

Antibiogram Study of Clinical Isolates of Enterococcus in a Tertiary Care Teaching Hospital

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ABSTRACT

Introduction: Excessive and non-specific use of broad-spectrum antibiotics has converted Enterococci from a normal gut commensal to opportunistic nosocomial as well as community acquired pathogen. The high level resistance to Aminoglycosides (HLAR) is long known and constantly increasing problem. Concomitant Vancomycin resistance (VRE) reduces the treatment options and increases potential risk of Vancomycin resistant gene transfer to *Staphylococcus aureus*.

Aim: To study the antibiogram of Enterococcus isolated at a tertiary care teaching hospital.

Method: A retrospective analysis was performed in microbiology laboratory of a tertiary care teaching hospital in Durg. All the Enterococcus isolated from urine, pus, blood and body fluids received between January, 2016 and June, 2017, were included in the study. The demographic details of patients were noted. All the isolates were identified and speciated by standard microbiological procedures.

Antimicrobial susceptibility was tested for all the isolates by modified Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines (2015).

Results: Among 178 Enterococcal species that were isolated and biochemically identified, 56% were *Enterococcus faecalis*, 40% were *E. faecium* and 4% were other species. Out of 178 isolates, most (60%) were from female patients. Most of the isolates (62%) were from urine followed by pus (25%), blood and others. Penicillin resistance and HLAR were seen in 50 and 58%, respectively. Three urinary isolates, all identified as *E. faecium*, were found to be resistant to Vancomycin by disk diffusion method. Among the urinary isolates, Nitrofurantoin resistance was seen in only 4.5% samples.

Conclusion: VRE were found to be rare in this area, but Penicillin resistance and HLAR were quite high. This may lead to increased use of higher antibiotics like Vancomycin and Linezolid, leading to increased selective pressure of these antibiotics to form resistance in near future.

Keywords: Antibiotic sensitivity, High level Gentamicin (HLG) resistance, Vancomycin resistance, VRE (Vancomycin resistant Enterococcus)

INTRODUCTION

Enterococci are a part of the normal human faecal flora and were traditionally regarded as low grade pathogens, but excessive and non-specific use of broad-spectrum antibiotics has converted them into opportunistic nosocomial and community acquired pathogen. They are being increasingly isolated as causative agent of nosocomial infections like urinary tract infections (UTIs), intra-abdominal and intra-pelvic abscesses, post-surgery wound infections [1] and blood stream infections (BSIs) [2,3]. Risk factors of nosocomial infections with Enterococci include longer duration of hospitalisation (specially stay in ICU), previous antimicrobial therapy (especially with multiple antibiotics), severity of illness, exposure to contaminated medical equipment, proximity to a previously known infected patient, or exposure to a nurse assigned to another known patient, haematological malignancy/bone marrow transplantation, parenteral/ oral Vancomycin and receipt of third-generation Cephalosporins

and drugs with activity against anaerobes [4-7].

Enterococci exhibit resistance, either intrinsic or acquired, to most of the commonly used antibiotic [8]. Intrinsic resistance expressed by enterococci include resistance to semisynthetic penicillinase resistant Penicillins, Cephalosporins, low level of Aminoglycosides and low level of Clindamycin, whereas acquired resistance includes resistance to Chloramphenicol, Erythromycin, high level of Clindamycin, Tetracycline, high level of Aminoglycosides, Penicillin, Fluroquinolones and Vancomycin [8-10]. High Level Resistance to Aminoglycosides (HLAR) & Vancomycin resistance (VRE) are of major concern. The high level resistance to Aminoglycosides (HLAR) is long known and constantly increasing problem. Concomitant Vancomycin resistance reduces the treatment options for treating serious infections caused by Enterococci [7,8,11]. Secondly, conjugation experiments have confirmed Vancomycin resistance gene transfer from Enterococci to *Staphylococcus aureus* [7, 11-13].

Many studies have been conducted in India to find out the magnitude of resistance in Enterococci isolated from clinical specimens, but very less work has been done in our region. Hence, this study was conducted at a tertiary care teaching hospital located in Durg, Chhattisgarh, to find out the resistance pattern of Enterococci isolated in our region.

OBJECTIVE

To study the antibiogram of Enterococcus isolated from clinical specimens at a tertiary care teaching hospital.

MATERIALS AND METHOD

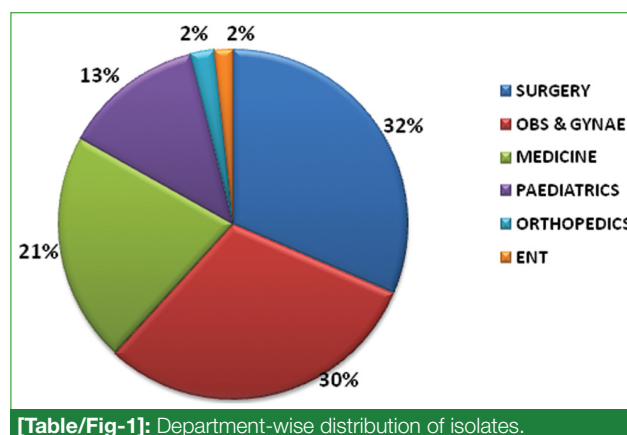
A retrospective analysis was conducted in Department of Microbiology at a tertiary care teaching hospital in Durg. Enterococcus isolates from all clinical specimens except from stool and sputum, received between January, 2016 & June, 2017 were included in this study. Enterococcus from commensal sites were excluded. The demographic details of patients were noted. Identification & speciation of isolates was done & they were tested for antimicrobial susceptibility by standard microbiological procedures. Isolates were confirmed to belong to genus Enterococcus by colony morphology on Blood agar & Mac Conkey agar, Gram stain, Catalase test, hydrolysis of Bile-esculin (BE), Pyrrolidonyl arylamidase (PYR) test, Heat tolerance at 60°C for 30 min in water bath & salt tolerance (6.5% NaCl) [14]. For speciation, Mannitol & Arabinose fermentation, Arginine hydrolysis & Potassium tellurite reduction test were performed [14]. Antimicrobial susceptibility testing was done by modified Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines-2015. *E. faecalis* ATCC 29212 was used as a sensitive ATCC control [15]. The Antibiotic sensitivity was tested for the following-

- Penicillin (10U), Vancomycin (30µg), Teicoplanin (30µg), Linezolid (30µg) & Gentamicin (120µg) (for HLAR determination) for all isolates
- Nitrofurantoin (300µg) & Norfloxacin (10µg) (only for urinary isolates)

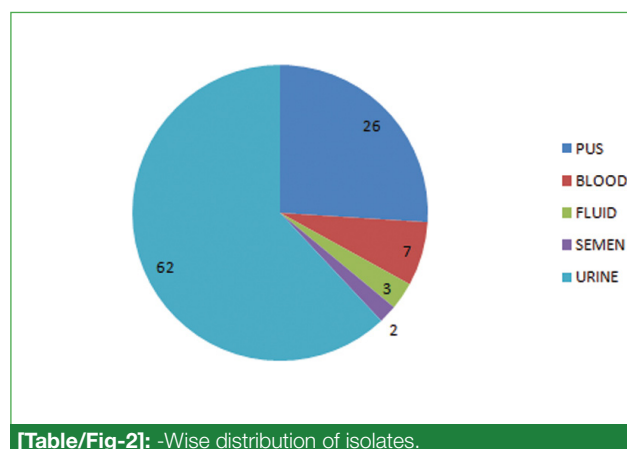
- Erythromycin (15µg) & Ciprofloxacin (5µg) (for isolates from specimens other than urine)

RESULTS

In the study, 178 Enterococcal species were isolated and biochemically identified. Out of them, 100 (56%) were *Enterococcus faecalis*, 71 (40%) were *E. faecium* and 7 (4%) were other species. Most of the isolates were from specimens



[Table/Fig-1]: Department-wise distribution of isolates.



[Table/Fig-2]: Specimen-wise distribution of isolates.

Antibiotics	Sensitive		<i>E. faecalis</i>		<i>E. faecium</i>	
	Number	%	Number	%	Number	%
Vancomycin*	175	98.3	100	100	68	96
Teicoplanin*	175	98.3	100	100	69	97
Linezolid*	177	99.4	100	100	70	99
Penicillin*	91	51	57	57	34	48
High Level Gentamicin*	74	41.6	46	46	27	38
Norfloxacin†	25	22.5	15	26.3	9	19
Nitrofurantoin‡	106	95.5	56	98.2	46	96
Erythromycin‡	26	38.8	18	42	8	35
Ciprofloxacin‡	23	34.3	14	32.5	9	39

[Table/Fig-3]: Antibiotic Sensitivity pattern of Enterococcus isolates.

* - Tested for all the isolates (Total= 178, *E. faecalis* =100, *E. faecium*= 71)

† - Tested for only urinary isolates (Total= 111, *E. faecalis* =57, *E. faecium*= 48)

‡ - Tested for isolates other than urine (Total= 67, *E. faecalis* =43, *E. faecium*= 23)

collected from female patients (60%). Majority of the isolates were from Surgery (32%) & Obstetrics & Gynaecology (30%) departments [Table/Fig-1]. Maximum isolates were from urine specimen (62%), followed by pus (26%), blood (7%) and others (5%) [Table/Fig-2]. Almost all isolates were sensitive to Linezolid (177/178) & most of urinary isolates were sensitive to Nitrofurantoin (106/111) [Table/Fig-3]. *E. faecium* was marginally more resistant as compared to *E. faecalis* [Table/Fig-3]. Three urinary isolates, speciated as *E. faecium*, were found to be Vancomycin resistant by disk diffusion method. All of these, three were sensitive to Linezolid and two to Teicoplanin & Nitrofurantoin.

DISCUSSION

Enterococci are part of the normal intestinal flora and were considered relatively harmless organisms. In recent times, Enterococci have been identified as the agents of nosocomial

infection with antimicrobial resistance, either intrinsic or acquired, to most of the currently approved agents [11]. Of major concern are High Level Resistance to Aminoglycosides (HLAR) & Vancomycin resistance (VRE) because they reduce the treatment options for treating serious infections caused by Enterococci [7, 8, 11] and also there is risk of resistance gene transfer to other organisms [7, 11-13].

Many studies have been conducted in India to find out the magnitude of resistance in Enterococci isolated from clinical specimens [Table/Fig-4], this study was conducted to find out the resistance pattern of Enterococci isolated in our region.

The study found that *E. faecalis* was the commonest species (56%) isolated, but count of *E. faecium* (40%) was higher as compared to that isolated in many other studies, [16-19] which showed isolation of about 15% of *E. faecium*. But a few other recent studies have shown an increase in isolation

Author & Year	Place	Isolates	Conclusions*	
Haritsa KB, et al.,15 Nov 2012 – Oct 2013	Bengaluru	90	Efs/ Efm - 84/14.5% Urine - 72%, Pus- 16% Pen R - 45.5% HLGR - 23.3%	Nit R - 8% VRE - 3 Efs Lz - 100% Sen Efm more resistant(exc.Va)
Barman J, et al.,16 Jul 2012 – Jun 2013	Assam	95	Efs/ Efm - 82/13% Urine - 88%, Pus- 5% Pen R - 100% HLGR - 53.7%	Nit R - 18% VRE - 1 Egal Lz & Tei - 100% Sen
Shanmukhappa, et al.,17 Jun 2002 – Nov 2003	Mysore	80	Efs/ Efm - 86/12.5% Urine - 50%, Pus- 33.7% Pen R - 52.5%	Er R - 55% Cip R - 38.75% Va - 100% Sen Efm more resistant
Bhakare U, et al.,18 Feb 2011 – Dec 2012	Nagpur	80	Efs/ Efm - 79/16% Urine - 56%, Pus- 11% Pen R - 52.5% HLGR - 25%	Nit - 100% Sen VRE - 3 Efs, 1 Efm Lz - 100% Sen
Manavalan J, et al.,19 Mar 2010 – Feb 2011	Puducherry	126	Efs/ Efm - 52/32.5% Urine - 57%, Pus- 41% Pen R - 85% HLGR - 5%	Nit R - 40% VRE - 4 Efm Lz - 100% Sen Efm more resistant
Sreeja S, et al.,20 Jan – Dec 2008	Bengaluru	128	Efs/ Efm - 76/24% Urine - 31%, Pus- 43% Pen R - 47% HLGR -47%	Cip R - 50% Va & Lz - 100% Sen Tei R - 32% Efm - No. increasing
Deshpande RV, et al., 21	Mumbai	291	Efs/ Efm - 70/30% Pen R - 76% HLGR - 73.5% Nit R - 40%	VRE - 38 Efs, 19 Efm Lz R - 2.4% Tei R - 11.3% Efm more resistant
Present study, Jan 2016 – Jun 2017	Durg	178	Efs/ Efm - 56/40% Urine - 62%, Pus- 25% Pen R - 50% HLGR - 58.5% Nit R - 4.5%	Er R - 61% Cip R - 65.7% VRE - 3 Efm Lz - ≈100% Sen Efm more resistant

[Table/Fig-4]: Review of Literature.

* Efs = Enterococcus faecalis, Efm = Enterococcus faecium, Egal = *E. gallinarum*, Pen = Penicillin, Nit = Nitrofurantoin, Lz = Linezolid, Va = Vancomycin, Tei = Teicoplanin, Er = Erythromycin, Cip = Ciprofloxacin, R = Resistance

rate of *E. faecium* up to 25-33% [20-22]. Increased isolation of *E. faecium* is important as it was found to be more resistant than *E. faecalis* in this & other studies [3, 16, 18, 20, 22].

In this study, majority of isolates were from urine (62%), followed by pus, blood and others. Similar results were obtained in many other studies conducted in India [16-20], but Sreeja et al., reported maximum isolation from pus (43%) followed by urine (31%) [21].

Resistance to Penicillin was 50% & HLGR was found to be 58.5%. Many other authors reported Penicillin resistance to be about 50% [16,18,19,21], while few authors reported higher resistance, ranging between 75-100% [17,20,22]. Most of the studies reported HLGR in the range of 25-55% [16,17,19,21]. This leads to failure of synergistic combination therapy of aminoglycosides with cell-wall active antibiotics (beta-lactams/ glycopeptides) for serious infections [13].

In this study, 95% urinary isolates were sensitive to Nitrofurantoin. Most other studies also showed similar results with low resistance (0-10%) against Nitrofurantoin [16,19], while Manavalan J et al., reported about 40% resistance against it [20]. Almost all isolates in this study were sensitive to Linezolid & Teicoplanin, similar results were obtained in other studies done in India [16-22]. High chances of sensitivity to these antibiotics justify them to be used as an alternative to Vancomycin.

In this study, three isolates were found to be Vancomycin resistant by disk diffusion method. All of them were isolated from urine and were speciated as *E. faecium*. Vancomycin resistance was not found to be a major resistance (1.7%) in this region, but VRE appears to be an emerging pathogen in India. This might be attributed to imprudent use of Cephalosporins and Vancomycin following the emergence of MRSA and poor infection control measures [19, 22]. In order to control the spread of Vancomycin resistant Enterococci, the CDC Hospital Infection Control Practices Advisory Committee (HICPAC) has given following recommendations [23]:

- i. Appropriate use of oral and parenteral Vancomycin and third generation Cephalosporins.
- ii. Educating the health care workers about the ways to prevent its spread and its impact on patient care outcome.
- iii. Early detection of patients colonized or infected with VRE using microbiology laboratory procedures effectively.
- iv. Implementation of infection control measures like use of gloves, gowns and isolation of patients.

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CONCLUSION

This study indicates that there is a change in epidemiology of enterococcal infections & *E. faecium* is now emerging as the predominant enterococcal isolate from human infections. Resistance to Vancomycin is rare but to Penicillin & HLA is quite high in this region. This may lead to increased use of higher antibiotics leading to increased selective pressure of these antibiotics to form resistance in near future. Hence, speciation of the Enterococcal isolates and regular monitoring for antibiotic resistance with special attention to Vancomycin and high level Aminoglycosides is warranted. To prevent increasing resistance, early detection by microbiology laboratory, greater adherence to infection control measures & prudent use of antimicrobials is vital.

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