1.3%) was E. avium. The maximum no. of isolates was from male patients, and pus samples yielded more Enterococci. HLGR was found in 25/75 (33.3%) strains and 3/75 (4%) strains showed Vancomycin resistance. Isolates had 100% sensitivity to Linezolid.

We found that sensitive strains and resistant strains of Penicillin was 35% and 65%, Ampicillin was 62% and 38%, Vancomycin was 94% and 6%, Gentamicin (10 µg) was 75% and 25%, high level gentamicin (120 µg) was 68% and 32%, Nitrofurantoin (for 18 urinary isolates) was 84% and 16%, Ciprofloxacin was 72% and 28%, Erythromycin was 21% and 79% and Linezolid was 100% respectively. Ahmed et al.[11] found that out the prevalence and risk factors for vancomycin resistant Enterococci in a leading

tertiary care centre. A total of 25 (6.3%) isolates of Enterococci were found to be vancomycin resistant, most of them recovered from the blood samples. E. faecium 16 (64%) was the predominant VRE isolated followed by E. faecalis 9 (36%). Factors like stay in an ICU, prior use of antimicrobials, placement of IV line and urinary catheter were associated with vancomycin resistant Enterococci (VRE) acquisition.

Shah et al.[12] determined the prevalence and susceptibility pattern of *Enterococci* in tertiary care hospital. Total of 92 enterococcal strains isolated from various samples were identified and speciated as per scheme of Facklam and Collins. Antibiotic susceptibility was determined for various drugs by Kirby bauer disc diffusion method. Results were interpreted as per CLSI guidelines and were even compared with Vitek2 automated system. 69 strains were *E. faecalis*, 21 were *E. faecium* and two were E. gallinarum. High level resistance to penicillin, ampicillin, gentamicin and streptomycin were observed. All strains were sensitive to linezolid and teicoplanin.8% strains showed vancomycin resistance which was detected by Vitek2 automated system.

LIMITATION OF THE STUDY: The shortcoming of the study is small sample size.

CONCLUSION

Authors found that more drug resistance to E. faecium isolates was found in the study, with a high rate of resistance to Aminoglycosides, Vancomycin, Penicillin, and Ciprofloxacin. This underscores the urgent need for more prudent use of antimicrobials and infection control.

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