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Original Research Article

AN INVESTIGATION OF THE DEVELOPMENT OF BIOFILM AND RESISTANCE TO VANCOMYCIN AMONG DIFFERENT SPECIES OF ENTEROCOCCUS BACTERIA IN A HOSPITAL THAT PROVIDES SPECIALIZED MEDICAL CARE.

Swathi. CM[1], D. Ramugoud[2], D. Sisira[3]

[1]Associate Professor, Department of Microbiology, Mallareddy Medical College for Women, Hyderabad, Telangana, India.
[2]Assistant Professor, Department of Emergency Medicine, Mallareddy Medical College for Women, Hyderabad, Telangana, India.
[3]Assistant Professor, Department of Microbiology, Mallareddy Medical College for Women, Hyderabad, Telangana, India.

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Corresponding Author:
Dr. Swathi. CM,
Associate Professor, Department of Microbiology, Mallareddy Medical College for Women, Hyderabad, Telangana, India.
Email: swathiarunarramraj@gmail.com

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ABSTRACT

Background: Vancomycin-resistant enterococci are a rapidly evolving bacterium that can cause serious or fatal infections acquired in hospitals, known as nosocomial infections. Therefore, the aim of this study was to determine the presence of enterococci in various clinical samples and assess their resistance to vancomycin, as well as their ability to form biofilms.

Materials and Methods: This analysis contained a total of 155 distinct Enterococcus species. The bacteria were isolated and identified using the conventional bacteriological methodology. The antibiotic susceptibility testing was performed in accordance with the recommended guidelines of the clinical laboratory. The biofilm production test using microtiter plate techniques.

Results: Among the 155 isolates, Enterococcus faecalis accounted for 68.39% whereas Enterococcus faecium accounted for 31.61%. The urine samples had the highest number of isolates. Vancomycin-resistant Enterococcus was detected in 5.98% of the isolates, with 17.23% of those isolates showing biofilm production.

Conclusion: Urine samples exhibiting a high prevalence of enterococci were found to be capable of producing biofilms. The prevalence of antibiotic resistance was greater in enterococcus isolates in comparison to non-biofilm producers.

Keywords: Biofilm, vancomycin, Enterococcus faecalis, Enterococcus faecium.

INTRODUCTION

Enterococci predominantly infect geriatric patients with severe medical conditions who are admitted to the hospital for prolonged durations, specifically in intensive care units. In addition, individuals with weakened immune systems and those receiving treatments that involve the use of invasive devices or broad-spectrum antibiotics are also at risk of developing enterococcal infections.[1,2] Concurrently with the increasing resistance to routinely utilised antibiotics, enterococci have surfaced as a pathogen accountable for severe and potentially fatal illnesses. This scenario is highly significant because enterococcus infections can be transmitted to individuals of all age groups, thus impacting a larger population. Enterococcus species have exhibited significant resistance to routinely prescribed antibiotics, which sets them apart as a differentiating feature.[1-3]

Enterococci exhibit a multitude of antibiotic resistances, enabling them to endure and multiply even when exposed to antibiotic therapies. Thus, these enterococci largely appear as the main sources of superinfection.[3-5]There exist around twenty distinct varieties of enterococcus. Enterococcus faecalis and Enterococcus faecium are the main organisms that cause infections in humans.

These bacteria commonly cause bacteremia and can also lead to severe infections such as hospital-acquired urinary tract infection, surgical site infection, and endocarditis.[4,5]

Through the conducted searches on E. faecalis and E. faecium, it has been discovered that these bacteria have the potential to develop antibiotic resistance.[5].In addition, enterococcus bacteria create virulence factors that contribute to the development of illness. Enterococcus species are harmful due to the presence of bacterial toxins such as hemolysin, hyaluronidase, gelatinase, hydrolytic enzymes containing serum protease, and biofilm.[5,6]The development of a biofilm, a sophisticated arrangement consisting of polymers, is regulated by both the environmental and genetic elements of bacteria. This biofilm is recognised as the underlying factor behind a multitude of enduring infections.[6,7]

The objective of this study was to investigate the capacity of Enterococcus species to form biofilms and their susceptibility to antibiotics, including vancomycin-resistant enterococci (VRE). The study focused on isolates obtained from various clinical specimens.[8,9]

MATERIAL AND METHODS

The present inquiry was carried out in the Department of Microbiology, Mallareddy Medical College for Women, Hyderabad, Telangana, India from April 2023 to March 2024. The study encompassed all clinical samples obtained within the specified time period and received approval from the Institutional Ethics Committee. The current investigation specifically examined catalase-negative cocci of the gram-positive type, derived from various clinical samples, while excluding catalase-positive gram-positive cocci and all gram-negative bacteria.[10]

Methodology

The microtiter plate method was used to test the clinical isolates of enterococci strains for biofilm development, following standard protocols with minor adjustments. Blood agar plates containing recently subcultured cultures of Enterococcus species were inoculated with 1 milliliter of tryptic soy broth (TSB) containing 1% glucose and left to incubate throughout the entire night at 37°C. 180 µl of new TSB medium and about 20 µl of the 24-hour-old bacterial culture were put to 96-well polystyrene microtiter plates, which matched the 0.5 McFarland turbidity standard. A well containing 250 µl of TSB medium was used as a negative control. For up to 24 hours, all of the isolates were cultured at 37°C after being injected in triplicate. Following incubation, each well's medium was removed and twice washed with cold, 1x phosphate-buffered saline (pH 7.4). After adding 150 µl of methanol and waiting for 15 minutes, the biofilm was cemented onto the surface. It was then cleaned and left upside-down to air dry for another 20 minutes. After that, 0.1% crystal violet was used to stain each well for 15 minutes. Following incubation, the wells were cleaned with tap water, allowed to dry naturally, and then destained with 150 µl of 33% glacial acetic acid, which was left in place for 20 minutes. At 570 nm, the optical density (OD) value was measured. For repeatability, the procedures were carried out three times for every isolate, and the average value was determined. The OD and the cut-off OD (average of all the ODs of the negative control) were calculated using the spectrophotometry reports read at 570 nm. Based on the OD in relation to the cut-off OD, the biofilm was quantified and categorized. For the isolated isolates, the formation of biofilm was quantified and estimated. The cut-off OD is the mean of all the ODs for the negative control. The OD values were categorized as weak, moderate, and strong biofilm producers after being compared to the cut-off OD.[10,11]

RESULTS

There were a total of 155 Enterococcus species identified, with E. faecalis representing 68.39% and E. faecium representing 31.61% of the isolates. The species were acquired from several clinical specimens, and the disparity in proportions was shown to be statistically significant ($p < 0.05$). Enterococcus bacteria were most prevalent in urine samples (29.45%), followed by pus samples (26.35%), and least usually identified in bodily fluids (9.30%). Enterococci were obtained from individuals spanning various age groups and genders. The male isolates constituted 62.75% of the total, whereas the female isolates accounted for 37.41%. The age cohort between 21 and 30 years had the highest number of isolates, constituting 20.41% of the overall total. In contrast, the age range of up to 10 years accounted for only 5.16% of the total number of isolates, the lowest proportion among all age groups. Teicoplanin exhibited the maximum susceptibility against Enterococcus species, with a sensitivity rate of 94.88%. Vancomycin followed closely with a sensitivity rate of 93.38%, while linezolid had a sensitivity rate of 92.88%. In contrast, they had the lowest susceptibility to penicillin (17.19%) and norfloxacin (18.75%). Overall, E. faecium shown reduced resistance to the investigated antibiotics in comparison to E. faecalis. Out of the 155 isolates, 35 (18.29%) were found to be capable of producing biofilms.

Enterococcus strains isolated from Pus samples demonstrated the highest capacity to produce biofilms, reaching a maximum of 35.13%. The blood and Urine samples exhibited biofilm development rates of 28.00% and 27.27% respectively, as indicated by the analysis of the samples. Nevertheless, the isolates collected from bronchoalveolar lavage fluid and pleural fluid

samples did not exhibit any potential to produce biofilm. Enterococcus isolates that produce biofilm shown a significant resistance to linezolid (88.95%), with teicoplanin (85.95%) and vancomycin (81.25%) following closely behind. Nevertheless, their response to norfloxacin was only 7.12%. Generally, isolates that form biofilms showed reduced susceptibility to antibiotics compared to isolates that do not form biofilms. A comparison study comparing VRE with vancomycin-sensitive Enterococcus found that males had a greater infection rate (62.75%), while the average age of the patients was 33 years. Enterococcus faecalis shown a greater degree of resistance (59.99%) to vancomycin in comparison to E. faecium (38.47%).

Table 1: Distribution of Enterococcus on a sample-by-sample basis (N = 155)

Sample	Enterococcus faecalis		Enterococcus faecium		Total	
	N	%	N	%	N	%
BAL fluid	7	4.52	2	1.29	9	5.81
Blood	15	9.68	10	6.45	25	16.13
Catheter tip	7	4.52	2	1.29	9	5.81
ET secretion	11	7.10	1	0.65	12	7.74
Body fluid	1	0.65	0	0.00	1	0.65
Pleural fluid	0	0.00	0	0.00	0	0.00
Pus	23	14.84	14	9.03	37	23.87
Sputum	8	5.16	2	1.29	10	6.45
TT aspirates	10	6.45	1	0.65	11	7.10
Urine	16	10.32	17	10.97	33	21.29
Vaginal swab	8	5.16	0	0.00	8	5.16
Total	**106**	**68.39**	**49**	**31.61**	**155**	**100**

Table 2: Distribution of Enterococcus species that create bio film on a per-sample basis

Sample	Total	Bio film	%
BAL fluid	9	0	0.00
Blood	25	7	28.00
Catheter tip	9	1	11.11
ET secretion	12	2	16.66
Fluid	1	0	0.00
Pleural fluid	0	0	0.00
Pus	37	13	35.13
Sputum	10	1	1.00
TT aspiration	11	1	9.09
Urine	33	9	27.27
Vaginal swab	8	1	12.50
Total	**155**	**35**	**100**

Table 3: An analysis comparing VRE with VSE

Variables	VRE (N =10)	%	VSE (N =145)	%
Sex				
Male	6	60	91	62.75
Female	4	40	54	37.41
Age distribution				
<10 years	2	20	6	5.16
11–50years	7	70	92	63.44
>50 years	1	10	47	32.41
OPD/IPD				
OPD	5	50	47	32.41
IPD	5	50	98	67.58
Species				
Enterococcus faecalis	4	40	89	61.37
Enterococcus faecium	6	60	56	38.62

DISCUSSION

Throughout our examination, Enterococcus faecalis was the predominant type of enterococci that we frequently found. The findings of Fernandes and Dhanashree, Jain et al., and Karmarkar et al,[10-12] corroborate these conclusions. Telkar et al. discovered that E. faecium was the species that appeared most frequently, which contradicts our own findings. Multiple comprehensive worldwide studies have repeatedly shown that Enterococcus faecalis is the predominant species of Enterococcus, and it is accountable for a diverse array of disorders. The present examination revealed that urine and pus samples exhibited the highest number of isolates, which is consistent with the findings of Kaur et al.'s study.[12]Consistent with our findings, Kaur et al. also noted that the age group between 21 and 30 years had the highest incidence of solitary

Enterococci. However, Bhatt et al. discovered that a significant number of Enterocooci species were responsible for infections in individuals who were 60 years of age or older. According to the current inquiry, Enterococcus accounted for 28% of the species detected in the urine samples.

Several research conducted in different nations consistently found that urine samples had the, highest occurrence of enterococci. Mathur et al,[13] Karmarkar et al,[12-13]and Udo et al observed success rates of 49%, 50%, and 37%, correspondingly. During the ongoing examination, it was shown that 6.7% of the isolates exhibited resistance to teicoplanin, whereas 4.87% showed resistance to vancomycin. The resistance of E. faecalis and E. faecium to the drugs vancomycin and teicoplanin showed no variation. Furthermore, several studies have revealed that whereas E. faecium constitutes a lesser percentage of clinical enterococcal isolates in comparison to E. faecalis, it exhibits significantly higher resistance to glycopeptides. The research undertaken by Deshpande et al,[13,14] discovered that less than 2% of E. faecalis strains exhibited resistance to vancomycin, a percentage slightly lower than our own findings.

Nevertheless, a study carried out in southern India revealed that 52% of isolates exhibited resistance to vancomycin, suggesting a notably elevated occurrence and intensity of glycopeptide resistance in that particular investigation. According to this study, 18.29% of individuals undergo the process of biofilm formation.

The Pus specimens exhibited the highest percentage of biofilm producers, comprising 35.13% of the total. Unlike our experiment, Khattak et al,[14,15] found that 33% of all enterococci isolates had the ability to produce biofilm. Of all the samples, the ones containing pus exhibited the greatest proportion of biofilm producers. The findings were consistent with our own studies. An independent study conducted in India found that 68% of the enterococci samples analysed exhibited the capacity to develop biofilms. Furthermore, the study revealed that 3.57% of the samples that produced biofilm exhibited resistance to teicoplanin, whereas 20.31% of the samples demonstrated resistance to vancomycin.[14-16]Our study's results contradicted the research conducted by Shridhar and Dhanashree,[17,18]which showed no presence of vancomycin resistance. The current analysis is constrained by several limitations as it did not specifically examine genes linked to the formation of biofilms and resistance to vancomycin.

CONCLUSION

Enterococci are a common pathogen that can cause many clinical problems. Enterococci pose a greater difficulty in treatment compared to other bacterial infections since they naturally resist routinely prescribed drugs, such as cephalosporins and aminoglycosides. Furthermore, the capacity to form biofilm and the inclination to acquire resistance to treatment exacerbate the management of enterococcal disease. Therefore, identifying the existence and resistance patterns of Enterococcus bacteria in a particular hospital or region would help choose more potent medicines to improve disease management and avoid the development of drug resistance in enterococci. The current analysis found that the prevalence rate of VRE is 6.7%, and biofilm producers have higher antibiotic resistance compared to non-biofilm producers. Tecoplanin, linezolid, and chloramphenicol have demonstrated efficacy against both biofilm-producing and non-producing enterococci. Thus, they can serve as practical treatments for enterococcal infections.

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