

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.612.281>

Study of Enterococci with a Special Reference to Species Distribution and Antibiotic Resistance Pattern

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ABSTRACT

Keywords

Enterococci, Disc diffusion, *Enterococcus faecalis*, Species distribution, Vancomycin resistant.

Article Info

Accepted:

17 October 2017

Available Online:

10 December 2017

Glycopeptide-resistant enterococci have become ascendant nosocomial pathogens. Vancomycin resistant enterococci (VRE) can significant increase morbidity and mortality in hospitalized patients. The emergence of VRE is of concern because infections due to these organisms can't be treated with currently available antimicrobial drugs. The present study was conducted with an aim to identify enterococci isolates to the species level and to assess their antibiotic susceptibility profile. Enterococcal isolates were identified as per standard microbiological protocol and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion technique. Majority of isolates were obtained from urine samples. *E. faecalis* followed by *E. faecium* were the predominant isolates. Vancomycin resistance was noted in a total of 21 (10.4%) enterococcal isolates. The high prevalence of isolation of *Enterococcus* spp. from clinical specimens and their ability to acquire resistance to the limited number of antibiotics available for treatment of enterococcal infections have highlighted the importance periodic surveillance programmes to identify the colonization with VRE and initiate prompt infection control measures.

Introduction

During the past 75 years, the “Antibiotic voyage” has met various major and minor turbulences of antibiotic resistance. The abuse of antibiotics like “pill for every ill”, self-medication, over the counter sell, starting broad spectrum antibiotics as the first line of therapy, treating viral fevers with antibiotics, excessive use of antibiotics in agriculture and animal husbandries and other types of misuses have lead to increased resistant rates by many multidrug resistant pathogens called as “Super Bugs” like methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus*, vancomycin resistant enterococci (VRE), extended

spectrum beta lactamase (ESBL) producers. In recent years, enterococci have emerged as important cause of health-care associated or nosocomial infections. In the US, enterococci are second most common cause of hospital associated urinary tract and wound infections and the number three cause of nosocomial blood stream infections (Schaberg *et al.*, 1991). Enterococci demonstrate both intrinsic and acquired resistance to most of commonly used antibiotics. Enterococci are inherently resistant to penicillinase-susceptible penicillin (low level), penicillinase-resistant penicillins, cephalosporins, lincosamides, nalidixic acid, low level of aminoglycoside and low level of

clindamycin (Murray, 1990). Resistance to penicillin by β -lactamases, high level aminoglycoside resistance (HLAR), vancomycin, chloramphenicol, erythromycin, high levels of clindamycin, tetracycline, and fluoroquinolone resistance are examples of acquired resistance (Murray, 1990).

Glycopeptide-resistant enterococci have become ascendant nosocomial pathogens. Like MRSA, VRE can significantly increase morbidity and mortality in hospitalized patients. The emergence of VRE is of concern because infections due to these organisms can't be treated with currently available antimicrobial drugs (HICPAC, 1995). Therefore present study was conducted with an aim to identify enterococci isolates to the species level and to assess their antibiotic susceptibility profile.

Materials and Methods

The present study was carried out in the Department of Microbiology of tertiary care hospital for the period of two years (January 2014 to December 2015). The study type was descriptive cross sectional. Enterococcal isolates from various clinical specimens were included in the study. Following were inclusion and exclusion criteria.

Inclusion criteria: Enterococcal isolates from clinical samples like blood, urine, pus, wound swab, catheter tip and other body fluids.

Exclusion criteria: Commensal enterococcal isolates from gastrointestinal tract, female genital tract and oral cavity.

The tests used for identification and speciation of enterococci included colony morphology on blood and MacConkey's agar, morphology on gram staining, catalase test, bile esculin test, growth in the presence of 65% NaCl, growth at 45⁰C and 65⁰C,

fermentation of sugars (1% glucose, mannitol, sorbose, sorbitol, arabinose, raffinose, lactose, and sucrose), arginine hydrolysis, tellurite reduction, H₂S production and pigment production.

Enterococcal isolates were tested for susceptibility against antibiotics like penicillin, ampicillin, chloramphenicol, erythromycin, amikacin, ciprofloxacin, linezolid, teicoplanin and vancomycin by the Kirby Bauer disc diffusion method. High level aminoglycoside resistance was tested against gentamicin (120 μ g) and streptomycin (300 μ g). Antibiotic discs were procured from HiMedia Laboratories Pvt. Ltd. The strength of discs and their zone size interpretative standards were as per the Clinical and Laboratory Standard Institute guidelines (CLSI, 2007). *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

Results and Discussion

During the study period, a total of 173 enterococci were isolated from various clinical specimens. As shown in figure 1, majority of isolates were from urine samples (58.9%) followed by pus (17.9%) and blood (10.9%).

The species wise distribution of enterococcal isolates is shown in figure 2, *E. faecalis* (n= 123, 71.1%) followed by *E. faecium* (22.5%) were the predominant isolates.

The sample and species wise distribution of enterococcal isolates is shown in table 1. *E. faecalis* was the major isolate from various clinical specimens except for blood. *E. faecium* was the predominant isolate from blood culture.

The antibiotic resistance pattern of *Enterococcus* spp. is shown in figure 3.

Among the enterococcal isolates, maximum resistance was noted to penicillin (91.3%) followed by erythromycin (77.4%) and amikacin (71.1%). A total of 63 (36.4%) isolates were resistant to high level gentamycin whereas, 65 (37.5%) isolates showed resistance to high level streptomycin. Vancomycin resistance was noted in a total of 21 (10.4%) enterococcal isolates.

A higher percentage of resistance to all antibiotics tested was noted among *E. faecium* isolates compared to *E. faecalis*. *E. avium*, *E. durans* and *E. gallinarum* were sensitive to penicillin, ampicillin, high level gentamycin, high level streptomycin, linezolid and vancomycin.

The comparison of antibiotic resistance pattern of *E. faecalis* and *E. faecium* is shown in figure 3. All isolates of *E. faecium* were

resistant to penicillin. Although the rate of vancomycin resistance was high among *E. faecium* isolates there was no significant difference observed between vancomycin resistance in *E. faecalis* and *E. faecium* isolates (Fischer Exact test, *P* value 0.167).

In recent years there has been a great deal of concern about the emergence of antimicrobial-resistant organisms. Bacteria and other microbial pathogens have shown a remarkable ability to develop resistance to virtually all class of antibiotics.

The development of vancomycin resistance in *Enterococci* is perhaps, the most impressive accomplishment of bacteria to date. In contrast to coliforms and other enteric bacteria, the enterococci are rather resilient and can survive for longer period in soil and water, and thus re-enter the food chain.

Table.1 Sample and species wise distribution of enterococcal isolates

Clinical specimen	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. durans</i>	<i>E. gallinarum</i>	<i>E. avium</i>	Total
Urine	78	21	02	01	-	102
Pus	23	05	01	-	02	31
Blood	08	09	-	01	01	19
Ascitic fluid	09	03	-	-	-	12
Pleural fluid	03	01	-	-	-	04
ET tube	-	-	01	01	01	03
CSF	02	-	-	-	-	02
Total	123	39	4	3	4	173

Fig.1 Specimen wise distribution of enterococcal isolates

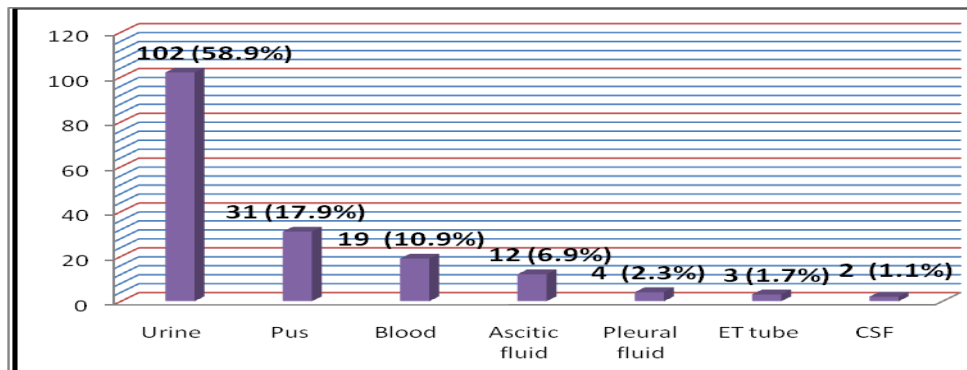


Fig.2 Species wise distribution of enterococcal isolates

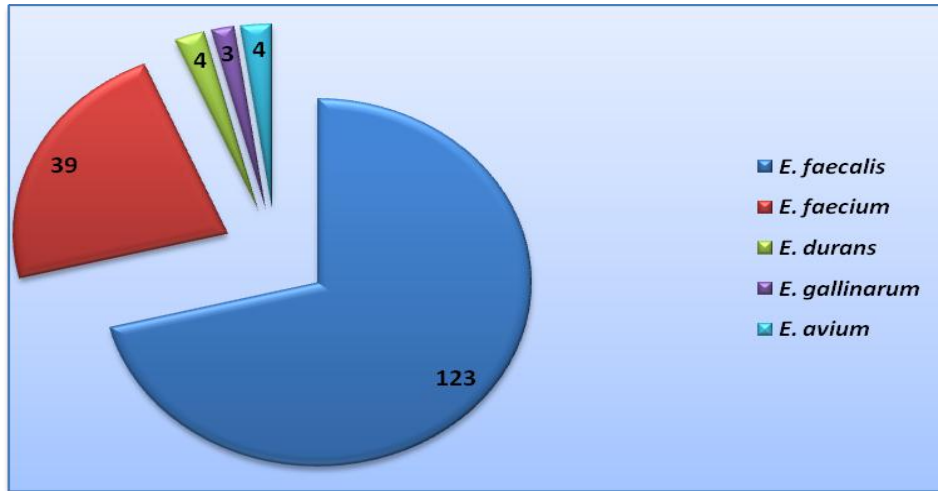
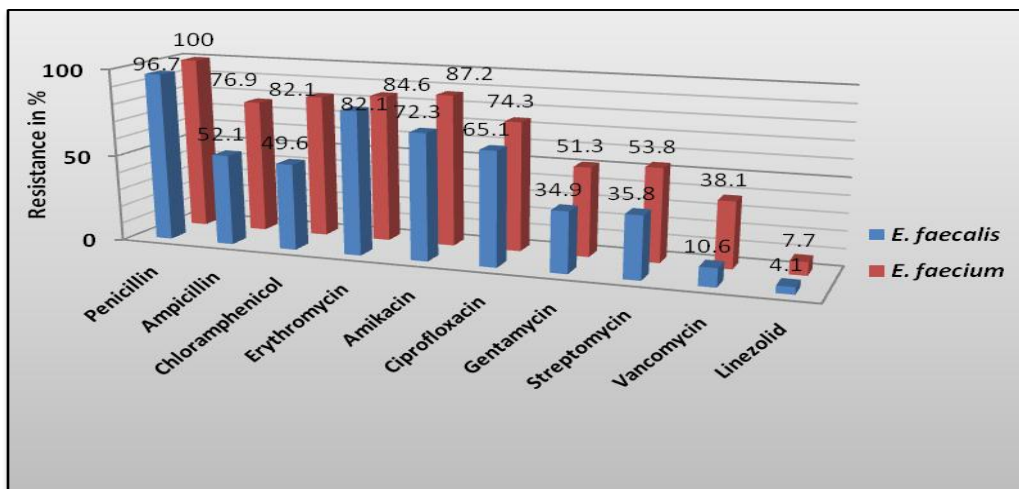
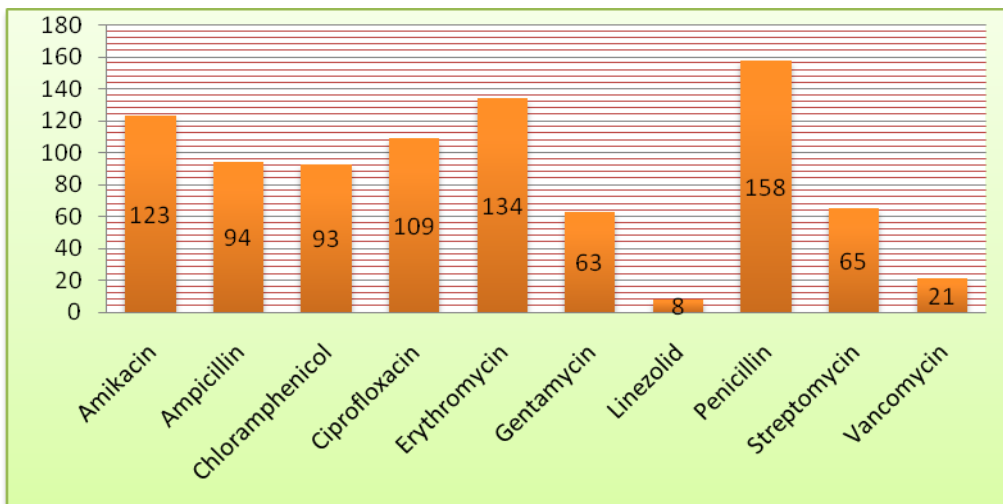


Fig.3 Antibiotic resistance pattern of *Enterococcus* spp



Despite relative lack of pathogenicity, enterococci have emerged as important health-care associated pathogens in many countries. One of the major reasons for increased isolation of *Enterococcus* spp. from health-care associated infections is their innate resistance to several classes of commonly used antibiotics and, perhaps more important, their ability to acquire resistance to currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons (Cleveland, 1990). The present study was conducted with an aim to identify the enterococcal isolates to the species level and to assess the antibiotic resistance pattern to widely prescribed antibiotics with a focus on vancomycin resistance.

In the present study, the maximum isolates were obtained from urine followed by pus and blood cultures. Our observation is in accordance to that of Gordon *et al.*, (1992), Udo *et al.*, (2002) and Chaudhary *et al.*, (2007). Nosocomial bacteriuria is the most common type of infection caused by *Enterococcus* spp (Jett *et al.*, 1994). The bladder, prostate, and kidneys are commonly infected by these bacterial pathogens, especially in patients with structurally abnormal urinary tract or presence of indwelling catheters (Jett *et al.*, 1994). Blood stream infections (BSI) due to *Enterococcus* spp. are much more common than enterococcal endocarditis (Murray, 1990). Severity of illness, patient's age, and use of broad-spectrum antibiotics are risk factors associated with enterococcal BSI (Green *et al.*, 1995).

Species identification plays an important role in investigation of nosocomial outbreak and selection of most appropriate antimicrobial agent for therapy (Gordon *et al.*, 1992). In severe infections (BSI or meningitis) species identification along with determination of

high level aminoglycoside resistance to gentamicin and streptomycin should be strongly encouraged because of variation in susceptibility profile to different classes of antibiotics by different *Enterococcus* spp (Gordon *et al.*, 1992). In the current study, *E. faecalis* (71.9%) and *E. faecium* (22.5%) were major species isolated from various clinical specimens. Similar findings were reported by other researchers. *E. faecalis* is found in higher numbers than *E. faecium* and other species in the faeces of most healthy adults. *E. gallinarum* was isolated from a total of 3 (1.7%) clinical samples. *E. gallinarum* is rarely recovered from clinical specimens. However Vandamme *et al.*, (1996) observed that 84% of the VRE species recovered from faecal specimens of critical patients were *E. gallinarum*.

In the present study, penicillin resistance was observed in 91.3% of enterococcal isolates. A higher percentage of resistance was noted among *E. faecium* (100%) compared to *E. faecalis* (96.7%). Similar finding was reported by Karmarkar *et al.*, (2004) and Mendiratta *et al.*, (2008). *E. faecalis* producing β -lactamase are resistant to anti-staphylococcal penicillins but are susceptible to ampicillin, amoxicillin, and piperacillin, combined with penicillinase inhibiting drugs like clavulanic acid, sulbactam and tazobactam. *E. faecium* do not produce penicillinase, yet confer high level resistance (Lin, *et al.*, 1991).

Ampicillin resistance was observed in a total of 94 (54.3%) isolates. Similar rate of ampicillin resistance in enterococcal isolates were reported by many other workers. Unlike other researchers, Karmarkar *et al.*, (2004) reported a higher percentage of ampicillin resistance among *E. faecalis*. In the present study, ciprofloxacin resistance was observed in 63% of the enterococcal isolates. Similar observation was reported by Mathur *et al.*, (2003) and Karmarkar *et al.*, (2004). *In vitro*

bactericidal effect of ciprofloxacin is highly inoculum dependent; when used alone or in combination with gentamicin in the treatment of experimentally induced endocarditis in rabbits, ciprofloxacin was significantly less effective than penicillin alone or in combination with gentamicin. Therefore ciprofloxacin may not be the best alternative for treating serious enterococcal infections (Gordon *et al.*, 1992).

In the present study, a total of 21 (10.4%) isolates showed resistance to vancomycin. The rate of vancomycin resistance in *E. faecalis* and *E. faecium* was 10.6% and 38.1% respectively. Uttley and colleagues (1988) were the first to report the isolation of vancomycin resistant *E. faecium* and *E. faecalis*. Vancomycin resistance in enterococcal isolates has been observed to generally be on the rise over the years. The emergence of vancomycin resistance in enterococci poses a serious challenge for physicians treating patients with infections due to these organisms.

In recent years, *Enterococcus* spp. have been identified as an important cause of a wide variety of infections, particularly UTI and wound infections. These bacterial pathogens have emerged as one of the major causes of health-care associated infections. The rising incidence of enterococcal infection along resistance to commonly prescribed antibiotics has highlighted the importance of adherence to the antibiogram provided by the clinical microbiologist to treating physicians.

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How to cite this article:

Satyendu Saha and Nagaraju Vanaparthi. 2017. Study of Enterococci with a Special Reference to Species Distribution and Antibiotic Resistance Pattern. *Int.J.Curr.Microbiol.App.Sci.* 6(12): 2432-2438. doi: <https://doi.org/10.20546/ijcmas.2017.612.281>