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Research Article

REGIONAL PREVALENCE AND ANTIBIOTYPES OF *ENTEROCOCUS* FROM A TERTIARY CARE HOSPITAL, KOCHI, KERALA, INDIA

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A B S T R A C T

Enterococci are considered to be important nosocomial pathogen and its intrinsic property of antibiotic resistance made treatment difficult. Hence a preliminary study was conducted on the prevalence of Enterococcal infections and its antibiotic sensitivity pattern in Sunrise Institute of Medical Sciences, a tertiary care hospital at Kochi. Various clinical specimens like blood, urine, abscess, vaginal swab etc. were analyzed and a total of 93 isolates were obtained. Four species like *E.faecalis*, *E.faecium*, *E.raffinosus* and *E.avium* were isolated and 38 % of total isolates were multi drug resistant strains.

INTRODUCTION

Enterococci normally inhabit the bowel. They are found in the intestine of nearly all animals from cockroaches to the humans. *Enterococci* are readily recovered from outdoor vegetations and surface water. (Jett *et al.,* 1994). Two species are common commensal organisms in human intestine: *Enterococcus faecalis* (90-95%) & *Enterococcus faecium* (5-10%). Enterococci are exceedingly hardy (Murray *et al.,* 2003). Though they are not capable of forming spores, *Enterococci* are tolerant of a wide range of environmental conditions. They can withstand a variety of growth conditions including temperatuture of 10ºc to 45ºc and hypotonic, hypertonic, acidic or alkaline environments. (Mark M Huycke *et al.,* 1998), As optimally defined by (Rice *et al.,* 2003) Enterococci can grew at 10ºC to 45ºCat pH 9.6, in 6.5 % NaCl broth and survive at 60ºc for 30 minutes. *Enterococcus faecalis* can adapt to adverse condition: Following pre exposure to sub lethal stress conditions, *Enterococcus faecalis* becomes less sensitive to normally lethal levels of Sodium Dodecyl sulphate, bile salts, hyper osmolarity, heat, ethanol, Hydrogen peroxide acidity and alkalinity (Flahaut *et al.,* 1996a).

Even though they are seen in normal flora of intestine they can cause various infections in human beings. They can cause wide variety of diseases like UTI, blood stream infections, endocardium, abdomen, biliary tract, burn and wounds etc. (Jett *et al.,* 1994).

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Enterococci accounts for approximately 110000 UTI, 25000 cases of bacterimia, 40000 wound infections, 1100 cases of endocarditis annually in US (Emori and Gaynes, 1993). Most infections occur in hospitals.

Enterococcal meningitis is a rare complication of neurosurgery. Up to 90% of enterococcal infections in humans are caused by E. *faecalis* (Guven Kayaoglu and Dag Orstavik, 2004). The majorities of the remaining are caused by E. *faecium*. The ability of E. faecalis to tolerate or adapt to harsh environments may act as an advantage over other species.

Sensitive strains of this bacterium can be treated with Ampicillin, Pencillin & Vancomycin. UTI can be treated specifically with Nitrofurantoin even in case of Vancomycin resistance (Zhanel *et al.,* 2001). An important feature of *enterococcus* is the high level of intrinsic antibiotic resistance. Some *enterococci* are intrinsically resistant to Beta lactam based antibiotics and many aminoglycosides. Unlike acquired resistance and virulence traits, which are usually transposon or plasmid codon, intrinsic resistance, is based on chromosomal genes, which typically non – transferable (Elango Padmasini *et al.,* 2014).

Also *enterococci* often acquire antibiotic resistance through exchange of resistance encoding genes carried on conjugative transposons, plasmids and other broad host range plasmids (Rice *et al.,* 2003). High level Gentamycin resistance occurred in 1979 and was quickly followed by numerous reports of nosocomial infections in 1980's. (Zervos *et al.,* 1987) Wide spread emergence and dissemination of Ampicillin &

Vancomycin resistance in *E. faecalis* would significantly confound a therapeutic dilemma (Mark M Huycke *et al.,* 1998) In the last two decades particularly virulent strains of *enterococcus* that are resistant to Vancomycin (Vancomycin Resistant *Enterococci* or VRE) have emerged in nosocomial infections (Kurup *et al.,* 2008).

In this study, a prevalence survey on enterococcal infections and its antibiotic sensitivity pattern was conducted in a tertiary care hospital for a period of 1 year.

MATERIALS AND METHODS

Prevalence study was conducted in Sunrise Institute of Medical Sciences for a period of one year. Various clinical specimens like urine, blood, pus, abscess and endo-tracheal aspirations were screened for the presence of *Enterococci*. All the samples were collected by aseptic methods.

Selective culture and biochemical identification. All samples were plated on two selective media, 5% sheep blood agar (Biomerieux) and on Bile Esculineazide agar. All plates were incubated at 37^0C for 48 hours. All colonies resembling *Enterococci* were initially identified by Gram staining and further identification were done by Vitek-2 compact system from Biomerieux SA, France.

Susceptibility testing. Antibiotic susceptibility testing was done by disc diffusion on Mueller Hinton agar (Himedia) plates according to the guidelines of Clinical Laboratory Standards Institute CLSI (2014). Along with the disc diffusion method susceptibility were also analysed using AST P 268 cards on Vitek-2 compact system.

RESULTS

Black coloured colonies on BEA agar plates and greyish white colored colonies on sheep blood agar were processed in Vitek-2 compact system for further identification. A total of 93 isolates of *Enterococci* were obtained from different clinical specimens. Four common species of *Enterococci* were isolated (Figure 1). *E faecalis* (84.94%) was the most abundant species, followed by *E faecium* (13.98 %), *E. raffinosus* (1.8%) and *E avium* (1.8%). Retrospective data of the isolates were as in Table 1.

Table 1 Retrospective data of the isolates

Specimen	No of Isolates			
	E faecalis	*E faecium*	*E. raffinosus*	*E avium*
Urine	47	4	-	-
Abscess	26	3	1	-
Vaginal Swab	4	-	-	-
Body Fluid	1	1	-	1
ET Secretion	1	-	-	-

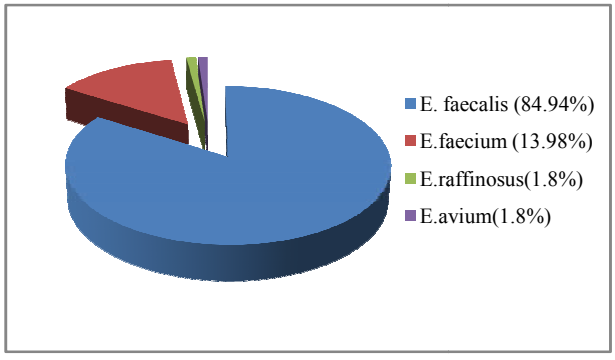

Figure 1 Retrospective data of the isolates

Susceptibility to antimicrobial agents: Out of the 93 isolates 38 % of the isolates were multi drug resistant strains (Figure 2), either high level amino glycoside resistant (HLG) or Vancomycin resistant (VRE). 6.45 % of the isolates were Vancomycin resistant *Enterococci* (VRE).

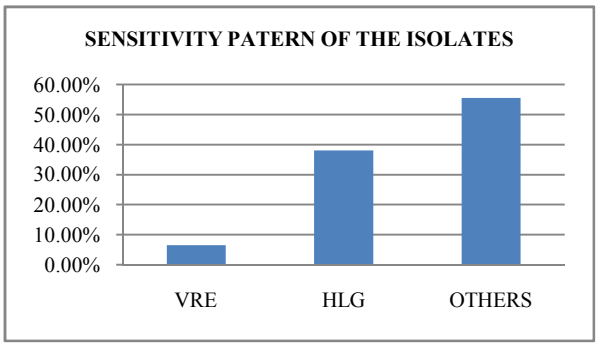

Figure 2

Isolated VRE strains were resistant to erythromycin, tetracyclin and tegicyclin (Table 2).

Table 2 Percentage of resistance of VRE

Antibiotic	% of Resistance
Ciprofloxacin	66.67
Erythromycin	100
Nitrofurantoin	66.67
GM 120	100
Levofloxacin	66.67
Linezolid	33.33
Penicillin G	66.67
Tetracyclin	100
Teicoplanin	50
Tegicyclin	100
Vancomycin	100

Percentage of resistance of VRE against teicoplanin was 50% and against linezolid was 33.33% (Figure 3).

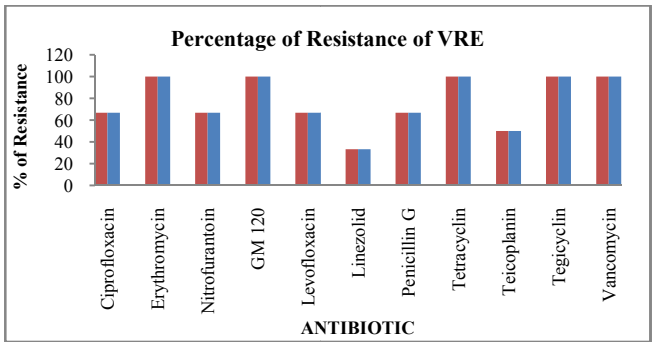

Figure 3

Antibiogram pattern of HLG resistant *enterococci* were detailed in the Table 3 and Figure 4.

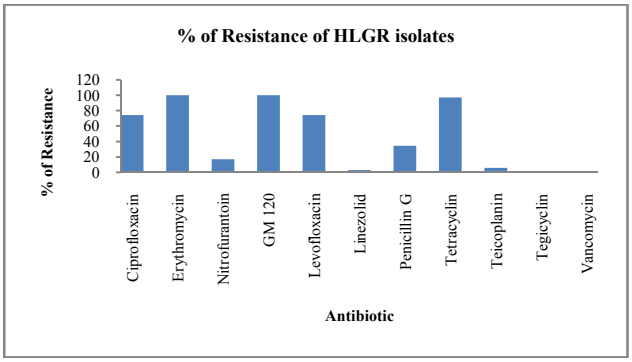

Figure 4

Table 3

Antibiotic	% of Resistance of HLGR
Ciprofloxacin	74.29
Erythromycin	100
Nitrofurantoin	17.14
GM 120	100
Levofloxacin	74.29
Linezolid	2.86
Penicillin G	34.29
Tetracyclin	97.14
Teicoplanin	5.71
Tegicyclin	0
Vancomycin	0

DISCUSSION

Multi drug resistant *Enterococci* have rapidly introduced in severe infections and may lead to severe nosocomial outbreaks. Proper infection control practices, proper education to health workers and continuous screening for multi-drug resistant infections are very essential to check such outbreaks (HICPAC, 1995). Hence the screening showed an increased prevalence of High Level Aminoglycoside resistant (HLGAR) and Vancomycin resistant *Enterococci* (VRE) strains.

In other studies *E. feacalis* is the most prevalent species in clinical infections, approximately 80 - 90% (Jones *et al.,* 1995 & Murray *et al.,* 2003). But Karmarkar, *et al.,* 2008 reported *E. faecium* as the most prevalent species. But in contrast to him we isolated approximately 85% *E. faecalis* from all types of clinical specimens as said by Jones, 1995 & Murray 1999 and only nearly 14% of isolates were *E. faecium*. *E. raffinosus* and *E. avium* were also isolated.

Out of the 93 isolates of *Enterococci,* 38% were multi drug resistant which is with high prevalence compared to other authors. In accordance with Mathai, 1994 we also found a high rate of High level aminoglycoside resistant (HLGAR) strains. Approximately 35% of isolates were HLGAR. Similar reports were obtained from other studies in India before (Mathai *et al.,* 1994). Even though the vancomycin resistance (VRE) incidence rate is low, the emergence of VRE is to be considered very seriously. The antibiotic resistance pattern for VRE isolates of our study reveals that the 50% of them were resistant to teicoplanin also. This is a significant pattern to be studied as most of the earlier studies showed lower teicoplanin resistance (Karmarkar *et al.,* 2004).

CONCLUSION

This study reveals an increased emergence of multidrug resistance in Enterococci and the molecular evaluation of the isolates could be done for detection of resistance factors. Such studies should be used to recognize the antibiotic policy in the area of interest in order to avoid the development of antibiotic resistance.

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