Section: Microbiology

Original Research Article

IDENTIFICATION AND SPECIATION OF ENTEROCOCCUS SPECIES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN VARIOUS CLINICAL SAMPLES

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ABSTRACT

Background: The aim is to isolate and identify Enterococcal species from various clinical samples and determine its antimicrobial susceptibility pattern. Background: Enterococci are a source of nosocomial and super infections in patients treated with antimicrobials. They can spread easily through water, animal meat, fomites, and healthcare workers. Most Enterococcal infections are caused by the patient's own commensal bacteria. **Materials and Methods:** A total 260 various clinical samples were collected from Patients attending the OPD and IPD. Enterococcal Isolates were identified by standard biochemical tests. Antimicrobial sensitivity testing was done on Mueller- Hinton agar by Kirby Bauer disc diffusion methods as per Clinical Laboratory Standards Institute (CLSI) guidelines. **Result:** Out of the total 260 clinical samples collected and processed from the patients suspected of bacterial infections, 108 (42%) showed growth of Enterococcus sp. Out of the total isolates of Enterococci 81% were E. faecalis and 19% were E. faecium. Antimicrobial susceptibility pattern of isolated Enterococcal species demonstrated 100% sensitivity to Vancomycin, teicoplanin, and linezolid. **Conclusion:** Among the total isolates, Enterococcus faecium was isolated in majority of patients 81% followed by Enterococcus faecium 19%. The most effective drugs were found to be vancomycin, teicoplanin, and linezolid.

INTRODUCTION

Genus Enterococci are aerobic, anaerobic, Gram-positive microorganism that are oval in shape and they appear in smears as pairs or single, or in short chain.[1] Intestinal Enterococcal sp are obligate anaerobes that can survive at high temperatures of (60°C) for short periods of time and can grow at high salt concentration. In the laboratory, they are characterized by their morphology on Gram stain and culture and by their hydrolysis of esculin in the presence of bile.[2] Enterococci related nosocomial infections have been on the rise in the last few years to the point where Enterococci are listed as one of the most isolated pathogens.[3] Enterococci are commonly thought of as commensal microorganisms found in the normal gut flora of humans and animals. However, due to their high isolation rates in community acquired as well as nosocomial infections associated with high mortality, Enterococci have become increasingly important.[4,5] Enterococci can survive on environmental surfaces for long periods of time and colonized patients are a potential vector for the transmission of these organisms to healthcare workers, the environment, and other patients.[6,7]

Most clinical Enterococcal isolates are multidrug resistant, giving them a selective edge in the hospital setting.[8,9] Many studies have shown that colonization is the most common cause of Enterococcal infections. Colonization can happen long before or shortly before infection, but colonization plays an important role in nosocomial infection.[10] Enterococci are also known as "opportunistic pathogens". They are a major contributor to nosocomial infections around the world.[9] This study was aimed at identification and speciation of Enterococcal species from various clinical samples and to determine its antimicrobial susceptibility pattern.

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MATERIALS AND METHODS

A cross sectional study was conducted in the Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow(UP). Out of 260 samples studied, Enterococci were isolated from 108 clinical samples from patients attending OPD and IPD of the hospital. Patients from all age-groups and sexes were included. The samples were inoculated on culture media after initial processing. All samples were inoculated on blood agar and MacConkey agar and CLED agar (cystine lactose electrolyte deficient agar) was used for the urine samples. Blood samples were inoculated in Blood culture bottles containing Brain heart infusion broth. All plates were incubated at 37°C for 18 -24hours to a maximum of 48 hrs under aerobic conditions.
Enterococccci were further identified based on colony morphology, Gram staining, catalase test, Bile Esculin hydrolysis and sugar fermentation. Isolates were identified by standard biochemical tests.[1] Antimicrobial sensitivity testing was done on Mueller- Hinton agar by standard disc diffusion methods as per Clinical Laboratory Standards Institute (CLSI) guidelines 2023.[13]

RESULTS

Out of 260 clinical samples of suspected bacterial infection Enterococcal species were isolated from 108 (42%) samples. Enterococccus faecalis was the major isolate (81%) while Enterococcus faecium made up only 19% of the total isolates. Out of the 108 samples highest number of Enterococci were isolated from urine sample (41%), followed by HVS (20%), pus (16%), blood (10%) and body fluids (2%). Higher number of Enterococci were isolated from samples received from OPD. Of the 108 Enterococci isolated, 37 were found in individuals of 21-30 years of age, 29 isolates were from age group of 31-40 years of age. Among 53 Enterococcal culture positive samples, most of the patient were from the Medicine Ward 30%. In the gender wise distribution; females were higher in number(76%).The Antimicrobial susceptibility pattern of isolated Enterococcal species reveal most effective Antibiotics were Vancomycin, Linezolid, and Teicoplanin which were 100% sensitive Followed by Doxycyclin (74.07%), HLG (40.75%), and Nitrofurantoin (27.77%)showing decreasing order of sensitivity respectively.

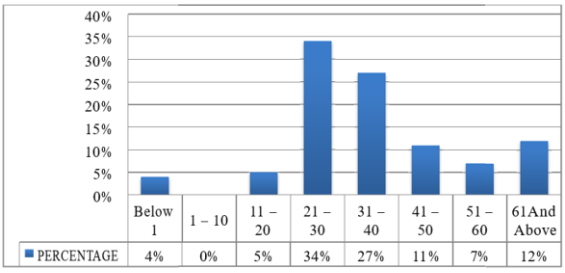

Figure 1: Age wise distribution

Table 1: Total Sample Studied

Total clinical sample studied	Isolated Enterococcus spp
260	108(42%)

Table 2: Isolated Enterococcus Spp from Various Clinical Samples

S.no.	Samples	No. Of isolates	Percentage
1	URINE	45	41%
2	PUS	17	16%
3	HVS	22	20%
4	TIP	4	4%
5	SEMEN	2	2%
6	TISSUE	3	3%
7	ASCITIC FLUID	2	2%
8	ET	2	2%
9	BLOOD	11	10%
TOTAL		108	100%

Table 3: Distibution according to the IPD & OPD ward

S.no.	Ward	Number of patients
1	OPD	55
2	IPD	53

Table 4: distibution according to the IPD ward

S.no.	Ward	Number	Percentage
1	ICU	8	15.09%
2	HIGH DEPENDENCY UNIT	4	7.55%
3	OBSTETRICS + LABOR ROOM	15	28.30%
4	SURGERY	9	16.98%
5	MEDICINE	16	30.19%
7	EMERGENCY	1	1.89%

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Table 5: Gender wise distribution of Enterococcus infection

S.no.	Gender	Number	Pecentage
1	Male	26	24%
2	Female	82	76%
Total		108	100%

Table 6: Speciation of Enterococcus species

S.no.	Species of enterococcus	No. Of isolates	Percentage
1	Enterococcus faecalis	87	81%
2	Enterococcus faecium	21	19%
Total		108	100%

Table 7: Antibiotic Susceptibility Pattern of Enterococcus

S.NO.	Antibiotics	Sensitive		Intermediate		Resistance	
		NO.	%	NO.	%	NO.	%
1	AMPICILLIN	26	24.07	33	30.55	49	45.37
2	CIPROFLOXACIN	8	7.40	30	27.77	70	64.81
3	VANCOMYCIN	108	100	0	0	0	0
4	LINEZOLID	108	100	0	0	0	0
5	TEICOPLANIN	108	100	0	0	0	0
6	DOXYCYCLIN	80	74.07	13	12.03	15	13.88
7	NITROFURATION (Urine)	30	27.77	69	63.88	9	8.33
8	LEVOFLOXACIN	5	4.62	42	38.88	61	56.48
9	NORFLOXACIN (Urine)	2	1.85	71	65.74	35	32.40
10	AMOXICLAV	22	20.37	79	73.14	7	6.48
11	PRISTINOMYCIN	13	12.03	25	23.14	70	64.81
12	HIGH LEVEL GENTAMYCIN	44	40.74	12	11.11	52	48.14

DISCUSSION

The present study was intended with the aim to identify Enterococcal species and to evaluate the antibiotic susceptibility pattern of these isolates. For this study, a total of 260 samples were included from patient's who attended the OPD and IPD of Era's Lucknow Medical College and Hospital.

In our study Enterococcus faecalis 81% were more common than Enterococcus faecium 19%. The finding is similar to the study by S. Sreeja et al. (2012) were Enterococcus faecalis 76% were more common than Enterococcus faecium24%.[14]

OPD samples made up 51% of isolates whereas 49% isolates were from the IPD. Interestingly these findings contrast with the result reported by Mohanty Srujana (2022) were 9%isolates where from OPD and 75.6% from IPD.[15]

The majority of the Enterococcus strains in our study were derived from the Urine sample 41%, followed by the pus 16% sample; which aligns with the results of Haritsa Kanthishree B. et al.(2014) who observed maximum number of isolates i.e72.2% from urine samples followed by pus 16.6%.[16]

Also in our study, higher number of Enterococci were isolated from female patients 76% compared to male patients 24%. This is consistent with an earlier analysis of Afroz Turin et al. (2024). In which among all participants, 37% were male, and 63% were female.[17]

Furthermore, the study conducted by Jada Sunil Kumar et al,[18] observed contrasting results showing more number of isolates i.e 55.06% among male compared to females (44.94%).

The age group from whom Enterococci were isolated in higher number was 21-30 years (34%) Contrasting to the study conducted by Abamecha Abdulhakim et al,[19] were higher number of isolates were from 28-55 age group (73.8%).

The present study was aimed to assess the impact of various antibiotics on Enterococcal isolates. It was observed that, all Enterococcal isolates shown 100% sensitivity to Vancomycin, teicoplanin, and linezolid, higher degree of resistance was observed for Doxycyclin 74.07%, HLG 40.75%, Nitrofuration 27.77%, Fluoroquinolones were the least effective. In the analysis done by Georges Martin et al, (2022) all tested isolates were 100% susceptible to Vancomycin, and linezolid, along with high level susceptibilty to Teicoplanin (97.5%), nitrofurantoin (90%), followed by ampicillin(84.1%), gentamycin (63.6%), and levofloxacin(56.85%).[20] On the other hand, the study by Afroz Turin et al, (2024) analyzed that ampicillin showed 74.07% susceptibility, followed by Levofloxacin 29.62% and Doxycyclin 14.81%.[17]

CONCLUSION

In the present study 260 clinical specimens from the patients suspected of bacterial infections were processed for culture and, 108 (41%) Enterococcus species were isolated. In terms of specimen distribution, the highest number of Enterococcus species was isolated from urine sample 42% followed by pus 16%. Among the total isolates, Enterococcus faecium was found in majority 81% followed by Enterococcus faecium 19%. Enterococcus isolates demonstrated that the Vancomycin, teicoplanin, and linezolid are 100% sensitive. Followed by Doxycyclin(74.07%), HLG(40.75%), Nitrofuration(27.77%) showing lower level of sensitivity. Fluoroquinolones were the least effective drugs.

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