Antimicrobial Resistance Profile of Enterococcus Faecalis Isolates from Wound Infection

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Abstract

Introduction: Enterococci have rapidly emerged as important nosocomial and community acquired pathogens. Enterococcus. faecalis has accounted for approximately 80-90% of the isolates causing human infection.

Aim & Objectives: To identify the etiology of wound infection and to determine the antibiotic resistance pattern of Enterococci isolates.

Materials & Methods: A total 61 bacterial isolates obtained from wound swabs over a period of six months were included in this study. Enterococci isolates were identified by colony morphology, gram staining, catalase test, growth in the presence 6.5% sodium chloride, aesculin hydrolysis in the presence of 40% bile and by biochemical reactions using Facklam and Collins scheme. Antimicrobial susceptibility testing was done according to the CLSI guidelines by the disc diffusion method of Kirby-Bauer. Vancomycin resistance and High level aminoglycoside resistance were tested by the disc diffusion method and the agar screen method and Minimum Inhibitory Concentration testing was done by the E test.

Results: The prevalence of Enterococcus. faecalis isolates from wound infection was found to be 16.39 %. By E test 3 Enterococcus. faecalis isolates were resistant to High level Gentamicin (≥512µg/ml), Minimum Inhibitory Concentration (MIC) of Teicoplanin was found in the range of 0.5-4 µg/ml and for vancomycin, MIC was in the range of 0.5-2 µg/ml. One isolate was found to be resistant to Linezolid with MIC of ≥8µg/ml.

Conclusion: Rapid and accurate susceptibility testing results, effective therapy and infection control measures are necessary to prevent the spread of multidrug resistant enterococci.

Keywords: Nosocomial infection, Multi Drug Resistant Enterococci, Minimum Inhibitory Concentration, Agar screen method, E test.
Introduction
Enterococci, recognised as opportunistic pathogens, are normal flora of respiratory, genital and gastrointestinal tract. Enterococci have become common cause of hospital acquired urinary tract infections, wound infections and bacteremia.[1] Enterococcus faecalis (80-90%) and Enterococcus faecium (5-10%) are the most frequently isolated strains from human infections. The second most frequent cause identified by Enterococci are intraabdominal and pelvic sepsis and surgical wound infections in which enterococci are part of mixed flora of colon organisms.[2] Hospital acquired wound infections are among the leading cause of morbidity and huge economic burden associated with prolonged hospital stay, readmissions and procedures.[1,2] Enterococci are intrinsically resistant to multiple antibiotics and their ability to acquire resistant genes to currently available antibiotics results in the selection and spread of Multi Drug Resistant (MDR) strains.[3] Enterococci isolates showing resistance to Vancomycin and Teicoplanin have been reported from many parts of the world.[4,5] Clinically resistance to vancomycin has been associated with persistent isolation of enterococci from primary site of infection, frequent episodes of bacteremia, increased frequency of endovascular infection and increased mortality. Both Enterococcus faecalis and Enterococcus faecium are intrinsically resistant to clinically achievable concentrations of aminoglycosides.[6] Widespread resistance to chloramphenicol, macrolides, kanamycin, streptomycin and tetracycline was found among isolates of Enterococcus. faecalis and Enterococcus. faecium. Antimicrobial resistance profile of Enterococci strains pose a significant challenge to physicians.[3,4]

Hence the present study was undertaken to know the etiology of wound infection and to, speciate and determine the antimicrobial resistant profile of Enterococci isolates.

Materials & Methods
A total of 61 bacterial isolates from wound infection collected between September 2017 to February 2018 were included in this study. The samples were cultured on Blood agar, Chocolate agar and Mac Conkey agar and incubated at 37º C for 18-24 hours. Bacterial isolates were identified by standard biochemical reaction and enterococci isolates were determined by colony morphology, gram staining, catalase test, growth in the presence 6.5% sodium chloride, aesculin hydrolysis in the presence of 40% bile and by biochemical reactions using conventional test scheme (Facklam and Collins).[7] To detect gelatin liquefaction, isolates were inoculated into tubes containing 4 mL of brain heart infusion broth with 4% gelatin and incubated at 35-37ºC for 24 h. The tubes were cooled at 4ºC for 30 min and the liquefaction of the medium was observed.[8]

Antibiotic susceptibility testing
Antibiotic susceptibility testing was performed for Enterococci isolates on Muller Hinton agar by Kirby Bauer disk diffusion method and the results were interpreted as per CLSI guidelines.[9] using Enterococcus faecalis 29212 as quality control. The following antibiotics were used: Amoxicillin/clavulanate(25μg/10μg),Penicillin(10units),High level gentamicin (120μg), Amikacin(30μg), Cotrimoxazole (25μg), Teicoplanin (30μg), Linezolid (30μg), Ciprofloxacin(5μg), Erythromycin (15μg).

Agar screen test for Vancomycin was done on Brain heart infusion agar containing 6 μg/ml vancomycin.[5,9] using Vancomycin sensitive strain ATCC 29212 E.faecalis as negative control and Vanomycin resistant strain ATCC 51299 E.faecalis as positive control.

Minimum Inhibitory Concentration for vancomycin and teicoplanin was determined by agar dilution method on Brain heart infusion agar supplemented with 2,4,8,16,32 μg/ml of antibiotics and were incubated at 37º C for 24 hrs and examined for growth. High level aminoglycoside resistance was determined by...
Minimum Inhibitory Concentration for Gentamicin was determined by agar dilution method (500,1000,2000 µg/ml of Gentamicin.) and E test.[9,10]

E test was performed to determine Minimum Inhibitory Concentration of vancomycin and teicoplanin. Enterococci isolates with MIC >32 µg/ml were considered as resistant; 8-16 µg/ml as intermediate resistant and 4 µg/ml as susceptible to vancomycin.

Isolates with MIC ≤ 8 µg/ml were considered as susceptible, 16 µg/ml as intermediate resistant and >32 µg/ml as resistant for teicoplanin.[5]

Enterococcus. faecalis ATCC 29212 and Staphylococcus aureus 25923 were used as control strains.[5,9]

Results
A total 61 bacterial isolates obtained from wound swabs over a period of six months were included in this study.

Among the Gram negative bacteria isolated, 18.03% were Pseudomonas spp, 16.39% were Klebsiella spp and 13.11% were E.coli. Among Gram positive isolates, 16.39% were Enterococcus faecalis followed by Staphylococcus aureus (13.11%). [Figure 1]

Out of the ten isolates of Enterococci studied, seven were Beta hemolytic and three produced Gamma hemolytic colonies on blood agar. None of these isolates liquefy gelatin. Seven isolates fermented mannitol, eight isolates hydrolysed arginine and three isolates were positive for sorbitol fermentation.

Out of 10 Enterococcus. faecalis isolates, 4 (40.00%) were resistant to Penicillin, 3(30.00%) to High Level Gentamicin, 6(60.00%) to Erythromycin, 4(40.00%) to Teicoplanin by Kirby Bauer disk diffusion method. One isolate was resistant to Linezolid. [Figure 2]
By agar dilution, one isolate was with vancomycin MIC ≥ 2µg/ml, three isolates showed high level gentamicin resistance with MIC ≥ 500µg/ml and one isolate with teicoplanin MIC ≥ 4µg/ml.

By E test 2 E. faecalis isolates were resistant to High level Gentamicin (≥1024 µg/ml), MIC of teicoplanin was found in the range of 0.5 - 4 µg/ml.

For vancomycin, Minimum Inhibitory concentration was found in the range of 0.5-2 µg/ml. [Table 1]

Of the resistant isolates studied, 3 were with MIC of ≥8 µg/ml for ciprofloxacin, levofloxacin and erythromycin respectively. MIC for Linezolid resistant isolate was found to be ≥8 µg/ml.

Table 1: Determination of Minimum Inhibitory Concentration by E test

<table>
<thead>
<tr>
<th>No of isolates(10)</th>
<th>Vancomycin</th>
<th>Teicoplanin</th>
<th>HLGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.5µg</td>
<td>1µg</td>
<td>2µg</td>
<td>≤ 0.5µg</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

HLGR: High Level Gentamicin Resistance

**Discussion**

Enterococci exhibits intrinsic resistance to some antimicrobial agents and have high ability to acquire antibiotic resistant determinants. Multiple antimicrobial resistant enterococci are emerging as the leading cause of hospital acquired infections, especially, Enterococcus faecalis and Enterococcus faecium. Combination of aminoglycosides with betalactam antibiotics or vancomycin is preferred in the treatment of severe life-threatening infections due to intrinsic and chromosomal resistance patterns.

The prevalence of Enterococcus faecalis isolates from wound infection was found to be 16.39 % in the present study. A study conducted by Chakraborty, et al from India have reported Enterococci isolation rate from wound samples as 4.53%. Of the total Enterococci isolates studied in our study, 40.00% were resistant to Penicillin, 30.00% to High Level Gentamicin, 60.00% to Erythromycin, 40.00% to Teicoplanin and 60.00% to Ciprofloxacin by Kirby Bauer disk diffusion method. One E. faecalis isolate was found to be resistant to Linezolid in contrast to other Indian studies, where 100% sensitivity was reported.

Linezolid is used in the treatment of infections caused by resistant gram positive bacteria particularly vancomycin-resistant enterococci.
Enterococcus faecalis\[^{[5]}\]. Linezolid inhibits ribosomal protein synthesis but at a different site from other agents that target the ribosome (chloramphenicol, macrolides, lincosamides, streptogramin, aminoglycosides, tetracycline). Consequently, existing mechanisms of resistance to these agents do not confer cross-resistance to linezolid.\[^{[16]}\]

In the present study by E test, Minimum inhibitory concentration for vancomycin was found in the range of 0.5-2 µg/ml and Minimum Inhibitory Concentration for teicoplanin was found in the range of 0.5-4 µg/ml. A study by Praharaj et al have reported 21.88 % VRE isolates from pus samples.\[^{[14]}\] In an Indian study by Suchitra et al from surgical site infection have reported 8.03 % VRE by broth dilution method.\[^{[17]}\] All the VRE isolates were resistant to penicillin. In our study one isolate with MIC of ≥2µg/ml for vancomycin was found to be resistant to other antibiotics tested viz Penicillin (≥32µg/ml), Ciprofloxacin (≥8µg/ml), Levofloxacin (≥8µg/ml), Erythromycin (≥8µg/ml), Linezolid (≥8µg/ml) and Teicoplanin (≥4µg/ml).This isolate was found to be sensitive to chloramphenicol and tetracycline. Three isolates showed high level resistance to Gentamicin (≥500µg/ml).In a study from North India by Mathur et al, 10.43 % isolates from pus sample showed high level resistance to gentamicin.\[^{[15]}\]

Enterococci belong to the normal flora of the human intestine and would be expected to contaminate wounds fairly frequently.\[^{[18]}\] In kidney and pancreas transplant recipients, bacteremia typically derives from wound or urinary tract infections.\[^{[19]}\] Knowledge of pathogenicity of Enterococci in wound and tissue infections, and their antimicrobial pattern , is of obvious importance to the physician.

Conclusion

This study illustrated multidrug resistance among Enterococci isolates from wound samples. Hence adequate measures should be taken to routinely identify enterococci to species level, test their antimicrobial susceptibility pattern and to implement a sound antimicrobial policy in every hospital to prevent further increases in resistance and the spread of enterococcal infections.

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