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High Level Gentamicin Resistance and Vancomycin Resistance in *Enterococcus* species at a tertiary care hospital in India

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A B S T R A C T

The *Enterococcus species* has emerged as a major nosocomial pathogen, and common difficulty faced in treatment of such infections are attributed to the presence of high level amino-glycosides resistance in them. Continuous monitoring of the antibiotics susceptibility pattern of *Enterococcus species* thus became inevitable for control of Hospital Acquired infection. The objective of the study was to isolate specie and determine the antibiotic sensitivity pattern of *Enterococcus species* from various clinical samples. Samples collected were processed under aseptic condition and the *Enterococcus species* were isolated by growth characteristics in culture plate, Gram staining, biochemical testing and speciated accordingly with the sugar fermentation test. Antibiogram of the resultant isolates were done in regards to the CLSI standards. Out of 96 isolates, *Enterococcus faecalis* accounted for 55, followed by *Enterococcus faecium* 35, *Enterococcus avium* 6. Total of 47 isolates showed resistant to high level Gentamicin (HLG) and 10 were resistant to Vancomycin. *Enterococcus species* are gram positive cocci which cause Nosocomial infection because of their resistance to many antibiotics. Thus, it is necessary for laboratories to provide accurate antimicrobial pattern for *Enterococcus species* so that effective therapy and infection control measures can be initiated.

Introduction

During past years, *Enterococcus species* have become increasingly resistant to many antibiotics in terms of both the level of resistance and multiplication of resistance to

many particular drugs.¹ The member of this species are natural habitat intestinal flora of humans but now they are also becoming as leading to cause of hospital acquired

infection due to higher antibiotics resistance.²

The most serious *Enterococcus species* infections are often difficult to treat since the organisms have a tremendous capacity to acquired resistance to vancomycin, penicillin and high concentration aminoglycosides.³The most common *Enterococcus species* responsible for infections are *Enterococcus faecalis* 80-90%⁴ and *Enterococcus faecium* for the remaining.⁵ Urinary tract infection (UTIs) is most frequent infection, which is caused by *Enterococci* and second most frequent infection is intra pelvic abscesses and intra abdominal or post surgery wound infections⁶. Epidemiological data suggested that *Enterococcus species* are most important reservoir for transmission of antibiotics resistant gene among different bacteria⁷.

Material and Methods

The study was conducted at Hind Institute of Medical Sciences, Safedabad, U.P, India during period of April 2015 to September 2015. Clinical specimens collected like Urine, Blood, Pus, Sputum and CSF were sent to the Microbiology laboratory. Samples were processed aseptically for direct microscopy, gram staining, growth in culture plates like Nutrient agar, MacConkey agar, Blood agar, CLED Agar for 37°C aerobically overnight. Later, the next day biochemical tests were done from

the growth obtained and speciated accordingly. Antibiotic sensitivity test was done by Kirby - Bauer disc diffusion method and reporting was done with respective to CLSI standards. Drugs like (ciprofloxacin, norfloxacin, ampicillin, high level gentamicin (120µg), vancomycin, penicillin, tetracycline and erythromycin) were used.

After that the identification of genus *Enterococcus*, specific test like Bile esculin hydrolysis test, growth on 6.5% NaCl, growth on Potassium tellurite agar (PTA) and Sugar fermentation test was done to speciate the obtained sample. *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434 were used as quality control strains.

Result and Discussion

Total 1266 samples were collected in which 718 were reported sterile, 452 were species like *E.coli*, *Pseudomonas*, and other than *Enterococcus species* 96 were isolated to be *Enterococcus species*.

The total no. of sample reported sterile were 718 and positive isolates were 548. Positivity percentage of male was 56.38 and females were 43.61. Out of 96 positive, 33 (34.37%) were males and 63 (65.6%) were females. There are total 96 isolates of *Enterococcus species* from different clinical specimens like urine 64 (66.7%), Blood 17(17.8%), Pus 9 (9.3%), and others 6 (6.2%).

Table no.1 Showing differentiation of *Enterococcus species* on the basis of sugar fermentation test

Acid Produced	<i>E.feacalis</i>	<i>E.faecium</i>	<i>E.avium</i>	<i>E.durans</i>
Mannitol	+	+	+	-
Sorbitol	+	+/-	-	-
Arabinose	-	+	+/-	-
Raffinose	+	+/-	+/-	-
Pyruvate	+	-	-	-

Table.2 Showing total positive male and female% (gender point)

Gender	Sterile Sample	Percentage	Positive Sample	Percentage
Male	366	50.97	309	56.38
Female	352	49.02	239	43.61
Total	718	100	548	100

Table.3 Showing positive male and female ratio difference in *Enterococcal* infection

Gender	Samples positive for <i>Enterococcus species</i>	Percentage
Male	33	34.37
Female	63	65.6
Total	96	100

Table.4 Showing total number and percentage of different isolated *Enterococcus species* in different specimen

Specimen	<i>E.facelis</i>	<i>E. faecium</i>	<i>E.avium</i>
Urine	35(63.63%)	26(72.22%)	3(60%)
Blood	10(18.18%)	5(13.88%)	2(40%)
Pus	6(10.90%)	3(8.33%)	00(00%)
Other	4(7.27%)	2(5.55%)	00(00%)
Total	55(100%)	36(100%)	5(100%)

Table.5 Showing Antibiotic resistivity pattern in the *Enterococcus species*

Antibiotics	<i>E.avium</i> [N=6]	<i>E.faecalis</i> [N=55]	<i>E.faecium</i> [N=35]
Ampicillin(10µg)	3	24	8
Norfloxacin(5µg)	3	29	12
Ciprofloxacin(5µg)	2	26	21
HLG(120µg)	2	32	12
Vancomycin(30µg)	2	5	3
Penicillin(10µg)	4	45	19
Tetracycline(30µg)	0	18	9
Erythromycin(15µg)	3	49	17

In the recent years, there has been a scenario of enhanced *Enterococcus species* infection due to their ability to cause nosocomial infection as well as increasing resistant to many antimicrobial agent⁸⁻¹⁰. This study has the total sample of 1266 in which 452 were

positive for other species only 96 were positive for *Enterococcus species* which 33(34.48%) were male and 63(65.62%) were female. In the present study the ratio of females infected with *Enterococcal* infection is more than ratio of male. The ratio

difference because *Enterococcus species* are frequently causative agent of the urinary tract infection which is more common in female this feature of ratio difference can be attributed to the factor that, the place where the study was conducted is a rural area hence females are not well educated enough to share their medical condition directly with the physicians. Out of 96 positive isolation of current study, 65 from urine, 17 from blood, 9 from pus, and 6 from other specimens like CSF, swab etc.

Findings in present study revealed *E.faecalis* as predominant species which was 57.29% followed by *E. faecium* 37.5%, our study results goes similar with study conducted in CSM medical college, lucknow, as their result were, out of 86 samples, 42 were *E. faecalis*, 33 were *E.faecium*, 5 were *E. avium*¹¹. 47 (48.95%) of the *Enterococcus species* showed HLGR in our study, which is lower than the study of Zervous and associates whose shows reported a prevalence of 55% of HLGR in *Enterococcus species* in US centres¹².

This study had HLGR in *E.faecalis* was 58.18% and *E.faecium* was 34.28% but in the result (Table No. 2) of prevalence of multidrug resistant *Enterococci* in tertiary care hospital in India, in which HLGR in *E.faecalis* was 44.4% and in *E.faecium* was 53.4%¹³. In our study out of 96 isolates 10 (10.41%) showed resistant to vancomycin which was 8.57% in *E.faecium*, 9% in *E.faecalis* in contrast to Islamic azad university zanzan branch Iran were the percentage of vancomycin in *E. faecalis* is 0% and in *E. faecium* is 10% (47.6%) respectively¹⁴.

Conclusion

To conclude *Enterococcus species* are gram positive cocci which are harmless

commensals, but cause nosocomial infection because their resistant to many antibiotics. The most frequent infections caused by them are urinary tract infection followed by blood stream infection. *Enterococcus* family poses therapeutic dilemmas for clinicians, due to their increase activity of resistance Species. Thus, providing accurate antibiogram results and periodic invigilation of hospital infection control program is inevitable to control the emerging infections.

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