



eISSN: 2321-323X
pISSN: 2395-0781

Research article

Antibiotic susceptibility pattern of enterococcal isolates from a tertiary care hospital

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Abstract

Enterococci have been regarded as pathogens that seldom cause serious infections. However, in the last decade they have emerged as an important cause of nosocomial infections, with an increasing frequency of multidrug resistance, including high-level resistance to gentamicin and resistance to ampicillin. It is noted that, 5–8 Glycopeptide resistance among enterococci has become increasingly common in many European countries and in North America. **Aim:** To study the Antibiotic susceptibility pattern of Enterococci and to detect Vancomycin resistant Enterococci (VRE) among the isolates. **Materials and Methods:** Two hundred (200) clinical isolates were collected from patients admitted from Government Rajaji Hospital, Madurai. Further, various specimens like Blood, Urine, Pus Swab and CSF were collected from the patients depending on the clinical symptoms. The antimicrobial susceptibility testing was carried out by the Kirby Bauer disk diffusion Method. The antibiotics used were Ampicillin, Erythromycin, High level Gentamycin, Ciprofloxacin, Doxycycline, Nitrofurantoin, Vancomycin and Linezolid. **Results:** Distribution of Enterococcus in this study showed that Enterococcus isolated from blood 20(55.5%), urine 14(38.8%), pus 1(2.7%) and wound swab 1(2.7%). On speciation, *E.faecium* 18(50%), followed by *E.faecalis* 15(41.6%) and *E.durans* 3(8.3%). **Conclusion:** This study demonstrates the increased prevalence of multidrug resistant enterococci with few isolates being resistant to all the antibiotics tested, thus posing a serious therapeutic challenge.

Keywords: Enterococci, antibiogram, Nosocomial infections

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1. Introduction

Enterococci are the most common aerobic and facultative anaerobic, gram positive cocci [1]. Enterococci have been considered as relatively low virulence but they are known to cause various clinical infections like urinary tract infections, endocarditis, intra abdominal and pelvic infections [2]. Enterococcus *faecalis* and Enterococcus *faecium* are most common species, both accounts up to 90% of clinical isolates [1]. Occasionally, human infections can be due to Enterococcus *raffinosis*, Enterococcus *casseliflavus*, Enterococcus *durans*, or Enterococcus *avium* [3].

The emergence of resistance to the most common anti-enterococcal antibiotics which include the β -lactam antibiotics like ampicillin, amino glycosides and most importantly glycopeptides like vancomycin besides being

inherently resistant to many others like cephalosporins and clindamycin has made the treatment of these infections a real challenge for clinicians. The high level resistance to aminoglycosides has made the therapeutic combination of penicillin and gentamicin ineffective [4]. With the increase in emergence of resistance in enterococci to vancomycin, treatment of these infections has become difficult especially in serious infections.

2. Materials and methods

This Prospective study was conducted at Government Rajaji Hospital, attached to Madurai Medical College after getting ethical clearance from the Human Ethical Committee, GRH, headed by the Dean. The study population consisted of 200 patients admitted in different

wards viz Paediatrics, General Surgery, Urology and Medicine at Government Rajaji Hospital, Madurai. Various specimens like Blood, Urine, Pus Swab and CSF were collected from the patients depending on the clinical symptoms. Blood was collected with strict aseptic precautions. Pus samples were collected in syringes or swabs. Urine samples were collected by sampling the mid stream flow by the clean catch technique. CSF was collected in cases with meningitis [5].

Gram's Positive cocci, Catalase negative, Salt tolerant, Bile esculin agar positive isolates, which were able to grow on MacConkey agar at temperatures of 10 > 45°C, and pH 9.6 were identified as *Enterococci* and selected for further speciation [6,7].

Speciation was done with fermentation of carbohydrates (Arabinose, Raffinose, Sucrose, Sorbitol and Mannitol), Arginine hydrolysis, Pyruvate fermentation, reduction of Potassium tellurite.

The different species of Enterococci were further subjected to antimicrobial susceptibility testing by the Kirby Bauer disk diffusion Method. The antibiotics used were Ampicillin, Erythromycin, High level Gentamycin, Ciprofloxacin, Doxycycline, Nitrofurantoin, Vancomycin and Linezolid. A few colonies of the isolates were inoculated into peptone water, which was standardized to a density equivalent to 0.5 McFarland opacity. The inoculum was then spread across the surface of Mueller Hinton agar plate of 9 cm. size to give a confluent growth. Filter paper discs of 6 mm size containing the specific concentration of the antibiotics were placed on the agar surface with 24 mm apart. Six discs were kept on the surface of the plate at a time. The plates were incubated over night at 37°C for 24-48 hrs. After incubation the diameter of the zone of growth inhibition was measured by a graduated ruler and the sensitivity and resistant pattern were identified following CLSI guidelines [8].

3. Result

Distribution of Enterococcus in this study showed that Enterococcus isolated from blood 20(55.5%), urine 14(38.8%), pus 1(2.7%) and wound swab 1(2.7%). On speciation, *E. faecium* 18(50%), followed by *E. faecalis* 15(41.6%) and *E. durans* 3(8.3%).

The susceptibility and resistance patterns of Enterococci were studied. Out of 18 *E. faecium* isolates, 17 (94.4%) were resistant to Ampicillin, 13 (72.2%) to Ciprofloxacin, 12 (66.6%) to Erythromycin, 12 (66.6%) to High Level Gentamycin, 11 (61.1%) to Doxycycline, 13 (12.2%) to Nitrofurantion. 1 (5.5%) showed Vancomycin resistance. All the isolates were sensitive to Linezolid.

Out of 15 *E. faecalis*, 13(86.6%) were resistant Ampicillin, 10(66.6%) to Doxycycline, 9(60%) to Nitrofurantion, 7(46.6%) to Erythromycin, 6(40%) to

High Level Gentamycin, 4(26.6%) to Ciprofloxacin. All the isolates were sensitive to both Vancomycin and Linezolid [9].

Out of 3 *E. durans*, 100% resistances were seen with Ciprofloxacin. Out of the 3 isolates 2(66.6%) were resistant to Doxycycline, 2 (66.6%) to Nitrofurantion, 1(33.3%) to Erythromycin, 1 (33.3%) to Ampicillin. All isolates were sensitive to High Level Gentamycin, Vancomycin and Linezolid [10].

Thus it was found that among all 36 isolates, 88.5% resistance to Ampicillin, and 50% resistance to High Level Gentamycin and 2.7 % resistance to Vancomycin. All the isolates were sensitive to Linezolid. It was noted that among the species, *E. faecium* showed more resistance than other species. The Antibiotic Sensitivity and Resistance pattern is given in Fig 3 and 4.

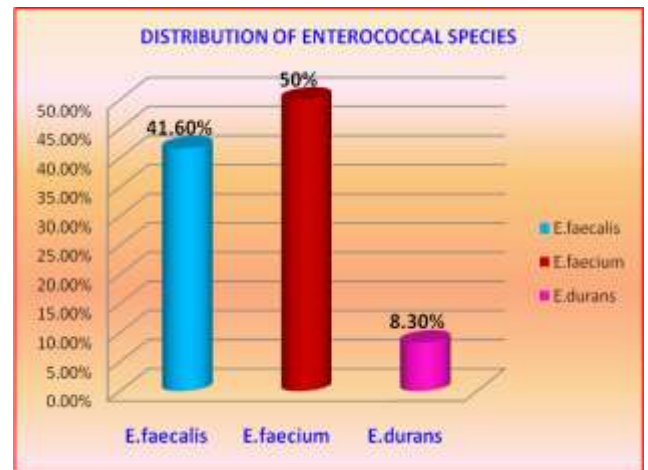


Fig-1

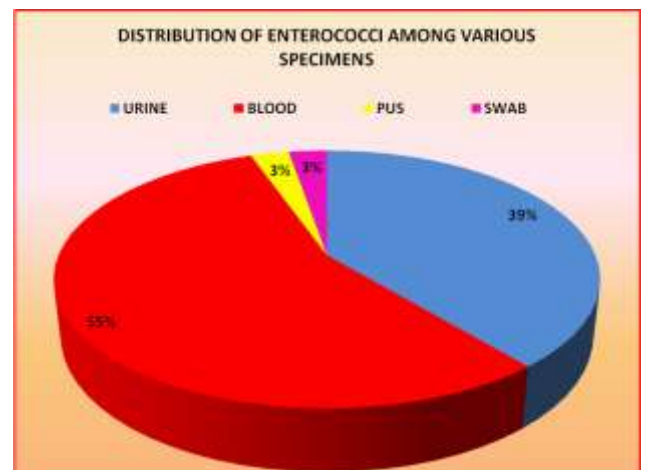


Fig-2

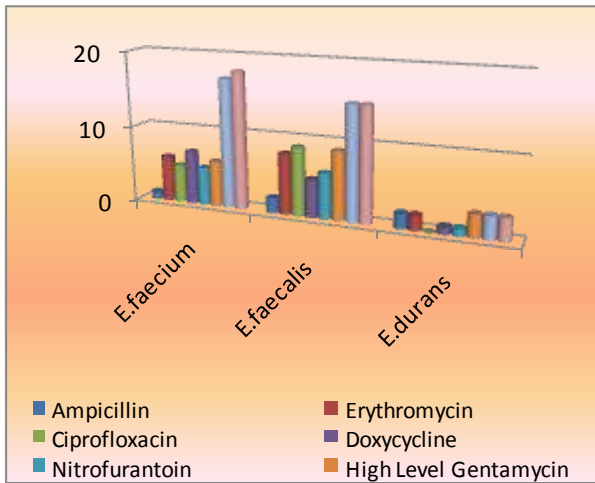


Fig 3 Antibiotic sensitivity pattern in enterococci species wise

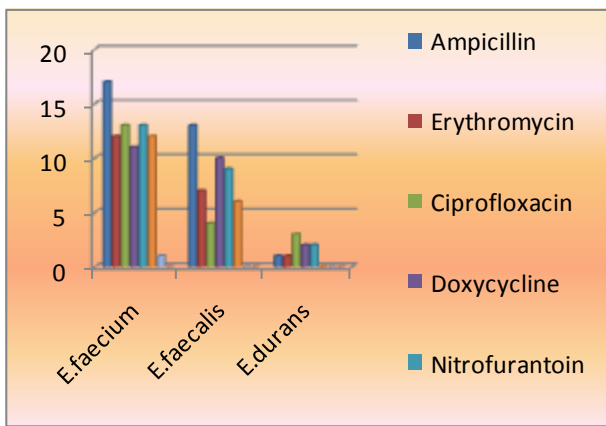


Fig 4 Antibiotic resistance pattern in enterococci -species wise

4. Discussion

The present study on antibiotic susceptibility pattern of Enterococcus species showed that E. faecium was resistant to common antibiotics like Ampicillin (94.4%), High Level Gentamycin (66.6%), Ciprofloxacin (72.2%), Doxycycline (61.1%), Erythromycin (66.6%), Nitrofurantoin (72.2%) and Vancomycin (5.5%). This is in accordance with the study [11] who showed E. faecium to have resistance to High Level Gentamycin (77.7%), Ciprofloxacin (81%) and Erythromycin (86%)⁹⁶ but resistance to Ampicillin was only 54%. [12] Have documented 85.5% resistance to Nitrofurantoin, 100% resistance to Gentamycin and 28.57% resistance to Vancomycin. During the period of study, antibiotics used in the pediatric ward were Ampicillin, Gentamycin, third generation cephalosporins and Vancomycin. All these antibiotics were given intravenously through the infusion lines in their lower extremities either twice a day or three times a day. Obviously the isolates were resistant to most of the antimicrobials as well as to third generation

cephalosporins which were already proved to be a resistant drug (intrinsically resistant). Imprudent use of antibiotics and colonisation pressure are the important causes of the drug resistance in enterococci [13].

Conclusion

This study demonstrates the increased prevalence of multidrug resistant enterococci with few isolates being resistant to all the antibiotics tested, thus posing a serious therapeutic challenge. This situation warrants the implementation of an efficient infection control program and regular surveillance of antimicrobial resistance of enterococci in order to establish a rational antibiotic policy for the better management of enterococcal infections.

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