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Original Research Article

Identification of *Enterococcus.faecalis* and *E.faecium* among *Enterococci* isolated from clinical samples in a teaching hospital Mandya Institute of Medical Sciences, Mandya

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ABSTRACT

Background: *Enterococci* are common commensal of gastrointestinal tract of human beings. They stand as one of the major causative agent in Nosocomial infections. *Enterococcus* has gained clinical importance due to their intrinsic and multidrug resistance. Speciation of *Enterococci* is important as faecalis being sensitive to vancomycin whereas faecium remains to be resistant, so isolating and treating *Enterococcus* has become an important task.
Objective: 1). To determine the species of Enterococci. 2). To determine the antibiogram of *Enterococci.*
Materials and Methods: A Prospective study was conducted at a tertiary care hospital in MIMS, Mandya. Standard protocols were followed for isolating & speciation of Enterococcus. As per CLSI guidelines antibiotic susceptibility testing was done.
Results: Out of 42 *Enterococcal* isolates majority were isolated from urine (59.52%) followed by pus (26.19%), blood (9.52%) and sterile body fluids (4.77%).
Enterococcus faecalis were (88.1%) and *Enterococcus faecium* were (11.9%). Among gender distribution, majority (54.77%) were females and (45.23%) were males. All the isolates were susceptible to Linezolid. Maximum resistance was seen against Penicillin and Tetracycline.
Conclusion: The appropriate infection control measures, use of antibiotics prescribed based on sensitivity obtained and avoid the empiric use of antimicrobials by clinicians can prevent the burden of drug resistance in *Enterococcus.*

1. Introduction

Enterococci are gram-positive organism that occurs in singles, pairs, and short chains. The cells look like coccobacilli if the Gram stain is prepared from agar plate growth. They are characterized by Lance field classification as group D streptococcus. [1]

Enterococci are facultative anaerobes that are able to grow under extreme conditions including 6.5% NaCl, high pH, 40% bile salts, and a temperature range of 10ºC-45ºC. [1]

The most accurate way to identify *Enterococcus* sp is to demonstrate that unknown is catalase-negative gram positive coccus, PYR (an abbreviation for L-pyrrolidonyl-β-naphthylamide) and LAP (leucine-beta-naththylamide) positive, bile esculin test, Arginine hydrolysis, mannitol fermentation positive. [1]

Enterococci are common commensals found in gastrointestinal, genital and urethral tract. It can be found in water, plants, soil, food, animals, birds and insects. [2]

Enterococus infections were considered to be acquired from the patient's own normal flora. It has emerged as nosocomial pathogen because of the development of

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antimicrobial resistance.[2]

Common clinical infections caused by *Enterococci* are urinary tract infections, endocarditis, pelvic infections, bacteremia and intra-abdominal, less commonly they can cause wound and soft tissue infections, meningitis, and respiratory infections.[1]

Enterococci are intrinsically resistant to antibiotics like penicillinase susceptible penicillin, penicillinase resistant penicillin, nalidixic acid, cephalosporin, clindamycin and aminoglycoside. *E.faecalis* is the predominant species implicated in infection followed by *E.faecium.*[3]

Recent evidence suggests that the prevalence vancomycin-resistant *E. faecium* is increasing. The resistance to vancomycin and ampicillin, is commonly associated with *E. faecium* than with *E. faecalis* Speciation of *Enterococcus* is important because vancomycin which is the drug of choice in *Enterococcus* infections cannot be used in faecium.[3]

The present study was undertaken for speciation of *Enterococci* and assessment of their antibiogram that will be helpful in choosing optimal empiric therapy and formulate antimicrobial policy.

2. Materials and Methods

A Prospective observational study was conducted in the Department of Microbiology culture laboratory in Mandya Institute of Medical Sciences, Mandya after getting ethical clearance from the institute.

2.1. Study period

Six months (July 2018 to December 2018).

2.1.1. Sample size

100.

2.1.2. Inclusion criteria

Enterococcus isolated from clinical sample like pus, urine, blood and sterile body fluid.

2.1.3. Exclusion criteria

Enterococcus isolated from clinical sample like sputum, stool, throat & GIT (Gastrointestinal tract).

2.2. Methods

Pus and Urine samples received in Microbiology laboratory for culture and sensitivity were inoculated onto MacConkey agar and blood agar and incubated at 37ºC for 18-24 hours. Inoculated culture plates were observed for growth and samples were processed according to CLSI standards.[4]Organisms which exhibited these characters such as catalase negative, gram positive, bile esculin agar positive, ability to grow in the presence of 6.5% sodium chloride and heat tolerance test were identified as *Enterococcus*.[4]

Speciation of *Enterococci* was carried out by Hippurate hydrolysis test and sugar fermentation test. a) Hippurate hydrolysis test- 0.1% solution of sodium hippurate media was prepared, a loopful of solid growth from blood agar plate were inoculated and incubate at 37ºC for 2 hours. 0.2ml of Ninhydrin solution was added and incubated further for 10 minutes at 37ºC. Test tube which turns Purple indicates glycine production on hydrolysis of hippurate.

b) Sugar fermtation test was carried out for Arginine, Sorbitol and Pyruvate. *Enterococcus* which fermented sorbitol and pyruvate was considered faecalis and isolate which fermented Arginine was considered faecium.[2,4]

Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method according to CLSI guidelines.[4]

The following antibiotics were tested- Ampicillin (10μg), Erythromycin (15μg), Chloramphenicol (30μg), Tetracycline (30μg), Ciprofloxacin (5μg), High level Gentamycin (120μg), Linezolid (30μg), Vancomycin (30μg),Norfloxacin (10μg), Nitrofurantoin (300μg), Nalidixic acid (30μg).[4]

3. Results

Out of 42 *Enterococcal* isolates maximum were isolated from urine 25 (59.52%) followed by pus11 (26.19%), blood 4 (9.52%) and sterile body fluids 2(4.77%) as shown in(Table 2). Out of 42 *Enterococcal* isolates *Enterococcu. faecalis* were 37(88.1%) and *Enterococcus. faecium* were 5(11.9%). Among the gender distribution, Out of 42 *Enterococcal* isolates majority 23 (54.77%) were males and 19(45.23%) were females as shown in-(Table 1).

Table 1: Gender wise distribution of Enterococcus species

Gender	Total	Percentage
Male (n=42)	23	54.77%
Female (n=42)	19	45.23%

Table 2: Distribution of Enterococcus species among clinical sample

S. No	Samples	Total (n=42)
1	Urine	25 (59.52%)
2	Pus	11 (26.19%)
3	Blood	4 (9.52%)
4	Sterile fluid	2 (4.77%)

Out of 42 isolates, all were sensitive to Linezolid, followed by Vancomycin showing 100% sensitive to faecalis and 82% sensitive to faecium, Teichoplanin showing 87.09% for E.faecalis and 90.90% for *E.faecium. E.faecalis* showed maximum resistance for Penicillin G with 85.29%, followed by Tetracycline with 80.64%. *E.faecium* also showed maximum resistant for Penicillin

Table 3: Antibiotic profile of Enterococcus species among urine samples

Antibiotics	**E. faecalis (n=31)**		**E. faecium (n=11)**	
All samples	Susceptible	Resistant	Susceptible	Resistant
Penicillin-G	2(14.71%)	29(85.29%)	3(27.28%)	8(72.72%)
Ampicillin	21(61.30%)	12(38.70%)	5(45.46%)	6(54.54%)
High level gentamicin	24(77.41%)	7(22.59%)	4(36.37%)	7(63.63%)
Erythromycin	11(35.48%)	20(64.51%)	5(45.46%)	6(54.54%)
Chloramphenicol	15(48.39%)	16(51.61%)	7(63.63%)	5(45.46%)
Teicoplanin	27(87.09%)	6(12.91%)	10(90.90%)	1(9.1%)
Ciprofloxacin	14(45.16%)	17(54.84%)	7(63.63%)	5(45.46%)
Tetracycline	6(19.36%)	25(80.64%)	3(27.28%)	8(72.72%)
Linezolid	100(100%)	0(0%)	100(100%)	0(0%)
Vancomycin	100(100%)	0(0%)	9(82%)	2(18%)
Extra drugs for Urinary isolates (n=25)	***E. faecalis (n=19)***		***E. faecium (n=6)***	
Nitrofurantoin	17(89.47%)	2(10.53%)	5(83.33%)	1(16.67%)
Norfloxacin	15(78.94%)	4(21.06%)	4(66.66%)	2(33.34%)
Nalidixic acid	16(84.21%)	3(15.79%)	4(66.66%)	2(33.34%)

G with 72.72% and Tetracycline with 80.64%. Urinary drug like Norfloxacin showed maximum resistance for both the species and Nitrofurantoin were sensitive as shown in the(Table 3).

4. Discussion

Escherichia coli, Pseudomonas and *Staphylococcus* remain to be the foremost cause of nosocomial infections. *Enterococcus* holding second place according to the data collected from CDC.[5] The spectrum of disease caused by *Enterococcus* varies from soft tissue infection, wound infection, UTI to bacteremia. It remains to be the second most cause of UTI and stands third place in causing bacteremia.[5]

In our study we observed maximum number of organisms were isolated from urine sample followed by pus sample, which is similar to those found in other studies.[6–11]

In the present study female patients were affected maximum (54.77%) when compared to male patients as observed in other studies.[8,12]

Only two species of *Enterococcus, E. faecium* and *E. faecalis* were isolated in our study which were comparable with other studies.[6,11,13] In our study we observed *E. faecalis* to be predominant isolate when compared to faecium as seen in similar studies.[7,8,14–17]

In the present study maximum resistance were noted to Penicillin-G, similar pattern were observed in the study conducted by Jain S et al., Thapa B et al., Trupti B et al and Devi PS et al.,

Our study showed all the isolates were sensitive to linezolid when compared with other studies.[6]

5. Conclusion

This study illustrates the prevalence and antibiotic pattern of *Enterococci* isolated from the patients in our region. We observed that linezolid resistant strains have not emerged during our study period in our region. Emerging drug resistance to *Enterococcus* acts as a reason to work on its antibiogram that can help us to formulate antibiotic policy for management of *Enterococcal* infection and helpful to the clinicians for empiric therapy.

6. Source of Funding

None.

7. Conflict of Interest

None.

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