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RESEARCH ARTICLE

Detection of Biofilm Production by Congo-red in Vancomycin Resistant Enterococci from various clinical samples

Muskan Bhardwaj[1], Siva Prasad Reddy[2], Akhileshwar Reddy Vangala[3]
[1]PhD Scholar, Department of Microbiology, National Institute of Medical Sciences and Research Jaipur, Rajasthan-303121
[2]Professor, Department of Microbiology, National Institute of Medical Sciences and Research, Jaipur, Rajasthan-303121
[3]PG Resident, Department of Community Medicine, National Institute of Medical Sciences and Research, Jaipur, Rajasthan-303121

*Corresponding Author
Dr Siva Prasad Reddy
(Bharadwajmuskan96@gmail.com)

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Abstract: Gram-positive bacteria known as enterococci are responsible for endocarditis, bloodstream infections, and urinary tract infections, among other severe nosocomial infections. The ability of enterococci to create biofilms—populations of cells that are permanently affixed to a variety of biotic and abiotic surfaces and covered in a hydrated matrix of proteins, polysaccharides, nucleic acids, and exopolymeric substances—is well-known. Bacterial pathogenicity is enhanced by biofilms in multiple ways. For instance, the bacteria can attach to silicone gastrostomy devices, biliary stents, and catheters (such as intravascular and urinary catheters) by adhesion, an early stage in biofilm formation. Furthermore, biofilms help bacteria become resistant to phagocytosis and antibiotics, which makes it very challenging to eradicate them. The bacterial cells in an established biofilm can withstand antibiotic concentrations 10–1000 times greater than those needed to destroy planktonic cells. ***Methods:*** The present study was an observational, cross-sectional study conducted at a tertiary care center from North India from June 2023 to May 2024 in which Enterococci isolates from different clinical specimen such as blood, pus, urine and other body fluids were included in the study. Biofilm formation was assessed using congo red agar. ***Results:*** Out of 259 enterococci isolates, 169 (65.25%) were identified as Enterococcus faecalis while the rest 90 (34.75%) were Enterococcus faecium. Among these, 10 were resistant to vancomycin. Biofilm production in E. faecalis (22.4%) is more as compared to E. Faecium (12.36%). ***Discussion:*** The present study reports Higher prevalence of Biofilm Production in E. faecalis (22.4%) is more as compared to E. Faecium (12.36%). In a Study on Biofilm Formation Among Enterococcus Isolates and Association With Their Antibiotic Resistance Patterns by Nair Pooja et.al a similar hospital based research in which Biofilm production was found more in Enterococcus faecalis as compare to Enterococcus faecium. ***Conclusion:*** E. faecalis (22.4%) biofilm production is more as compared to E. Faecium (12.36%). There is increased literature evidence showing that multi drug resistance is prevalent among the Enterococci around the world.

Keywords: Enterococcus; antibiotic susceptibility pattern; antimicrobial resistance; vancomycin resistant Enterococci

INTRODUCTION

Gram-positive bacteria known as enterococci are responsible for endocarditis, bloodstream infections, and urinary tract infections, among other severe nosocomial infections.[1]The ability of enterococci to create biofilms—populations of cells that are permanently affixed to a variety of biotic and abiotic surfaces and covered in a hydrated matrix of proteins, polysaccharides, nucleic acids, and exopolymeric substances—is well-known.[2] Bacterial pathogenicity is enhanced by biofilms in multiple ways. For instance, the bacteria can attach to silicone gastrostomy devices, biliary stents, and catheters (such as intravascular and urinary catheters) by adhesion, an early stage in biofilm formation.[3] Furthermore, biofilms help bacteria become resistant to phagocytosis and antibiotics, which makes it very challenging to eradicate them. The bacterial cells in an established biofilm can withstand antibiotic concentrations 10–1000 times greater than those needed to destroy planktonic cells.[4] The VRE rate in India ranges between 5–10%, with North India having a rate of 7.9%. Through cell adhesion to the surface matrix, a biofilm—a collection of microorganisms—causes health issues for people who have indwelling medical devices.[5] It makes microorganisms more resistant to antimicrobial agents and causes infections in humans. By entering the host tissue, adhering, and consuming nutrition, these factors give the virus an advantage. When drug-resistant bacteria are present, the infection gets worse.[6] Recognizing the complex pathophysiology of the organism and selecting the optimal treatment option from the limited options may be aided by knowledge of prevalence and biofilm formation.[7] The current study serves as a foundation for interventional research aimed at lowering the burden of enterococcal infections as well as a starting point for vancomycin resistance and enterococci phenotypic expression.[8]

MATERIAL AND METHOD

The present study was an observational, cross-sectional study conducted at a tertiary care center from North India from June 2023 to May 2024 in which Enterococci isolates from different clinical specimen

such as blood, pus, urine and other body fluids were included in the study. Gram's staining was used to link the culture isolates with all specimens except urine, which underwent wet mount inspection to identify the kind and amount of cells, including pus cells (≥104 CFU/ml corresponded with pyuria). Gram-positive cocci that gathered in pairs on Gram's staining were identified as enterococci.

In compliance with CLSI guidelines M100, the Antimicrobial Susceptibility test (AST) was performed using Muller Hinton agar (HiMedia Laboratories, India) and the Kirby Bauer disk diffusion method. The antimicrobial discs used for disc diffusion testing were: Ampicillin (10µg), High level gentamicin (120µg), Erythromycin (15µg), Vancomycin (30µg), Teicoplanin (30µg), and Linezolid (15µg). For urine isolates, antibacterial discs containing Ampicillin (30µg), High level gentamicin (120µg), Levofloxacin (5µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Vancomycin (30µg), Teicoplanin (30µg), and Linezolid (30µg) were used. The plates were stored at 37°C for the full day before being read under transmitted light.

Vancomycin resistance was determined when the isolate's zone size around the antibiotic was less than 14 mm. Vancomycin screen agar, which was created by combining brain heart BHI agar with 6 µg/ml vancomycin, was also used to check for vancomycin resistance. It was believed that the growth of one or more Enterococcus spp. colonies indicated vancomycin resistance. During culture and AST, suitable controls were used using strains of E. faecalis ATCC 29212 and E. faecium ATCC 51559 that were available in the laboratory.

Biofilm formation was detected using Congo Red agar (HiMedia Laboratories, India) which is based on the principle of the ability of the Congo red dye to stain the polysaccharides black. The media was prepared by adding 50g/L sucrose and 0.8g/L Congo red stain to the Brain Heart Infusion agar. Enterococcal strains were inoculated on the Congo Red Agar plates and incubated at 37°C for 24 hours. Black, dark or dark pink colonies indicated strong, moderate and weak biofilm production respectively.

RESULT:

A Total of 259 Enterococci isolates were extracted from clinical specimens. With 59.46% of the patients being male and 40.5% being female, the patients' mean age was 44.3 years (range: 2–83 years). 4.6% of the patients were OPD patients, while 95.4% were the hospitalized patients.

Out of these 259 isolates, 169(65.25%) were identified as *Enterococcus faecalis* while the rest 90(34.75%) were *Enterococcus faecium.*

Table1: Detection of BIOFILM in Enterococcus species

Organism	Biofilm	
	Number (n)	Percentage (%)
E. Faecalis	58	22.4
E. Faecium	32	12.36
Total	90	34.75

In above table which shows detection of BIOFILM in Enterococcus species, we found that in E. faecalis (22.4%) biofilm production is more as compared to E. Faecium (12.36%).

In above table which shows detection of BIOFILM in clinical samples, we found that maximum percentage of biofilm production is seen in urine sample (19.3%), but only 0.4% in ET & body fluids.

Table 2: Detection of BIOFILM in clinical samples

Sample	Biofilm	
	Number (n)	Percentage (%)
Urine	50	19.3
Blood	20	7.72
CSF	1	0.4
Pus	17	6.6
Body fluid	1	0.4
ET	1	0.4
Total	90	34.75

Table3: Antimicrobial susceptibility and detection of BIOFILM in Enterococcal species.

AST		Biofilm	
		Number (n)	Percentage (%)
P	R	56	21.62
	S	34	13.13
AMP	R	32	12.36
	S	58	22.39
VA	R	05	1.93
	S	85	32.82
LZ	R	02	0.8
	S	88	33.98
TEI	R	02	0.8
	S	88	33.98
TE	R	09	3.5
	S	81	31.27
HLG	R	22	8.5
	S	68	26.25
CIP	R	11	4.25
	S	79	30.5
LE	R	09	3.47
	S	81	31.3
FO	R	13	5.02
	S	37	14.3
NX	R	20	7.72
	S	30	11.6
NIT	R	10	3.86
	S	40	15.44
E	R	27	10.42
	S	13	5.02
DO	R	30	11.58
	S	59	22.78
MI	R	13	5.02
	S	27	10.42
C	R	12	4.63
	S	28	10.81
TGC	R	03	1.16
	S	17	6.6

Above table shows antimicrobial susceptibility and detection of biofilm, we observed that biofilm production is maximum in Enterococcal species resistance (21.62%) to penicillin as compared to sensitive (13.13%) to penicillin. Maximum number of biofilm production we observed is in 34.36% sensitive to linezolid then in teicoplanin 33.98%, while in Vancomycin we observed 1.93% resistance and 328% sensitivity but with other antibiotics mainly it is present in sensitive as compared to resistance towards antibiotic.

DISCUSSION

The present study reports Higher prevalence of Biofilm Production in E. faecalis (22.4%) is more as compared to E. Faecium (12.36%). In a Study on Biofilm Formation Among Enterococcus Isolates and Association With Their Antibiotic Resistance Patterns by Nair Pooja et.al a similar hospital based research in which Biofilm production was found more in Enterococcus faecalis as compare to Enterococcus faecium[9] Similar findings were reported by V Silva et al [10].

In terms of biofilm detection and antimicrobial sensitivity, we found that enterococcal species that are resistant to penicillin (21.62%) produce the most biofilm, but those that are sensitive to the antibiotic (13.13%) do not. The highest percentage of biofilm formation that we saw was 34.36% sensitive to linezolid and 33.98% sensitive to teicoplanin, in vancomycin 1.93% resistance and 32.8% sensitivity were found, nevertheless, with other antibiotics. Similar findings were reported by Kumar D et al.[11]

CONCLUSION

E. faecalis (22.4%) biofilm production is more as compared to E. Faecium (12.36%). There is increased literature evidence showing that multi drug resistance is prevalent among the Enterococci around the world. In order to successfully treat patients, particularly those who are hospitalized, it is suggested that there should be ongoing or sporadic surveillance of the dynamics of enterococci-caused illnesses at least in hospitals at all levels, it is mostly seen in sensitive as opposed to resistant groups. Prevention and management of the spread of drug-resistant The hospital's departments must work together to prevent enterococcal infections, which can only be accomplished by training hospital employees, using antibiotics with vigilance, having laboratories detect and report infections early, and putting in place the right infection control measures right away.

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