

A paradigm shift in Enterococcal infections: A cause of concern

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Received: 21st August, 2018

Accepted: 1st September, 2018

Abstract

Introduction: Infections due to Gram-positive bacteria are on a rise, of which those due to *Enterococcus* spp. form a major part. The shifting pattern of infections caused by *Enterococci* from novel urinary tract infections to soft tissue and wound infections are increasingly reported.

Objective: The aim of the present study is to isolate, speciate *Enterococci*, determine the antibiotic resistance pattern and to detect vancomycin resistance by E-test.

Materials and Methods: All wound swabs, pus samples sent for bacterial culture, irrespective of the diagnosis of the patients which were received to the department of microbiology for a period of one year were included in the study. The samples were processed for isolation, identification and speciation of *Enterococci*. Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method. Vancomycin resistance was determined by E-test.

Results: A total of 3000 pus samples were received over a period of one year. Out of which 43 isolates were detected as *Enterococcus* species. Among 43 isolates, 26 were *E.faecalis*, 17 were *E.faecium*. In present study, ampicillin resistance was seen in 88% followed by resistance to erythromycin (86%) and clindamycin (72%). High level gentamicin resistance was seen in 28% cases. Vancomycin resistance was not detected in any of the samples.

Conclusion: The shifting spectrum of infections due to *Enterococcus* is gaining inquisitiveness among health care workers especially in the trauma care units. Thus there is a prerequisite to isolate speciate and determine the resistance that helps in holistic health care.

Keywords: *Enterococci*, Soft tissue infection, Antibiotic resistance, Vancomycin resistance, E test.

Introduction

Enterococci, traditionally known as commensal bacteria are now well established as organisms of causing dreaded infections.¹ *Enterococci* is assuming the position of a nosocomial pathogen because of its capacity to attain and transfer the resistance genes. Intrinsic and acquired resistance to all antibiotics currently in use has assisted the bacteria to thrive even in the harsh environment. *Enterococci* show intrinsic resistance to penicillins, cephalosporins, aminoglycosides and lincosamides. *Enterococci* have gained much attention of researchers and health care personnel because of its ability to cause variety of infections. Dramatic change in role of Enterococcal infections from conventional urinary tract infections to wide spread wound infections has alarmed the need to isolate the bacteria. Soft tissue and wound infections due to *Enterococcus* spp. are increasing worldwide emphasizing the need to study the microbiological aspects of the bacteria.² In addition, there is paucity of data regarding the soft tissue and wound infections due to *Enterococcus* spp. and its resistance pattern in India. Hence, the present study was taken to determine the dissemination of *Enterococcus* spp. in wound and soft tissue infections as well as its antibiotic resistance pattern.

Materials and Methods

The present study was conducted in department of Microbiology, Mysore Medical College and Research Institute, Mysore. All wound swabs, pus samples sent for bacterial culture during the study period to the department of Microbiology were included in the study. On receiving the sample, the gross appearance of the specimen whether it is clear, turbid or contains blood and colour was noted. Smears were prepared from each specimen and stained with Gram's stain and acid fast stain as per requirement. Gram's stained smears were examined, first under low power (10 x objectives) to determine the presence and type of cells in the specimen and then observed under oil immersion lens. The relative number of micro organisms and their morphology was studied and recorded. All the samples were inoculated on to the culture media to appreciate the cultural characteristics. The speciation of the *Enterococci* is done by following the standard proforma.^{1,3} Antimicrobial susceptibility testing was done by Kirby-bauer disk diffusion testing. The groups tested were Ampicillin (10mcg), High level gentamicin (120mcg), Tetracycline (30mcg), Clindamycin (2mcg), Erythromycin (15mcg), Chloramphenicol (30mcg), Tigecyclin (15mcg), Linezolid (30mcg), vancomycin (30mcg). The results were interpreted as per CLSI guidelines. E-test was done to detect the vancomycin resistance. MIC testing was done by E- strip method

using Mueller Hinton Agar. A strip with concentration gradient of 0.016 to 256µg/ml was used. The results were interpreted as per CLSI guidelines.⁴

Results

Total of 3000 pus samples were processed in a year, of which 43 *Enterococcus* spp. were isolated. The clinical cases were, 20 (46%) diabetic foot, 05(11%) post operative wound infection, 15(35%) traumatic ulcer, 03(6%) abscess as shown in table 1. Among 43 isolates, 26(60%) were *Enterococcus faecalis* followed by 17(40%) *Enterococcus faecium* as shown in table 2. Among the isolates, male preponderance was seen with male to female ratio being 1.8: 1 as shown in table 3. In the present study, ampicillin resistance was seen in 88% of cases, followed by erythromycin 86%, clindamycin 72%, 28% showed resistance to high level gentamycin. All the isolates were sensitive to linezolid. Vancomycin resistance was not seen in any of the isolates as shown in table 4. All the isolates were subjected to E test for the detection of vancomycin resistance. MIC of vancomycin of all the samples was less than 4mcg. The cultural characteristics of Enterococci on blood agar and MacConkey agar are as depicted in Fig. 1a and 1b respectively. Biochemical tests performed for the identification and speciation of Enterococci are as shown in figure 2a and 2b respectively. E test done for vancomycin is as shown in Fig. 3.

Table 1: Isolation of *Enterococci* from different patients

S. No	Diagnosis	No. of Isolates
1	Diabetic foot	20
2	Traumatic ulcer	15
3	Post op infection	05
4	Abscess	03

Table 2: Number of *Enterococcus* species isolated

Species	No. of Isolates	Percentage
<i>E.faecalis</i>	26	60.5
<i>E.faecium</i>	17	39.5
Total	43	100

Table 3: Number of *Enterococci* in relation to sex group

Gender	No. of Cases	Percentage
Male	28	65
Female	15	35
Total	43	100
Male: Female	1.866:1	

Table 4: Antibiotic susceptibility pattern of *Enterococci*

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	12	88
Clindamycin	28	72
High Level Gentamycin	72	28
Tetracyclin	77	23
Chloramphenicol	77	23
Erythromycin	14	86
Tigecyclin	72	28
Linezolid	100	00
Vancomycin	100	00

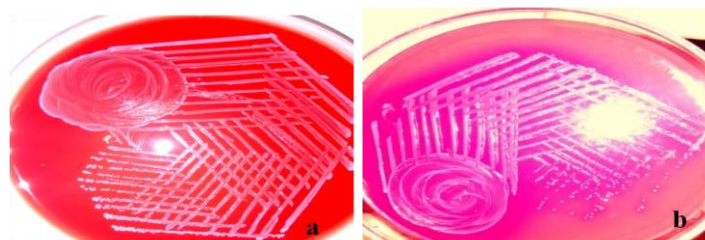


Fig. 1: Photograph showing the cultural characteristics a) on blood agar b) magenta pink colonies on MacConkey agar

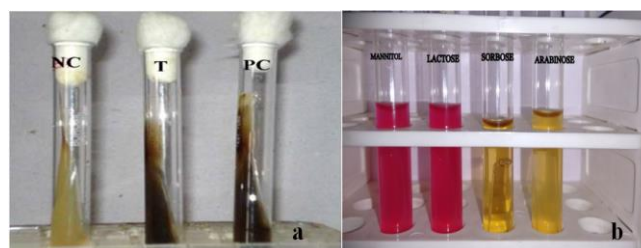


Fig. 2: Photograph showing biochemical tests a) bile esculin hydrolysis b) sugar fermentation tests



Fig. 3: E-test for detection of vancomycin resistance

Discussion

Enterococci, a group of facultative organisms have become increasingly isolated in various hospital settings.⁵ Of late, they have become major cause of concern in causing nosocomial infections, especially of the bloodstream, urinary tract and surgical sites.

Enterococci are second leading cause of nosocomial infections and the third commonest cause of hospital acquired infections in United States.^{1,6} The major infections caused by *Enterococci spp* apart from novel urinary tract infections (UTI) include wound infections, intra-abdominal infections, cholecystitis, bacteremia, endocarditis, and rarely meningitis. Though there is limited literature, wound infections are increasingly reported next to urinary tract infections.^{7,8}

Enterococcus has the ability to grow even in the extreme environmental conditions because of their several intrinsic characters. Furthermore, intrinsic resistance to multiple antimicrobials and the ability of exogenous acquisition of resistance has empowered the bacteria to become a successful pathogen. This in turn generated an additional need for typing the isolates as a means of assisting infection control and epidemiological studies.

In our study out of 3000 pus samples received, 43 samples yielded *Enterococci* in concurrence with the study done by Rajkumari N et al.² Male to female ratio in our study was 1.8:1 which was similar to the study done by Rajkumari N et al.² The male preponderance can be attributed to the occupation and the higher prevalence of trauma in males than in females.

In our present study, two *Enterococcus* species were identified, 60.5% *E. faecalis* and 39.5% *E. faecium* which is in concurrence with the trend reported worldwide. *E. faecalis* was reported to be responsible for about 80 to 90% infections followed by *E. faecium* that is found in 5–10% of Enterococcal infections.^{9,10}

The emergence of multi drug resistant *Enterococci* has lead to a scenario which is almost as precarious as the pre antibiotic era since many of these multi-drug resistant (MDR) strains have developed resistance to practically all available antibiotics.¹¹ In our present study, ampicillin resistance was seen in 88% followed by resistance to Erythromycin (86%) and clindamycin

(72%). High level gentamycin resistance was seen in 28% cases. Intrinsic capability to show resistance, ability to acquire resistance and capacity to transfer it are the major reasons that can be attributed to the emergence of multidrug resistance in *Enterococci*. Efficient control of multiple-drug resistant *Enterococci* can be achieved by comprehensive study on the pattern of the resistance and hence implementing the antibiotic policy which helps in judicious administration of the antibiotics curbing the transmission of the resistance.¹²

In our study resistance to vancomycin is not seen in any of the isolates. The MIC of the vancomycin among 43 isolates was less than 4mcg. The prevalence of VRE in India is much lower when compared to western world.¹³ Vancomycin resistance is a paramount issue because of the difficulty in identification and treatment. Further, it poses a major threat because of its potential to transfer the plasmid mediated resistance to other microorganisms.

A coordinated effort by various departments should be made in educating the hospital staff regarding the problem of drug resistance, the vigilant use of antimicrobials by physicians, prompt reporting. Usage of appropriate procedures by laboratories and an immediate implementation of the appropriate infection control measures also help in curbing the spread of infection right at its source. There should be a coordinated approach among the clinical departments and the laboratories to tackle such superbugs. This eventually helps to prevent the emergence of vancomycin resistant *Enterococcus* and can also reduce the burden of multidrug resistant *Enterococcus*. Changing spectrum of Enterococcal infections and their emergence as nosocomial pathogens emphasizes the importance of isolation and determination of resistance pattern to formulate efficient antibiotic policy and infection control protocol for the holistic health care.

Conclusion

Enterococci have long been recognized as low virulence bacteria occurring as commensals in the human intestine. Currently their role is better defined in causing wound and soft tissue infections in trauma care facilities, especially in the ICUs. Thus it becomes necessary to identify, speciate and manage infections caused by *Enterococci* at various levels.

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How to cite this article: Archana R. K, Ravindranath C. A paradigm shift in Enterococcal infections: A cause of concern. *Indian J Microbiol Res*. 2018;5(4):442-445.