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ORIGINAL RESEARCH

Resistance pattern of enterococcal isolates, phenotypic characterization of vancomycin resistant isolates from various clinical samples in a tertiary care hospital

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Abstract

Introduction: Enterococci cause a multitude of infections and emergence of vancomycin resistance is of special concern, as it is the primary alternative drug to penicillin for treating Enterococcal infections.

Objectives: The present study was taken up to know the species predominance, resistance pattern and various phenotypes of vancomycin resistant Enterococci in a tertiary care hospital in south India.

Material & methods: The Data collected from the department of Microbiology at Krishna Institute of Medical Sciences, Secunderabad from January 2012 to December 2015 was retrospectively analysed. 226 Enterococci were speciated, antibiograms were analyzed and phenotyping of VRE was done based on standard guidelines.

Results & discussion: The predominant species was found to be *E. faecalis* (51.3%) followed by *E. faecium* (43.3%), *E. gallinarum* (2.2%), *E. durans* (1.32%), *E. casseliflavus* (0.88%) and *E. raffinosus* (0.88). *E. faecalis* showed less resistance (30.39%) to antimicrobials than *E. faecium* (50.64%). The resistance pattern was less in out-patient samples when compared to in-patient samples. Vancomycin resistant Enterococci were 13(5.75%), all isolated from inpatients. Among the 13 isolates, 6(46.15%) were VanA, 4(30.76%) were VanB and 3(23.07%) were VanC phenotypes. VanC phenotype was observed in *E. casseliflavus* and *E. gallinarum* due to intrinsic resistance.

Conclusion: The species predominance of *E. faecalis* and *E. faecium* reflects the change in recent decade. Drug resistance pattern and phenotyping is correlating with other studies, VanA being the commonest. Monitoring for vancomycin resistant Enterococci is critical in hospital infection control and for effective treatment.

Keywords: phenotyping; antibiogram; vancomycin-resistant Enterococci; infection control

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Introduction

The term 'Enterococcus' originates from the Greek *enteron* meaning 'the gut or intestine' and *kokkos* meaning 'a berry or kernel'. This is helpful as multidrug resistant Enterococci are associated with the gastrointestinal tract and have become 'kernels' of antimicrobial resistance [1].

Enterococci, previously classified as group D Streptococci, are normal part of enteric microbiota. On Grams stain they appear typically as Gram positive oval cocci of size approximately 1µm arranged in pairs at angles, or in short chains. They are usually nonhemolytic, grow in presence of bile, hydrolyze esculin, grow well in 6.5% NaCl and grow well between 10°C and 45°C (Characteristics that differentiate Enterococci from Streptococci).

Most common sites of infection are the urinary tract, wounds, biliary tract and blood. They are also associated with meningitis and bacteremia in neonates and endocarditis in adults. Among the total species of Enterococci identified till date, only one third are implicated in human disease. Although *Enterococcus faecalis* was the most common species isolated earlier, a shift to *Enterococcus faecium* is observed in the last few decades [2].

Enterococcus is an important pathogen because of its intrinsic as well as acquired antibiotic resistance. The intensive use of broad spectrum antibiotics in hospitals provides selective pressure favoring growth of intrinsically drug-resistant commensal organisms, one of them being Enterococcus. The first case of Vancomycin Resistant Enterococci (VRE) was reported in England in 1988 by Uttley et al; since then observed all over the world [3]. In India there are reports from New Delhi, Chandigarh and Mumbai [4-6].

Several genes, including VanA, VanB, VanC, VanD, and VanE, contribute to resistance to vancomycin in Enterococci. Acquired resistance is mediated by VanA and VanB phenotypes whereas intrinsic resistance is VanC mediated.

Newer agents such as linezolid, daptomycin, quinupristin- dalfopristin and tigecycline (among others) are used for the treatment of Vancomycin Resistant Enterococci infections [7].

The importance of VRE is because of the few options available for treatment, the difficulty to eradicate once established and the possibility of transfer of vancomycin resistance to *Staphylococcus aureus* and *Listeria monocytogenes* [8].

Material and methods

Study area: Department of Microbiology at Krishna Institute of Medical Sciences, Secunderabad

Study population: All patients admitted to KIMS and visiting out-patient departments were included.

Inclusion criteria: 1) All age groups, 2) IP and OP patients of all departments, 3) Both males and females are included, 4) Percentage Identification more than 89% in VITEK 2 compact (bioMerieux).

Exclusion criteria: Percentage identification less than 89%in VITEK 2 compact (bioMerieux)

Study design: Retrospective lab based observational study of antibiotic resistance of Enterococci. Phenotyping of Vancomycin resistant Enterococci in our hospital.

Study duration: 1 January 2012 to 31 December 2015

Method of measurement of outcome of interest

All clinical samples of patients which showed growth of Enterococci were analyzed. In the study, 226 enterococcal isolates were speciated by VITEK 2 compact (with percentage identification > 89%).

Antibiogram was analysed according to Clinical Laboratory Standards Institute (CLSI) guidelines [9] prevailing at that point of time.

Isolates showing resistance to Vancomycin were phenotyped into VanA, VanB, VanC, based on MIC values of Vancomycin and Teicoplanin, according to CDC guidelines [8, 10].

Results

A total number of 226 isolates of Enterococci were identified during the study period, among which 13 were Vancomycin Resistant (VRE). All Vancomycin Resistant Enterococci were isolated from inpatient

Table 1: Characteristics of phenotypes of glycopeptide resistant Enterococci

S. No	*Phenotype*	*Van MIC (µg/ml)*	*Tei MIC (µg/ml)*	*Common species*	*Location*	*Genetic determinant*	*Expression*	*Transferable*
1	VanA	> 128	≥16	*E. faecium, E. faecalis*	Plasmid	Acquired	Inducible by Vancomycin & Teicoplanin	Yes
2	VanB	16-64	≤1	*E. faecium, E. faecalis*	Plasmid/ Chr	Acquired	Inducible by Vancomycin	Yes
3	VanC	2-16	≤0.5	*E. gallinarum, E. casseliflavus*		Intrinsic	Constitutive	No
4	VanD	64-128	04-8	*E. faecium*	Chr	Acquired		No
5	VanE	64-128	04-8	*E. faecalis*	Chr	Acquired		No

Abbreviations: Chr: Chromosomal.

samples only. When vancomycin phenotypes were further analyzed, 6 were found to be VanA, 4 were VanB and 3 were VanC.

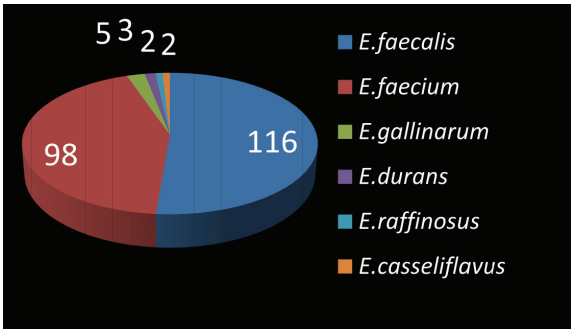

Figure 1: Distribution of various species of Enterococci isolated.

Abbreviations: AM: Ampicillin; CIP: Ciprofloxacin; FT: Nitrofurantoin; HLG: High Level Gentamicin; HLS: High Level Streptomycin; LEV: Levofloxacin; LNZ: Linezolid; P: Penicillin; TE: Tetracycline

Discussion

A total of 226 enterococcal isolates could be identified by VITEK 2 compact (bioMerieux). The predominant species was found to be *E. faecalis* (116, 51.3%), followed by *E. faecium* (98, 43.3%). Ratio of *E. faecalis* and *E. faecium* is 1.18: 1. This supports the study saying that there is a progressive decline in the ratio in recent years, ie, progressive increase in *E. faecium* infection [3].

All together 11 (6.87%) isolates were identified as non- *E. faecalis* non- *E. faecium* in the present study, comparable to other studies [11, 12]. Other species isolated include *E.gallinarum* (5, 2.2%), *E. durans* (3, 1.32%), *E. casseliflavus* (2, 0.88%) and *E. raffinosus* (2, 0.88%). Most of the isolates were

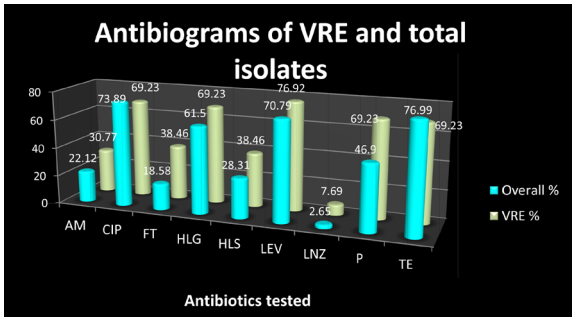

Figure 2: Resistance pattern of van phenotypes.

from inpatients (70.79%) especially so in case of *E. faecium* (84.69%). No gender difference was noted in isolation of different species.

Enterococci have adequate intrinsic and acquired resistance to many antibiotics [13]. They are intrinsically resistant to penicillinase resistant penicillins and cephalosporins; have acquired resistance to chloramphenicol, erythromycin and high level resistance to aminoglycosides, penicillin, fluoroquinolones and vancomycin [14]. *E. faecium* and *E. faecalis* were more resistant when compared to other species. Among the two, *E. faecalis* is less resistant (30.39%) to antimicrobials than E .faecium (50.64%). Antimicrobial resistant Enterococci are being reported with increasing frequency in United States and other parts of the world [15]. Their number in Asian subcontinent is comparatively less.

The phenotypic classification scheme used usually corresponds well to the genotypic classification. It is an inexpensive method as it uses information that is derived simply in a laboratory. However there are certain limitations [10].

Thirteen Vancomycin resistant phenotypes were isolated (5.75%), majority of them from urine

Table 2: Various enterococcus species & van phenotypes in different samples.

S.No	*Enterococcus species*	*Inpatient*	*Outpatient*	*Total number*	*VanA*	*VanB*	*VanC*
1	*E. faecalis*	66	50	116	2	2	0
2	*E. faecium*	83	15	98	4	2	0
3	*E. gallinarum*	4	1	5	0	0	1
4	*E. durans*	3	0	3	0	0	0
5	*E. casseliflavus*	2	0	2	0	0	2
6	*E. raffinosus*	2	0	2	0	0	0
	Total number (n)	160	66	226	6	4	3

Table 3: Antibiotic resistance pattern of *E. faecium*, *E. faecalis* & VRE.

S.No	*Antibiotic*	*E. faecium (n=98)*			*E. faecalis (n=116)*			*VRE isolates (n=13)*
		IP	*OP*	*Total*	*IP*	*OP*	*Total*	
1	Ampicillin	48.19	40	46.93	6.06	0	34.4	30.77
2	Ciprofloxacin	93.97	86.66	92.85	65.15	0.66	65.5	69.23
3	Nitrofurantoin (urine isolates only)	34.93	26.66	33.67	12.12	2	7.75	38.46
4	High Level Gentamicin Resistance	73.49	66.66	72.44	62.12	54	58.6	69.23
5	High Level Streptomycin Resistance	43.37	40	42.85	21.21	16	18.9	38.46
6	Levofloxacin	90.36	80	88.77	65.15	60	62.93	76.92
7	Linezolid	1.2	0	1.02	7.57	0	4.31	7.69
8	Penicillin	83.13	0.6	89.79	28.78	18	24.13	69.23
9	Quinupristin – Dalfopristin	12.04	13.33	12.24	-	-	-	-
10	Tetracycline	78.31	0.8	78.57	87.87	78	83.6	69.23
11	Vancomycin	7.28	0	6.12	6.06	0	3.44	-
12	Teicoplanin	4.81	0	4.08	3.03	0	1.72	-

Table 4: Vancomycin phenotypes of vancomycin resistant enterococcus – Details.

S.No	*Organism*	*Sample*	*VAN MIC (µg/ml)*	*TEI MIC (µg/ml)*	*Phenotype*
1	*E. faecium*	Blood	≥128	≥32	VanA
2	*E. faecium*	CSF	≥128	≥32	VanA
3	*E. faecium*	Tissue	≥128	≥32	VanA
4	*E. faecium*	Urine (Catheter catch)	≥128	≥32	VanA
5	*E. faecium*	CSF	≥32	<1	VanB
6	*E. faecium*	Urine (Catheter catch)	≥32	<1	VanB
7	*E. faecalis*	Central line tip	≥128	≥32	VanA
8	*E. faecalis*	Urine (Catheter catch)	≥128	≥32	VanA
9	*E. faecalis*	Urine (Catheter catch)	≥32	≤ 0.5	VanB
10	*E. faecalis*	Urine (Catheter catch)	≥32	<1	VanB
11	*E. casseliflavus*	Urine (clean catch)	4	0.5	VanC
12	*E. casseliflavus*	US guided fluid	4	0.5	VanC
13	*E. gallinarum*	Pus	4	0.25	VanC

Abbreviations: Van: Vancomycin; Tei: Teicoplanin; MIC: Minimum Inhibitory Concentration.

samples. VanA is the commonest (6 out of 13), closely followed by VanB (4 out of 13) and then VanC (3 out of 13). When the resistance pattern is looked into, majority of the isolates were found to be sensitive to Linezolid. Nitrofurantoin though useful in vancomycin susceptible isolates, 5 out of 13 (38.46%) VRE isolates were resistant to it. VRE were highly resistant to levofloxacin, penicillin, tetracycline and ciprofloxacin.

The overall resistance of VRE isolates was higher than non-VRE isolates except for ciprofloxacin and tetracycline. Even though tigecycline is reported to have good activity against VRE infections, it could not be tested as majority isolates were from urine. Use of tigecycline for the treatment of Urinary Tract Infections (UTIs) has been questioned because of low peak serum concentrations and limited excretion into urine [16, 17].

Conclusion

The present study concludes that the overall incidence of vancomycin resistant enterococci among all infections is 5.75%, majority being urinary tract infections. Although *Enterococcus faecalis* was the predominant species, the percentage prevalence in our study is consistent with the change in trend mentioned by other authors. Drug resistance of E.faecium was more as compared to *E. faecalis*. VanA is the commonest phenotype observed in our study as is the case in all other studies. Linezolid remains a promising drug to combat VRE till date. Nitrofurantoin is a good alternative for vancomycin resistant enterococcal urinary tract infections. The finding that all VRE are isolated from inpatients highlights the role of Hospital Infection Control in controlling the drug resistance. Early detection and reporting of VRE plays an important role in initiating effective infection control practices. However, there is a continued need for the development of new antimicrobial agents for treating VRE infection, as well as a regimen that would eradicate VRE colonization.

Limitations of study

- The study would have been better had it been prospective
- Clinical history, treatment given and outcome of patients are not known
- Phenotypic classification system used in the study is not fool proof
- Genotypic confirmation of Van phenotypes using PCR was not done

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Conflicts of interest

Authors declare no conflicts of interest.

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