www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v6i6.164

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Antimicrobial Resistance Profile of Enterococcus Faecalis Isolates from Wound Infection

Authors
Reena Rajan[1], C.Abhirami[2], C. Amirtha[3], G. Sasikala[4], V.Anandi[5]
[1]Senior Lecturer, Department of Microbiology, Penang International Dental College, Vinayaka Mission 's Research Foundation (Deemed to be University), Salem, Tamil Nadu
[2]Senior Resident, Department of Dermatology, Indira Gandhi Medical College and Research Institute, Puducherry
[3]Post Graduate student, Department of Obstetrics & Gynaecology, Sree Balaji Medical College & Hospitals, Chennai, Tamil Nadu
[4]Consultant Microbiologist, VIMS Hospital, Salem
[5]Professor, Department of Microbiology, Vinayaka Mission's Medical College, Vinayaka Mission 's Research Foundation (Deemed to be University) Karaikkal, Tamil Nadu

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 infection caused 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 Enterococus 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 37^0 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 37^0 C for 24 hrs and examined for growth. High level aminoglycoside resistance was determined by

screen agar containing 500µg/ml gentamicin.[6,9]. 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

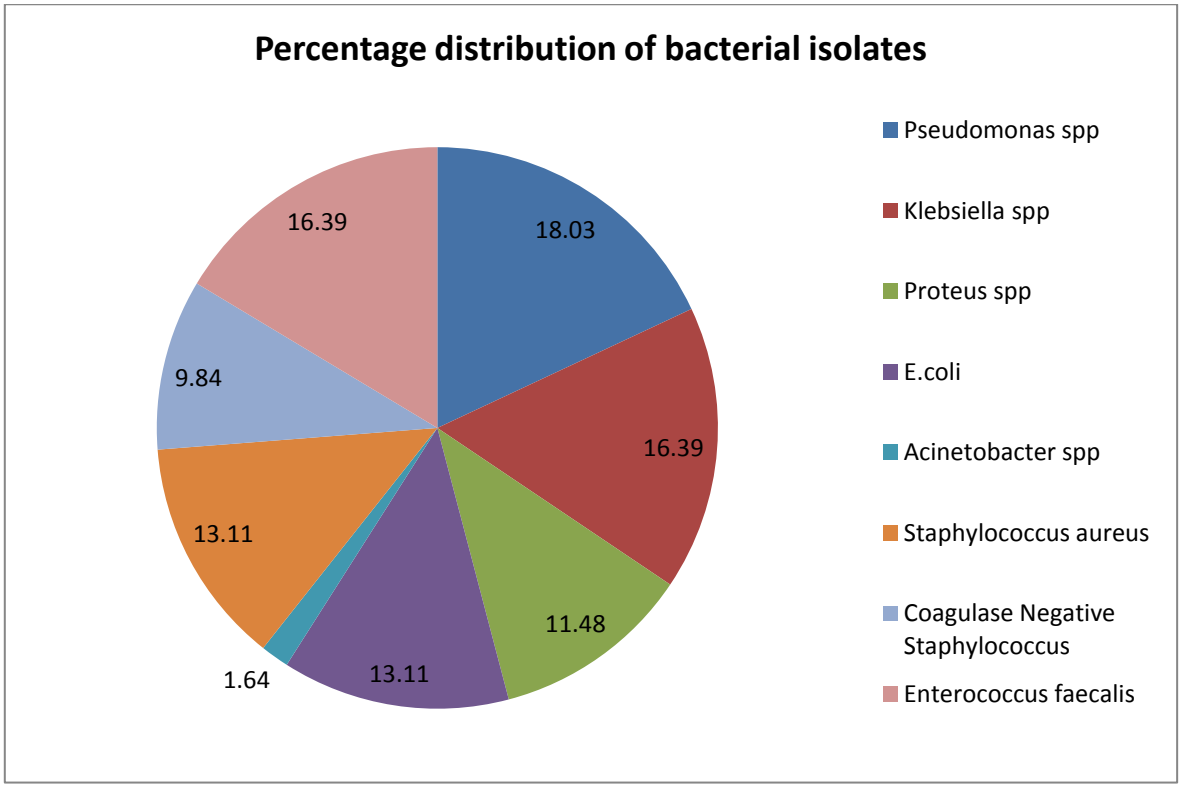
Isolates with MIC ≤ 8 µg/ml were considered as susceptible, 16 µg/ml as intermediate resistant and >32 µg/ml as resistant for teicoplanin.[5]

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.

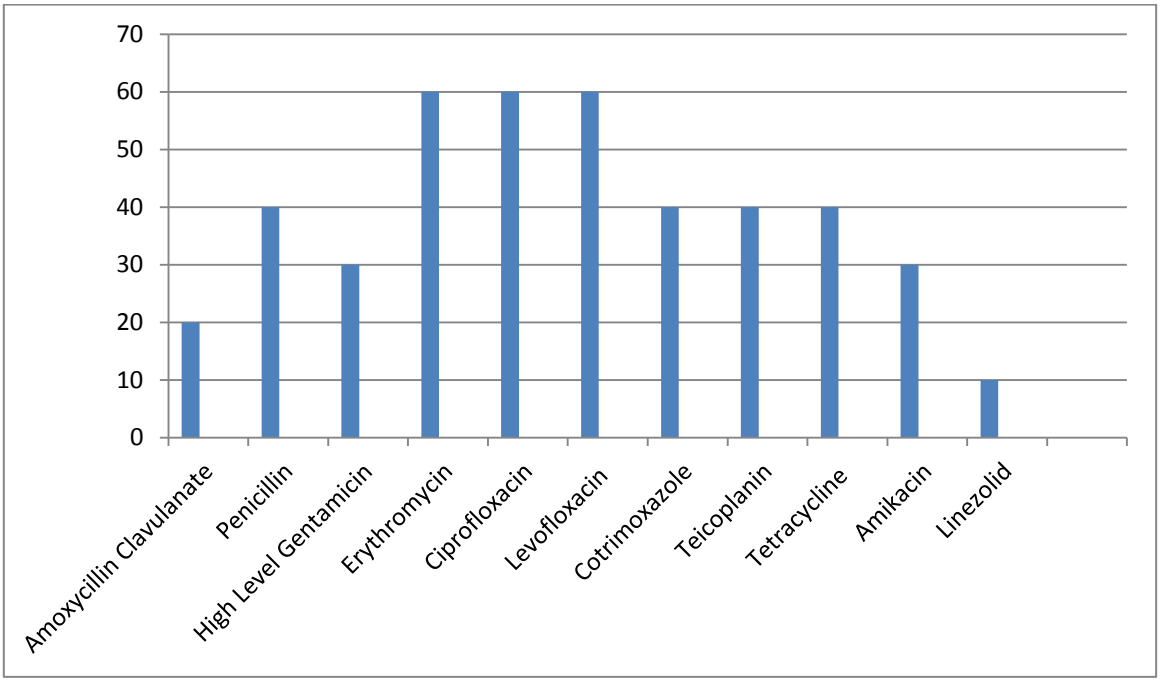
Among the Gram negative bacteria isolated, 18.03% were Pseudomonas spp, 16.39% were Klebsiella spp and 13.11 % were E.coli .Among Gram positive isolates,16.39% were Enterococcus faecalis followed by Staphylococcus aureus (13.11%). [Figure 1]



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]

Figure 2: Antimicrobial resistance pattern of Enterococcus faecalis by Disk diffusion method



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

No of isolates(10)	Vancomycin			Teicoplanin		HLGR	
	≤ 0.5µg	1µg	2µg	≤ 0.5µg	4µg	512µg	1024µg
	7	2	1	9	1	1	2

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.[11] 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[12].

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%[13]. 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. [14,15]. Linezolid is used in the treatment of infections caused by resistant gram positive bacteria particularly vancomycin- resistant

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.

References

1. Brian .L. Hollen beck, Louis. B. Rice. Intrinsic and acquired resistance mechanisms in Enterococcus. Virulence journal. 2012;3(5):421-33.
2. William. R. Miller, Jose. M. Munita, Cesar. A. Arias. Mechanism of antibiotic resistance in Enterococci. Expert Review of Anti Infective Therapy. 2014; 12(10): 1221-36.
3. Cesar. A. Arias, Barbara. E. Murray. The rise of the Enterococcus: beyond vancomycin resistance. Nature Review Microbiology. 2013;10(4):266-78.
4. Y .A.Marothi, H.Agnihotri, D.Dubey. Enterococcal resistance –An overview. Indian Journal of Medical Microbiology 2005;23(4):214-93
5. S.Sujatha, Ira Praharaj. Glycopeptide resistance in gram positive cocci :A Review. Interdisciplinary perspectives on Infectious diseases 2012;1-10
6. Joseph .W. Chow. Aminoglycoside resistance in Enterococci. Clinical Infectious Diseases. 2000;31:586-9
7. R.R. Facklam and M.D Collins. Identification of Enterococcus species isolated from human infections by a conventional test scheme. Journal of Clinical microbiology. 1989;27(4):731-745.
8. Marra A, Dib-Hajj F, Lamb L, Kaczmarek F, Shang W, Beckius G, Millici AJ, Medina I, Gootz TD. Enterococcal virulence determinants may be involved in resistance to clinical therapy. Diagnostic Microbiology and Infectectious Diseases .2007;*58*: 59-65.
9. Clinical and Laboratory Standard Institute (CLSI). Performance standards for

antimicrobial susceptibility testing .26th ed. Wayne USA .CLSI ,2016

10. Martha .L.Sanchez,Mary S Barrett ,Ronald N Jones .Use of E test to Predict High Level Resistance to Aminoglycosides Among Enterococci. Journal of Clinical Microbiology . 1992;30(11):3030-3032
11. Yeliz Tanriverdi Cayci, Keramettin Yanik, Adil Karadag, Hava Yilmaz, Saban Esen, Murat Gunaydin. Changes in antimicrobial resistance of Enterococcus spp. Over a few years. Biomedical Research 2016; 27 (1): 6-10
12. Amelework Yilema, Feleke Moges, Sisay Tadele, Mengistu Endris, Afework Kassu, Wondwossen Abebe and Getnet Ayalew. Isolation of enterococci, their antimicrobial susceptibility patterns and associated factors among patients attending at the University of Gondar Teaching Hospital. BMC Infectious Diseases .2017; 17:276
13. Atreyi Chakraborty, Nishith K. Pal, Soma Sarkar ,Manideepa Sen Gupta .Antibiotic resistance pattern of Enterococci isolates from nosocomial infections in a tertiary care hospital in Eastern India. Journal of Natural Science, Biology and Medicine . 2015:6 (2):394-397
14. Ira Praharaj, S. Sujatha ,Subash Chandra Parija. Phenotypic and genotypic characterisation of vancomycin resistant Enterococcus isolates from clinical specimens. Indian Journal of Medical Research 2013;138:549-556.
15. Purva Mathur, Arti Kapil, Rachna Chandra, Pratibha Sharma & Bimal Das. Antimicrobial resistance in Enterococcus faecalis at a tertiary care centre of northern India. Indian Journal of Medical Research 2003;118: 25-28.
16. Smith PF, Birmingham MC, Noskin GA, et al. Safety, efficacy and pharmacok-inetics of linezolid for treatment of resistant Gram-positive infections in cancer patients with neutropenia. Annals of Oncology. 2003;14:795-801.
17. J. B. Suchitra and N. Lakshmidevi. Vancomycin resistant enterococcus causing surgical site infections in a tertiary care hospital. Journal of Microbiology and Antimicrobials , 2009;1(1): 009-011
18. Richard A. Horvitz and Alexander Von Graevenitza .Clinical Study of the Role of Enterococci as Sole Agents of Wound and Tissue Infection. The yale journal of biology and medicine. 1977 ;50:391-395
19. Joels CS, Matthews BD, Sigmon LB. Clinical characteristics and outcomes of surgical patients with vancomycin-resistant enterococcal infections. The American Journal of Surgery. 2003;69:514-519.