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Prevalence and Antibiotic Susceptibility Pattern of *Enterococcus spp.* from Various Clinical Samples of Patients at a Tertiary Care Hospital, Patna

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A B S T R A C T

Enterococci are opportunistic human pathogen and are gaining importance due to their high level of resistance against antimicrobials (betalactams, aminoglycosides, glycopeptides). The present study aims to estimate the burden of *Enterococcal* infection and determine its antibiogram at our multi-speciality hospital. This hospital based retrospective study was carried out in the Dept. of Microbiology, IGIMS, Patna. Isolates of *Enterococci* from various clinical samples were subjected to antibiotic susceptibility testing and speciation. Laboratory procedures were followed as per CLSI recommendation. From a total of 13,046 sample processed, 286 *Enterococcus* spp.were isolated, of which 234(83%) were from IPD and 52(17%) from OPD patients. Maximum number of *Enterococcus* spp. were recovered from urine (246) followed by pus(16). Isolates were found to be resistant to ciprofloxacin (92%), levofloxacin (85%),and high-level gentamicin (60%).Only one of the isolates showed resistance to linezolid. Sensitivity to vancomycin and nitrofurantoin (in urine) were 96% and 78%respectively. Antibiotic resistance is an ever-increasing problem which complicates management in patients with *Enterococcal* infection. Prudent use of antibiotics is a fundamental strategy to avoid such problems.

Introduction

Enterococci are ubiquitous gram positive, non-spore forming, facultative anaerobic cocci belonging to group D Streptococci as characterized by Lancefield in 1938, whose taxonomy has changed considerably in last few years (Parmeswarappa *et al.*, 2013). They are part of normal intestinal flora in humans, but are also responsible for causing opportunistic infections (Murray *et al.*, 2000). Earlier they were considered pathogen of low virulence but during the last three decades, their impact in nosocomial settings has gained our concern. They primarily affect debilitated and immunocompromised patients and exhibit resistance to many antimicrobials. *Enterococci* affects multiple anatomic sites and produce a

variety of infections like bacteremia, endocarditis, UTIs, abdominal and pelvic infections, skin and soft tissue infections, bone and joints infections, CNS infections and pulmonary infections. In the Indian scenario, *Enterococci* are emerging as nosocomial pathogens which are difficult to treat. The clinical importance of *Enterococci* is directly related to its changing antibiogram. Prolonged stay in hospital, empirical use of antibiotics, lack of sufficient knowledge and poor implementation programs to control the rapid spread of Enterococci has led to increased mortality caused by these infections (Bhatt *et al.*,2015 & Arias *et al.*, 2012). *Enterococci* are regarded as "tough bugs" capable of withstanding many environmental stressors and surviving for long periods. They are tolerant of a wide range of environmental conditions, extreme temperature (10^0-45^0 C), pH(4.5-10.0) and high sodium chloride concentrations, enabling them to colonize a wide range of niches (Arias *et al.*,2014). Till date, literature describes 12 *Enterococcal* species which are pathogenic to human, most common being *Enterococcus faecalis* and *Enterococcus faecium*.

Enterococcus spp. are intrinsically resistant to a number of antimicrobials including cephalosporins, trimethoprim sulfamethoxazole and also exhibit low level resistance to beta lactams and aminoglycosides (Mulla *et al.*, 2012 & Tripathi *et al.*, 2016). This intrinsic resistance to several commonly used antimicrobials is the main reason for their survival despite aggressive treatment. *Enterococci* are also capable in acquiring resistance either through chromosome transfer of plasmid or by acquisition of transposons. Their capability of transferring determinants of antibiotic-resistant genes between the species, as well as other bacteria (*Staphylococcus aureus*), is a global cause of concern. Dramatic increase in antibiotic resistance of *Enterococcus* species worldwide highlights the need for greater understanding of this genus.

Materials and Methods

This retrospective study was carried out in the department of Microbiology, Indira Gandhi Institute of Medical Sciences(IGIMS), Patna, India. Aim was to determine the prevalence of *Enterococci* in various clinical samples of patients coming to the institute for treatment. All the clinical samples like blood, CSF, pleural fluid, peritoneal fluid, urine, wound swabs and pus swabs received from January 2018 to December 2018 showing growth of *Enterococci* in culture were included in the study.

Samples were processed and the pathogen identified as per routine laboratory protocol. Standard guidelines issued by Clinical Laboratories Standards Institute (CLSI) were used for testing the anti-microbial susceptibility pattern of all the isolates(CLSI 2016).

Samples were subjected to gram's staining and then cultured onto 5%sheep blood agar, chocolate agar and MacConkey agar plates. Urine samples were also cultured on CLED plates. They were incubated for18- 24 hours at 37 ^{0} C and growth on culture plates were described. On sheep blood agar plate colonies were as 0.5-1mm in diameter and non-hemolytic. On MacConkey agar they appeared as small dark-red magenta colored colonies.

Growth were subjected to Gram staining procedure, in which it showed gram positive cocci in pairs. Colonies were confirmed as *Enterococci* with the help of various biochemicals like negative catalase test, positive bile esculin test, growth in 6.5%NaCl broth(Konemann's Color Atlas 2006). Further speciation was done on the basis of pigment production, motility test and standard

biochemical reactions as per standard microbiological guidelines.

All *Enterococcal* isolates were then subjected to modified Kirby-Bauer antibiotic susceptibility test using standard techniques as per CLSI recommendations (Facklam *et al.,* 1989 & Murray *et al.,*). The panel of antibiotic discs that were used for susceptibility testing of *Enterococcal* isolates were ampicillin (10mcg), ciprofloxacin (5mcg), gentamicin high content (120mcg), vancomycin (30mcg) and linezolid (30mcg).

For urine isolates, an additional nitrofurantoin (300mcg) disc was used. With the help of a straight inoculating wire, 3–5 well-isolated colonies of the same morphology were picked and transferred into a tube containing 5 ml of Mueller Hinton broth. The broth culture was incubated at 35°C until the turbidity of 0.5 McFarland standard was achieved.

The comparison was done visually in adequate light against Wickerham card..After achieving the required turbidity sample for AST was taken using a sterile cotton swab dipped into inoculum suspension. Any excess inoculum was removed by rotating and firmly pressing it against the inner wall of the tube above the fluid level. Lawn culture was done over the entire surface of sterile Mueller Hinton agar plate.

Thereafter, antimicrobial discs supplied by HiMedia Laboratories, India, were dispensed evenly onto the surface of inoculated agar plates which were incubated within 15 minutes for 16–18 hours at 35°C.Eventually, the results were read and interpreted.After16-18 hours of incubation, plates were viewed with unaided eye using reflected light for the presence or absence of zones of inhibition around the antibiotic discs. Strain of ATCC *Enterococcus faecalis* 29212 was used as control.

Statistical Analysis

Microsoft Excel and Microsoft word (version 8.1) were used to generate the tables and figures. Results are based on descriptive statistics.

Results and Discussion

A total of 13,046 considering exudate and urine samples were received in our laboratory. Among them, 1822 (14%) showed growth on culture plates. *Enterococcus* spp. were isolated and identified in 286 isolates, of which 234(83%) belonged IPD and 52 (17%) to OPD. Speciation was done using the standard laboratory protocol using phenotypic method of identification. Of 286 *Enterococcus* isolates, 264 (92.3%) were *E.faecalis* and 22 (7.6%) were *E.faecium* (Table 1). Male and female ratio was 1:1.17(Figure1). Age wise distribution showed greater no. of cases in the age group of 40-49(27%) followed closely by 20-29(20%) (Table 2). Majority of isolates were recovered from urine (uropathogen) i.e. 246 (86%) (Figure 2).

Isolates were found to be resistant to ciprofloxacin(92%), levofloxacin (85%), high level gentamicin(40%) and ampicillin(39%). A single isolate from urine showed resistance to linezolid which was from a patient admitted to ICU. Urinary isolates were moderately sensitive (78%) to nitrofurantoin. Resistance to vancomycin(VRE) was found in 4% of urinary samples, isolated from IPD patients.

Enterococci have now emerged as an increasingly important nosocomial pathogen. These infections are recognized by 3 ts - tough, tenacious and often times troublesome (Edwards *et al.*, 2000). CDC in a survey on nosocmial diseases, indicated that *Enterococci* accounted for 13.9% infections, being next to *Escherichia coli*(CDC 1989-93).

Table.1

Specimen	*E.faecalis*	*E.faecium*
Urine	228	18
Pus	14	02
Ascitic fluid	08	00
Pleural fluid	02	00
HVS	04	00
Conj.swab	02	00
Tissue	02	00
BAL	04	02

Table.2

Nature Of Specimen Recovered From Different Age-Groups									
AGE	**A/F**	**BAL**	**CONJ.SW**	**HVS**	**P/F**	**PUS**	**TISSUE**	**URINE**	**Grand Total**
10-19						2		16	**18**
20-29	2			4				52	**56**
30-39	2	2	2		2	4		44	**56**
40-49	4	2				8	2	62	**78**
50-59								32	**32**
60-69		2				2		22	**26**
70-80								18	**18**
Grand Total	**8**	**6**	**2**	**4**	**2**	**16**	**2**	**146**	**286**

Fig.1

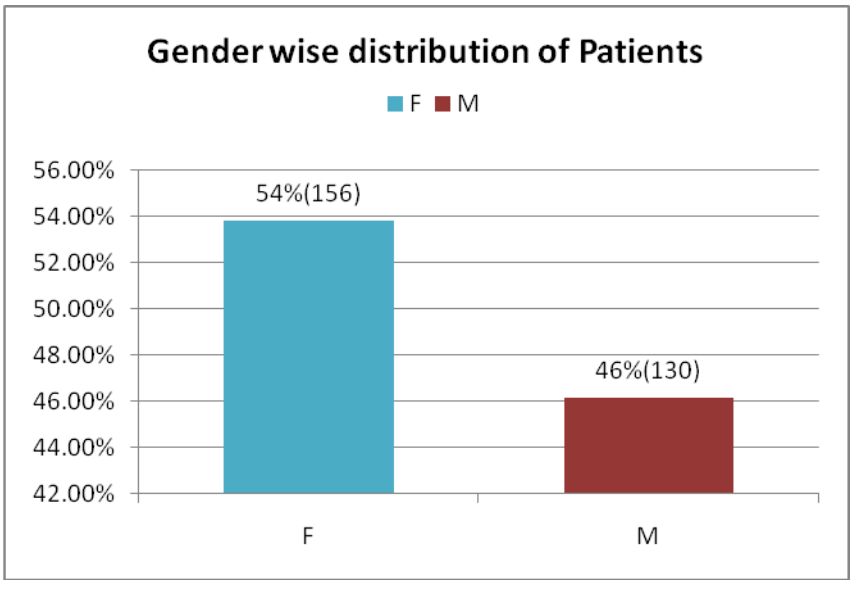


Fig.2

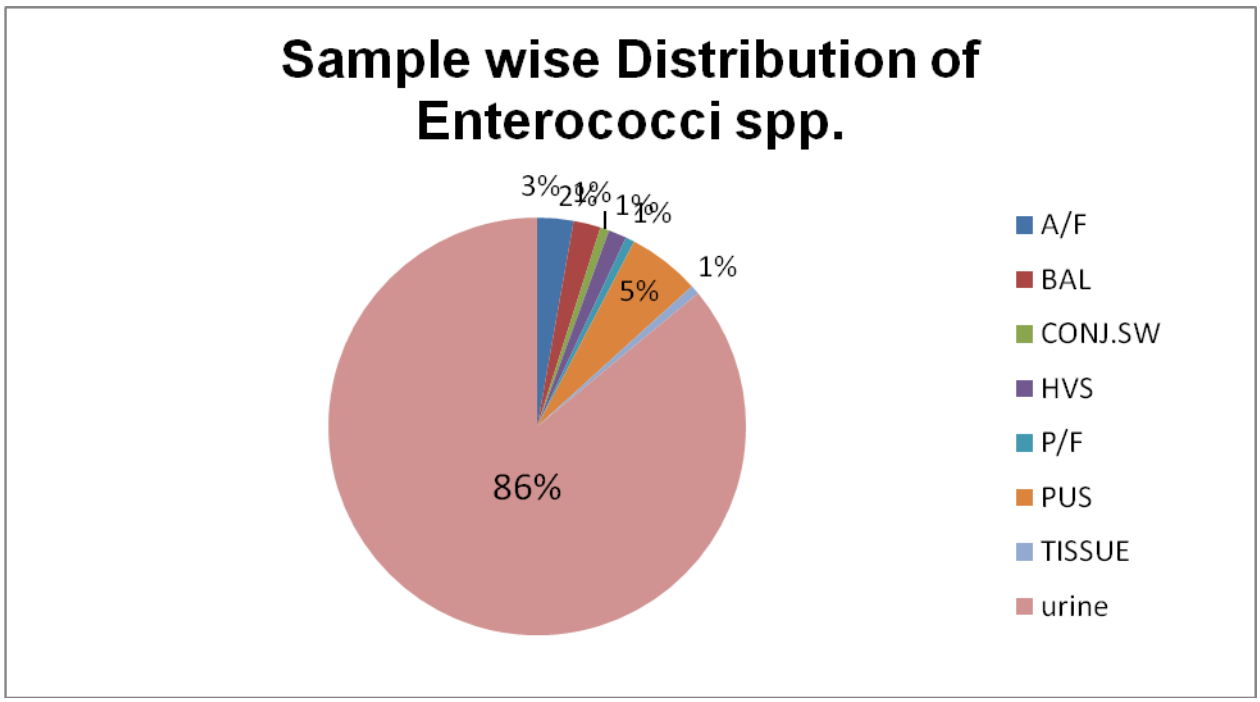
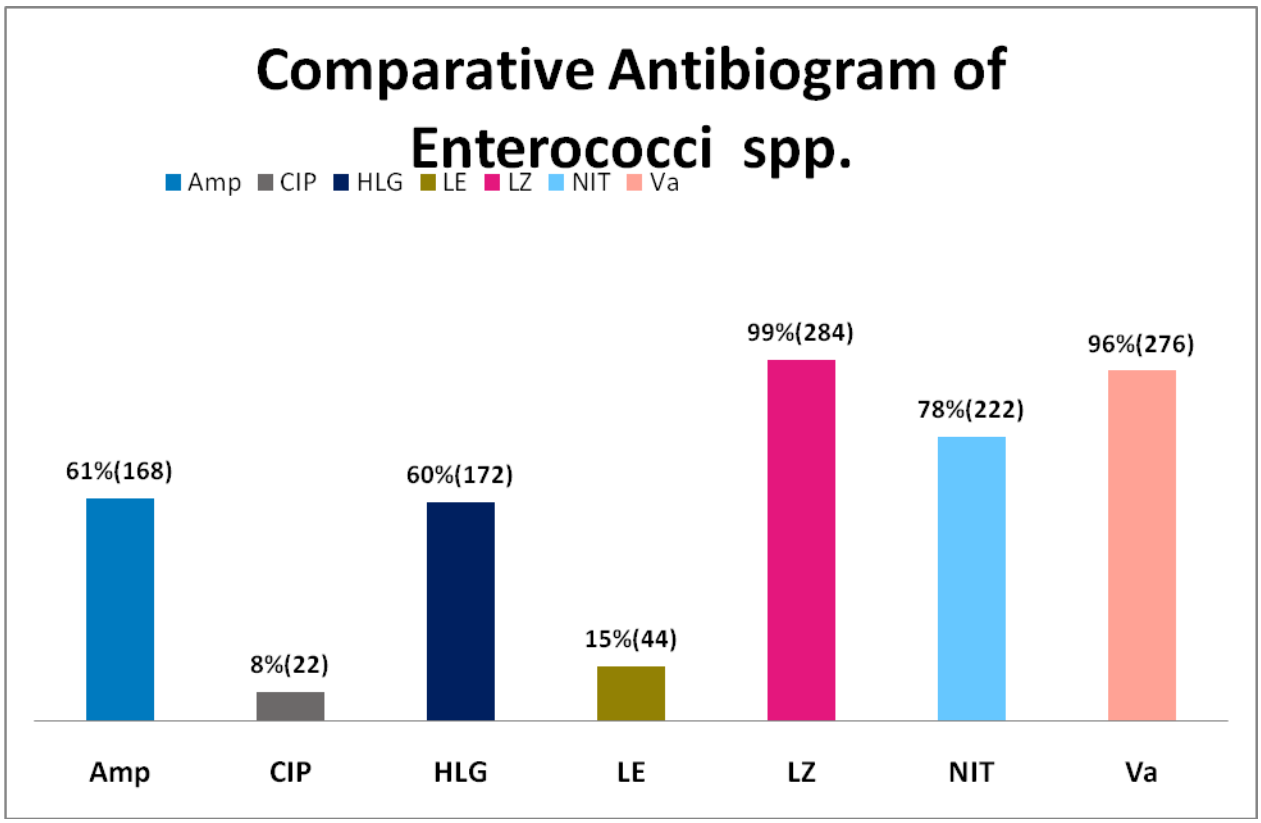

Sample wise Distribution of Enterococci spp.
3%
2%
1%
1%
1%
5%
86%
A/F
BAL
CONJ.SW
HVS
P/F
PUS
TISSUE
urine

Fig.3


Comparative Antibiogram of Enterococci spp.
Amp
CIP
HLG
LE
LZ
NIT
Va
61%(168)
8%(22)
60%(172)
15%(44)
99%(284)
78%(222)
96%(276)
Amp
CIP
HLG
LE
LZ
NIT
Va

Hence, it is important to know the changing pattern of its antibiogram. Frequently considered to be commensals, *Enterococci* has come into picture due to their ability to cause infections especially in immunocompromised and hospitalized patients. In our study most of the isolates were recovered from urine followed by pus and blood. Studies done ones estimating the burden of Enterococcal infections support our findings (24). This study also reveals a drastic increase in resistance of the organism to fluoroquinolones which again is comparable to the study conducted in Bangalore (Sreeja *et al.,* 2012). Resistance to ciprofloxacin was found to be 92%. Similar pattern of high resistance to ciprofloxacin was reported by Butch *et al.,* and Subbalaxami *et al.,* (Butcu *et al.,* 2011 & Subbalaxmi *et al.,* 2010). High resistance to ciprofloxacin probably could be because of its irrational use as over-the-counter drug. Study by Clara Sinel showed that since fluoroquinolones are extensively prescribed, sub-inhibitory concentrations(SICs)are likely to occur *in vivo,* with potential effects on bacterial metabolism with subsequent modulation of opportunistic traits (Sinel *et al.,*2017).

Resistance of our urinary isolates against nitrofurantoin was 22% which is in concordance with other studies(Kaur *et al.,* 2003 & Meena *et al.,* 2017).

The drug of choice for serious *Enterococcal* infections is an aminoglycoside in combination with a cell wall active agent. However, high-level aminoglycoside resistance (HLAR) is responsible for loss of synergy between cell wall active agents and aminoglycoside (Bekhit *et al.,* 2011). Studies conducted in various countries also report varying rates of resistance to HLG. A study from India(Shah *et al.,* 2012) reported 40%, 53%, 68%, and 8% resistance rates for ampicillin, high-level gentamicin, high-level streptomycin, and vancomycin respectively. Another study done in 2007 by Gupta *et al.,* reported 75%resistance rate for high-level gentamicin. In our study, resistance to HLG was found to be around 40% similar to study by Mendiratta *et al.,*(10).

Most recently, there has been reports of rising rates of resistance in *Enterococci* especially to Vancomycin (Chaudhary *et al.,* 2007 & Devi *et al.,*2002). In our study 4% isolates were VRE, which shows significant similarity to results (1.7-20%) from studies done in other parts of India(9-11). Study at Apollo hospitals Chennai(2012) showed similar findings with prevalence of VRE of about 4%(Vidyalakshmi *et al.,*2012).Research work from Southern part of India revealed higher percentage of VRE (Parmeswarappa *et al.,* 2013 & Karmarkar *et al.,* 2004). Emergence of VRE has been attributed to the indiscriminate use of vancomycin, the colonization pressure and noncompliance with the infection control measures(Tripathi *et al.,* 2016,Vidyalakshmi *et al.,* 2012, Biswas *et al.,* 2016)

Antibiotic resistance is an ever-increasing problem complicating therapy in patients with bacterial infections, especially *Enterococcal*. They pose a major therapeutic challenge because of having both intrinsic and acquired resistance to various antibiotics. Widespread use, misuse and easy availability of these antibiotics over the counter without any prescription is considered to play a major contributory role. Change in pattern of antibiotic resistance in *Enterococcus* spp., with the emergence of vancomycin resistance (VRE) and high-level aminoglycoside resistance is of major concern. Currently, specific recommendation for antimicrobial prescription of VRE is lacking. Moreover, at most of the clinical settings, the protocol for follow-up surveillance is inadequate (Addisu *et al.,* 2020). Increased prevalence of MDR Enterococci constitute a major health issue,

leading to high rates of morbidity and mortality, economic loss and limited treatment options. To encounter this alarmingly increasing health problem, there is urgent need for evidence-based research, focusing on the identification of the factors facilitating the transmission of *Enterococcal* antimicrobial resistance within the hospital environment. Prudent use of antibiotics is a fundamental strategy to deal with the problem of antibiotic resistance.

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