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Isolation and Identification of *Enterococcus* Species from Various Clinical Samples and Their Antimicrobial Susceptibility Pattern at a Tertiary Health Care Hospital in Agra

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A B S T R A C T

The present study was conducted on various clinical samples, in the Department of Microbiology, S.N medical college, Agra from February 2018 to July 2018. 72 *Enterococcus* isolates were identified. Maximum samples from which *Enterococcus* spp. was isolated is urine i.e. 39 (54.17%), followed by pus and blood i.e. 22 (30.55%) and 5 (6.94%) respectively. Maximum isolate is *E. faecalis* i.e. 62 (86.11%) followed by *E.faecium* i.e. 10 (13.89%). On Antimicrobial Susceptibility testing, Ampicillin, Ciprofloxacin, Levofloxacin, Tetracycline and Doxycycline show 70.83%, 38.88%, 30.55%, 58.33% and 52.77% resistance respectively. Among the 33 Enterococcal isolates tested by disc diffusion method, Erythromycin shows 57.57% resistance. Nitrofurantoin and Norfloxacin show 15.38% and 76.92% resistance respectively, among the 39 Enterococcal isolates obtained from urine. Vancomycin shows 4.16% resistance and High Level Gentamycin shows 18.05% resistance. The emergence of Vancomycin resistance and High Level Aminoglycoside resistance in Enterococci poses a serious threat to patient's safety as it leaves fewer options for disease management. In the present study, Teicoplanin and Linezolid were found to be 100% susceptible for all the Enterococcal isolates, hence these drugs can be prescribed even for Vancomycin Resistant Enterococci (VRE).

Introduction

Enterococci are indigenous flora of the intestinal tract, oral cavity and the vagina. They are relatively avirulent in healthy individuals, but have emerged as nosocomial pathogens in spite of the low levels of their virulence (Sreeja *et al.,* 2012). The genus *Enterococcus* includes more than 17 species, but only a few cause clinical infections in humans (Sunil Kumar and Karthika, 2012). The common species of *Enterococcus* which cause human infections are *E. faecalis* (80-90 %) and *E. faecium* (5-10%), but now there is an increase in the isolation rate of *E. faecium* which is a problem, as its intrinsic resistance may lead to treatment failure (Sreeja *et al.,* 2012). Factors that determine the virulence of *Enterococcus* species include, their ability to colonize the gastrointestinal tract which is the normal habitat; ability to adhere to a range of

extracellular matrix proteins, including thrombospondin, lactoferrin and vitronectin; and ability to adhere to urinary tract epithelia, oral cavity epithelia and human embryo kidney cells. Most Enterococcal infections are believed to be endogenous, by translocation of bacteria through the intestinal epithelial cells, which then cause infection via lymph nodes and thus spread to other cells within the body (Fisher and Phillips, 2009). The most frequent infections caused by enterococci are urinary tract infections (UTI), with intra - abdominal and intra - pelvic abscesses or post-surgery wound infections being the second most frequent (Low *et al.,* 2001). Blood stream infections are the third most frequent infections followed by other infections such as central nervous system (CNS) and neonatal infections that are caused with lower frequency. Respiratory tract infections, osteomyelitis, or cellulitis are rarely caused by Enterococci. Enterococci are currently ascendant nosocomial pathogens, that have become the second most common organisms recovered from nosocomial UTI and wound infections and the third most common cause of nosocomial bacteraemia in the United States (Sood *et al.,* 2008). Further, the emergence of high level aminoglycoside resistance (HLAR), β-lactamase production and glycopeptide resistance including vancomycin resistant enterococci (VRE), is posing a therapeutic challenge to the physicians due to the ease of acquiring and transferring antimicrobial drug resistance (Murray, 1998).

Materials and Methods

The study was conducted, in the Department of Microbiology, S.N Medical College, Agra from February 2018 to July 2018. It was done on various clinical samples of IPD and OPD patients attending the department of Microbiology during this period. Complete data about the patients such as name, age, sex, date, time of collection, source of specimen and details about the clinical history was recorded before the specimen was processed. Various clinical samples like urine, pus, blood, Ascitic fluid, Pleural fluid, CSF and Endotracheal tube tip were collected by aseptic technique in sterile container except blood which was collected in blood culture bottle.

Processing in laboratory

Direct microscopy

Smears were made from the specimens and Gram staining was done to look for pus cells & Gram positive cocci arranged in pairs and short chains.

Culture

After collection of samples, these were inoculated on the nutrient agar, blood agar, Mac Conkey agar. For urine culture, Cysteine Lactose Electrolyte Deficient (CLED) medium was used. Such inoculated plates were incubated at 37^0C for overnight. On nutrient agar, colonies were circular, translucent, smooth, convex, 1-2mm in diameter, with regular margin, on blood agar, colonies were circular, translucent, smooth, convex, 1-2mm in diameter, with regular margin showing either alpha or non-hemolytic colonies. On Mac Conkey agar, colonies were small, 0.5-1mm, magenta colored while on CLED agar, small, orange-yellow colonies were seen. In case of urine specimen, colony counting was done and only the significant counts were further processed.

Presumptive identification of *Enterococcus* genus was done by colony morphology, Gram's stain and biochemical reactions as per standard protocol (Facklam and Collins, 1989) like catalase test, bile esculin test, salt tolerance test, heat tolerance test and PYR test in which Enterococci are Catalase negative,

hydrolyse the aesculin to aesculitin, tolerate the salt concentration of 6.5%, tolerate the temperature of 60°C for 30 minutes and PYR test positive, respectively. Further *Enterococcus* species were identified by pyruvate utilization test, potassium tellurite reduction test, arginine dihydrolase test, motility testing and sugar fermentation tests including glucose, arabinose, raffinose, mannitol, sorbitol, sucrose, lactose.

Antimicrobial susceptibility testing was done on Mueller Hinton agar plate by Kirby Bauer Disc diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). The antibiotic discs used for *Enterococcus spp.* were: Ampicillin (10ug), Ciprofloxacin (5ug), Levofloxacin (5ug), Tetracycline (30ug), Doxycycline (30ug), Erythromycin (15ug), Norfloxacin (10ug-for urine), Nitrofurantoin (300ug-for urine), Vancomycin (30ug), Linezolid (30ug), Teicoplanin (30ug) and High Level Gentamicin (120ug).

Results and Discussion

In the present study, 72 *Enterococcus* isolates were identified. Maximum samples from which *Enterococcus* was isolated is urine i.e. 39 (54.17%), followed by pus and blood i.e. 22 (30.55%) and 5 (6.94%) respectively. Similarly, (Nautiyal *et al.,* 2016) and (Mukherjee *et al.,* 2016) in their study reported maximum number of isolates from urine, followed by pus and blood. (Triveda *et al.,* 2016) also in their study reported maximum isolates from urine, followed by pus and blood. Out of the 72 Enterococcal isolates, maximum isolate is *Enterococcus faecalis* i.e 62 (86.11%) followed by *Enterococcus faecium* i.e. 10 (13.89%). This is comparable with the study of (Suddhanshu Bharadwaj *et al.,* 2013) who reported 86% *E.faecalis* and 14% *E.faecium* in their study. Similar findings of 86.25% *E.faecalis* and 12.5% *E.faecium* were reported by (Shanmukhappa *et al.,* 2015). Among urine isolates, 34 are *E.faecalis* and 5 are *E.faecium* while among pus isolates, 19 are *E.faecalis* and 3 are *E.faecium*. Among blood isolates, 4 are *E.faecalis* and 1 is *E.faecium* whereas in Ascitic fluid isolates, 1 isolate is of *E.faecalis* and 1 isolate is of *E.faecium*. 2 isolates of *E.faecalis* are obtained from pleural fluid, 1 isolate from CSF and 1 isolate from Endotracheal tube tip respectively.

Maximum number of patients are in age group of 21-30 years i.e. 29 (40.27%) followed by 31-40 years i.e 14 (19.44%), >60 years i.e. 9 (12.5%), 51-60 years i.e. 7 (9.72%), 11-20 years i.e. 6 (8.33%), 41-50 years i.e. 4 (5.55%) and least from age group of 0-10 years i.e. 3 (4.16). In this study, maximum number of patients are in age group of 21-30 years i.e. 29 (40.27%) and least from age group of 0-10 years i.e. 3 (4.16). This is similar to the study of (Bose *et al.,* 2012) who reported maximum number of patients in age group of 21-30 years and least from age group of 0-10 years while, in another study (Shanmukhappa *et al.,* 2015) have reported high prevalence of Enterococcal infection in the age group ≥61 years and least from age group of 41-60 years. No. of males are higher i.e. 42 (58.33%) as compared to females i.e. 30 (41.67%), which may be due to the fact that more importance and priority is given to the health of males in our society. Maximum patients are from IPD i.e. 45 (62.5%) followed by OPD i.e. 27 (37.5%). Linezolid and Teicoplanin show 100% susceptibility each, by disc diffusion method, in the present study. Ampicillin shows 70.83% resistance which is comparable with the study of Mukherjee *et al.,* (2016) who reported 70% resistance for Ampicillin among the Enterococcal isolates. Ciprofloxacin, Tetracycline and Erythromycin show 38.88%, 58.33% and 57.57% resistance respectively among the Enterococcal isolates (Fig. 1 and 2; Table 1–8).

Fig.1 Gender wise distribution of patients

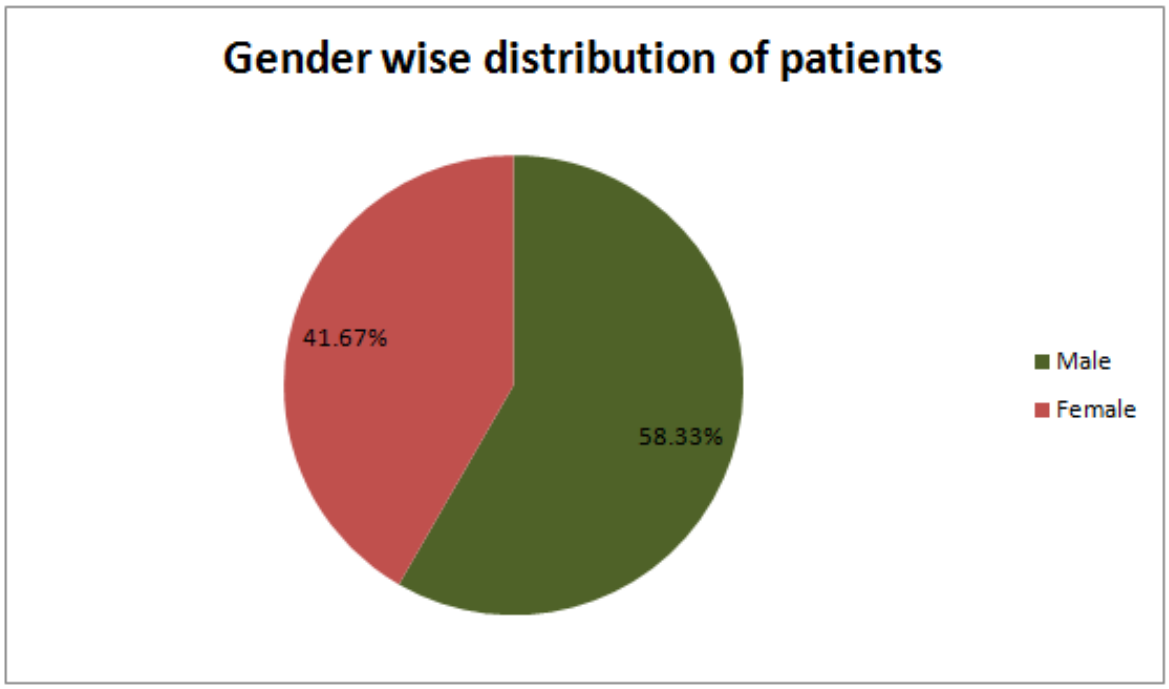

Fig.2 Distribution of patients according to OPD and IPD

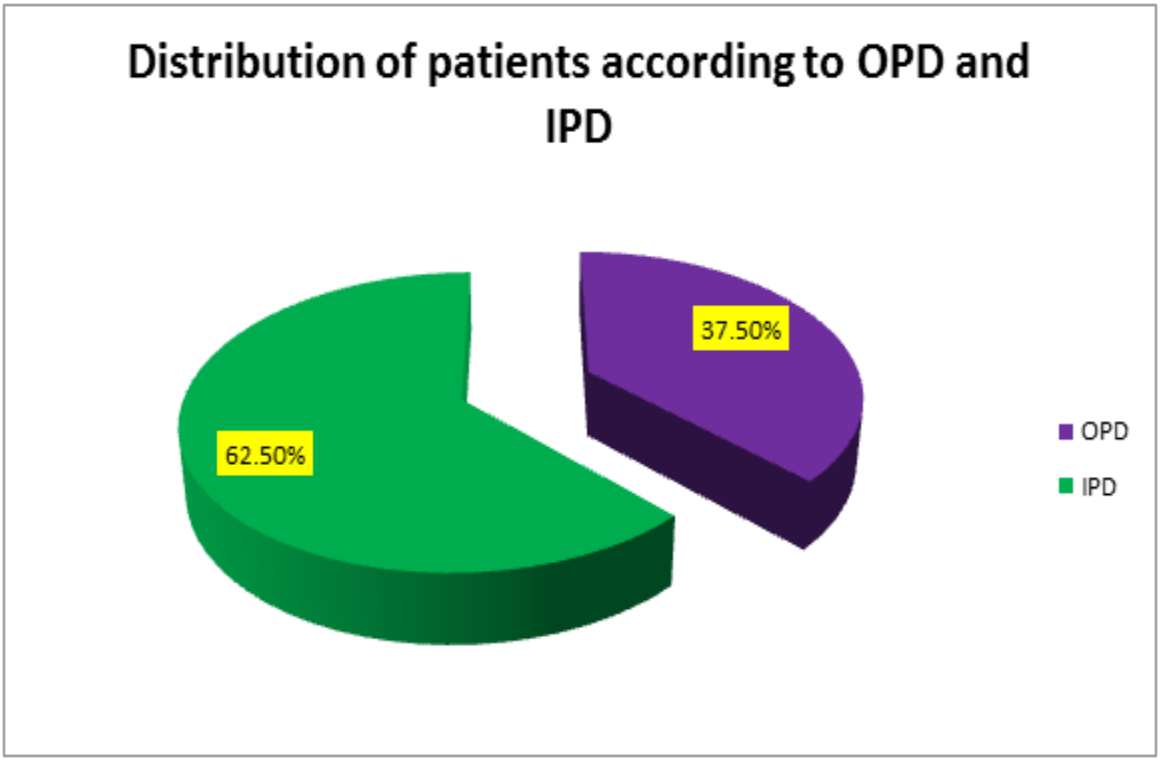

Table.1 Distribution of patients according to age & sex

Age groups (years)	Male	Female	Total	
			No.	%
0-10	2	1	3	4.16
11-20	4	2	6	8.33
21-30	16	13	29	40.27
31-40	8	6	14	19.44
41-50	3	1	4	5.55
51-60	3	4	7	9.72
>60	6	3	9	12.5

Table.2 Distribution of Enterococcal isolates in different clinical samples

SAMPLES	No.	(%)
Urine	39	54.17
Pus	22	30.55
Blood	5	6.94
Ascitic fluid	2	2.78
Pleural fluid	2	2.78
CSF	1	1.39
Endotracheal tube tip	1	1.39
Total	**72**	**100**

Table.3 Distribution of *E. faecalis* and *E. faecium* in the total Enterococcal isolates

E.faecalis		*E.faecium*	
No.	%	No.	%
62	86.11	10	13.89

Table.4 Distribution of *E.faecalis* and *E.faecium* in various clinical samples

SPECIMEN	*E. faecalis*	*E. faecium*
Urine	34	5
Pus	19	3
Blood	4	1
Ascitic fluid	1	1
Pleural fluid	2	0
CSF	1	0
Endotracheal tube tip	1	0
Total	**62**	**10**

Table.5 Antibiotic Susceptibility Pattern of the total Enterococcal isolates

Antibiotics	Sensitive		Resistant	
	No.	%	No.	%
Ampicillin (n=72)	21	29.16	51	70.83
Ciprofloxacin (n=72)	44	61.11	28	38.88
Levofloxacin (n=72)	50	69.44	22	30.55
Tetracycline (n=72)	30	41.66	42	58.33
Doxycycline (n=72)	34	47.22	38	52.77
Erythromycin (n=33)	14	42.42	19	57.57
Nitrofurantoin (n=39)	33	84.61	6	15.38
Norfloxacin (n=39)	9	23.07	30	76.92
Vancomycin (n=72)	69	95.83	3	4.16
Linezolid (n=72)	72	100	0	0
Teicoplanin (n=72)	72	100	0	0
High Level Gentamicin (n=72)	59	81.94	13	18.05

Table.6 Antibiotic Resistance Pattern of *Enterococcus spp.* isolated from urine samples

Antibiotics	Resistance	
	No.	%
Ampicillin (n=39)	26	66.66
Ciprofloxacin (n=39)	16	41.02
Levofloxacin (n=39)	13	33.33
Tetracycline (n=39)	24	65.53
Doxycycline (n=39)	19	48.71
Nitrofurantoin (n=39)	6	15.38
Norfloxacin (n=39)	30	76.92
Vancomycin (n=39)	2	5.12
Linezolid (n-39)	0	0
Teicoplanin (n=39)	0	0
High Level Gentamicin (n=39)	8	20.51

Table.7 Antibiotic Resistance Pattern of *Enterococcus spp.* isolated from Clinical samples other than urine

Antibiotics	Resistance	
	No.	%
Ampicillin (n=33)	25	75.75
Ciprofloxacin (n=33)	12	36.36
Levofloxacin (n=33)	9	27.27
Tetracycline (n=33)	18	54.54
Doxycycline (n=33)	19	57.57
Erythromycin (n=33)	19	57.57
Vancomycin (n=33)	1	3.03
Linezolid (n=33)	0	0
Teicoplanin (n=33)	0	0
High Level Gentamicin (n-33)	5	15.15

Table.8 Antibiotic Resistance Pattern of *E.faecalis* and *E.faecium* in the total Enterococccal isolates

Antibiotics	Resistant		*E.faecalis*		*E.faecium*	
	No.	%	No.	%	No.	%
Ampicillin (n=72)	51	70.83	44	70.96	7	70
Ciprofloxacin (n=72)	28	38.88	23	37.09	5	50
Levofloxacin (n=72)	22	30.55	18	29.03	4	40
Tetracycline (n=72)	42	58.33	37	59.67	5	50
Doxycycline (n=72)	38	52.77	34	54.83	4	40
Erythromycin (n=33)	19	57.57	15	53.57	4	80
Nitrofurantoin (n=39)	6	15.38	5	14.7	1	20
Norfloxacin (n=39)	30	76.92	26	76.47	4	80
Vancomycin (n=72)	3	4.16	2	3.22	1	10
Linezolid (n=72)	0	0	0	0	0	0
Teicoplanin (n=72)	0	0	0	0	0	0
High Level Gentamicin (n=72)	13	18.05	11	17.74	2	20

This is comparable with the study of (Shanmukhappa *et al.,* 2015) who reported 38.75%, 58.75% and 55% resistance respectively for Ciprofloxacin, Tetracycline and Erythromycin. Nitrofurantoin shows 15.38% resistance among the 39 Enterococcal isolates obtained from urine which is comparable to the study of (Paul *et al.,* 2017) who reported 16.4% resistance for Nitrofurantoin. In this study, Vancomycin shows 4.16% resistance and High Level Gentamycin shows 18.05% resistance. Three Enterococcal isolates which were found to be resistant against Vancomycin by disc diffusion method, were further confirmed by E-test, which showed two isolates resistant against Vancomycin and one isolate sensitive. It is therefore necessary to confirm all VRE isolates by confirmatory test such as, E-test to eliminate the false positive isolates of VRE detected by disc diffusion method. The overall antibiotic resistance was higher in *E.faecium* as compared to *E.faecalis* except for Tetracycline and Doxycycline which showed higher resistance in *E.faecalis* as compared to *E.faecium*. This is comparable with the study of (Jain *et al.,* 2011) and (Saraswathy *et al.,* 2013) who reported higher resistance for tetracycline in *E.faecalis* as compared to *E.faecium* in their study.

The emergence of Vancomycin resistant Enterococci poses a serious threat to patient's safety as it leaves fewer options for disease management and there is a potential risk of the Vancomycin resistance gene transfer from the *Enterococci* to *Staphylococcus aureus*. Resistance to aminoglycoside is also of great concern, as it eliminates the synergy of aminoglycosides with beta lactam antibiotics, which is the treatment of choice for most of the enterococcal infections, thus limiting the therapeutic options.

There has been an increase in the rate of infection and antibiotic resistance in *Enterococcus* species. There is also a change in pattern of antibiotic resistance in *Enterococcus* species, with the emergence of Vancomycin resistance and High Level Aminoglycoside resistance. Enterococci can survive in the hospital environment due to their intrinsic resistance to several commonly used antibiotics and their ability to acquire

resistance to all currently available antibiotics through plasmid or by mutation.

Therefore, it is of utmost importance to implement infection control measures in the hospital, screening of health care workers, surveillance cultures in intensive care units along with constant monitoring of the antibiotic susceptibility pattern, that will help in formulating the local antibiotic policies.

In the present study, Teicoplanin and Linezolid were found to be 100% susceptible for all the Enterococcal isolates, hence these drugs can be prescribed even for Vancomycin Resistant Enterococci.

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