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Original Research Article

Prevalence of Vancomycin Resistant *Enterococcus* in Various Clinical Specimens in Tertiary Care Hospital

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ABSTRACT

Introduction - *Enterococci* form a part of the normal flora of the intestinal tract, oral cavity and the vagina. (1) Vancomycin resistant *Enterococci* (VRE) have caused hospital acquired infections, outbreaks worldwide which have been dramatically amplified in recent years. Most common infections caused by *Enterococci* are endocarditis, bacteraemia, intra abdominal and urinary tract infections etc.
Aim & Objective - To determine the prevalence and the Antibiotic Susceptibility pattern of *Enterococci* isolated from various clinical specimens & To determine the MIC of Vancomycin resistant *Enterococci.*
Material and Method - The present study was conducted in the Department of Microbiology, Government Medical College, Azamgarh, UP, India. The samples which included were urine, pus, blood wound swab, throat swab and other body fluids. Presumptive identification of *Enterococcus* was done on the basis of colony characteristics, Gram's staining & Catalase test. Confirmation was done by growth in 6.5% NaCl, & Bile esculin hydrolysis. Antibiotic Susceptibility Testing done by Kirby Bauer Disc Diffusion Method. MIC was done by the E test for all the *Enterococci* isolates which showed resistance Vancomycin by the Kirby Bauer disc diffusion method.
Result - A total of 53 isolates of *Enterococcus* were obtained from various clinical samples. Out of 53, 32 (60.3%) were *Enterococcus faecalis* and 21 (39.6%) were *Enterococcus faecium*. Among 53 isolated *Enterococcus*, 16 isolates (30%) were Vancomycin resistant. Conclusion - This study demonstrates the increased prevalence of multidrug resistant *Enterococci*, thus posing a serious therapeutic challenge.

Key Words – E-Test, Vancomycin resistant Enterococcus, MIC, Bile esculin

INTRODUCTION

Enterococci form a part of the normal flora of the intestinal tract, oral cavity and the vagina. (1) Vancomycin resistant *Enterococci* (VRE) have caused hospital acquired infections, outbreaks worldwide which has been dramatically amplified in recent years, because of widespread abuse and misuse of antibiotics, leading to increase in infections caused by these strains. (2) Among 20 species of *Enterococcus*, the two most common human pathogens of this bacterium are *Enterococcus faecalis* (85-90%) and *Enterococcus faecium* (5-10%). (3) Most common infections caused by *Enterococci* are endocarditis, bacteraemia, intra abdominal and urinary tract infections etc. Nowadays, it is a challenge to treat infections caused by *Enterococcus* because

of their increasing resistance to different antimicrobials which include β lactam antibiotics, aminoglycosides and most importantly glycopeptides like vancomycin. [(4)] So infections caused by Vancomycin Resistant *Enterococcus* is a threat to people & challenge to treat. Treatment of VRE is also very important due to high risk of mortality.

AIM AND OBJECTIVES

- To determine the prevalence and the Antibiotic Susceptibility pattern of *Enterococci* isolated from various clinical specimens.
- To determine the MIC of Vancomycin resistant *Enterococci*

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, Government Medical College, Azamgarh, UP, India. The samples which included were urine, pus, blood wound swab, throat swab and other body fluids which were collected from patients of all age group admitted in the various Departments. All the Specimens received in the bacteriology laboratory were inoculated on Blood agar and MacConkey agar plates except Urine, which was inoculated on CLED agar & incubated at 37^{o} C for 24-48 hours. Presumptive identification of *Enterococcus* was done on the basis of colony characteristics, Gram's staining & Catalase test. Confirmation was done by growth in 6.5% NaCl, & Bile esculin hydrolysis. Antibiotic Susceptibility Testing done by Kirby Bauer Disc Diffusion Method. Muller-Hinton agar (MHA) plates were overlaid with the inoculums turbidity equivalent to that of a 0.5 McFarland Standard using the following commercially available Antimicrobial discs Ampicillin (10 µg), Penicillin (10µg), Vancomycin (30 µg), Gentamicin (120µg), Teicoplanin (30µg), Linezolid (30µg), Piperacillin (100µg) & Ciprofloxacin from (Hi media) were tested on Mueller Hinton agar along with a control strain of ATCC *E. faecalis* 29212, as per the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2017. [(5)] Vancomycin MIC was done by the E test for all the *Enterococci* isolates which showed resistance Vancomycin by the Kirby Bauer disc diffusion method.

RESULT

A total of 53 isolates of *Enterococcus* were obtained from various clinical samples. Out of 53, 32 (60.3%) were *Enterococcus faecalis* and 21 (39.6%) were *Enterococcus faecium*. Among 53 isolated *Enterococcus*, 16 isolates (30%) were Vancomycin resistant. Among 16 resistant isolates, 8 were from urine samples, 5 from pus samples, 2 from throat swabs followed by 01 from Blood. In antibiotic susceptibility testing *Enterococcus* showed the maximum resistance towards Ciprofloxacin while Linezolid & Teicoplanin showed the maximum sensitivity.

Fig. 1: Bile Esculin Hydrolysis (Positive)

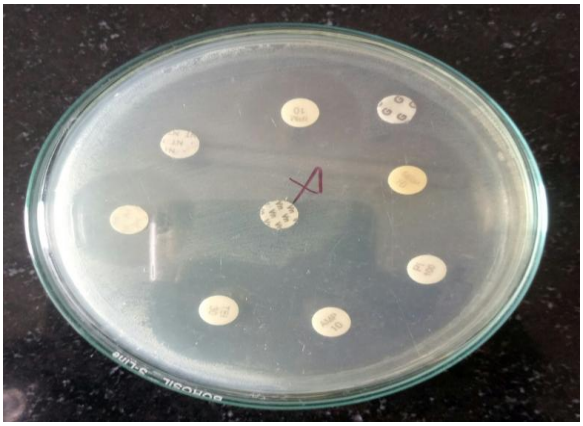

Fig. 2: Antibiotic Susceptibility Test (Kirby Bauer disk diffusion method) showing Vancomycin Resistance.

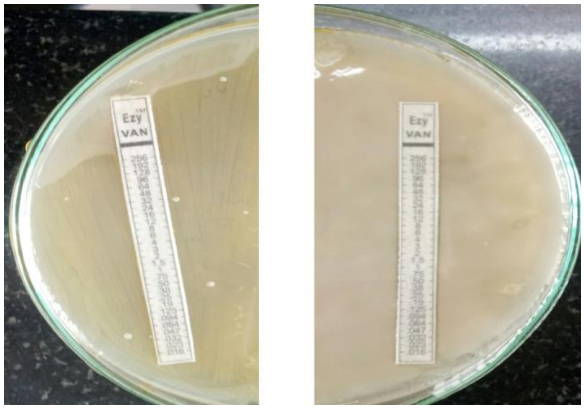

Fig. 3: E Test (MIC Test for VRE) Enterococcus is showing the >256 mcg/ml

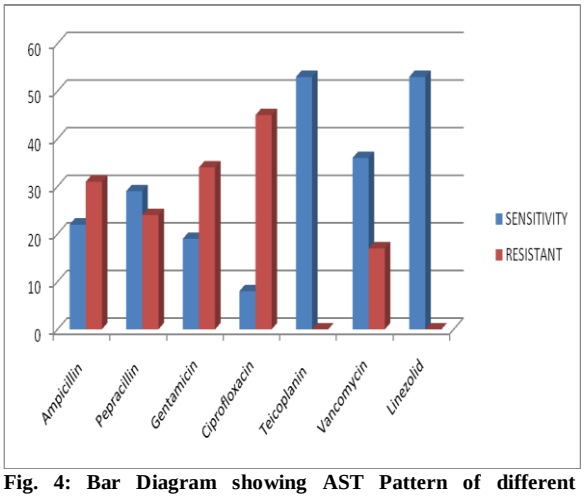

Fig. 4: Bar Diagram showing AST Pattern of different antimicrobials

Table 1: Distribution of *Enterococcus* species isolated.

S.No	Isolate	Number of isolates	Percentage (%)
1.	E. *feacalis*	32	60.3
2.	E. *faecium*	21	39.6
	Total	53	100

Table 2: Specimen-wise distribution of isolated *Enterococcus* & Vancomycin resistant *Enterococcus* & their percentage

S.No	Sample	Number of *Enterococcus* isolated (Total 53)	Percentage (%)	Vancomycin resistant *Enterococcus* (Total 16)	Percentage (%)
1.	Urine	35	66.0	08	50
2.	Pus	09	16.9	05	31.2
3.	Other body fluids	04	7.5	0	0
4.	Throat swab	04	7.5	02	12.5
5.	Blood	01	1.8	01	6.2

Table 3: Showing resistance towards different Antimicrobials

S. No	Antibiotics	E. *faecalis* (n = 32) (Resistance)	E. *faecium* (n= 21) (Resistance)	Total (n= 53)
1.	Ampicillin	19 (59.3%)	12 (57.1%)	31 (58.2%)
2.	Pepracillin	15 (46.8%)	09 (42.8%)	24 (45.2%)
3.	Gentamicin	23 (71.8%)	11 (52.3%)	34 (64.1%)
4.	Ciprofloxacin	27 (84.3%)	18 (85.7%)	45 (84.9%)
5.	Teicoplanin	0	0	0
6.	Vancomycin	04 (12.5%)	12 (57.1%)	16 (30.1%)
7.	Linezolid	0	0	0

DISCUSSION

VRE is the most important cause of hospital acquired infections in patients who have weakened immune system. Widespread abuse and misuse of antibiotics, leading to increase infections caused by these strains. *Enterococci* have become increasingly important because of their ability to cause serious infections like endocarditic, bacteremia, intra-abdominal and urinary tract infections and due to their increasing resistance to different antimicrobials which include β lactam antibiotics, aminoglycosides and most importantly glycopeptides like vancomycin. Serious *Enterococcal* infections are often refractory to treatment with a high mortality rate. [(6-7)] In the present study, the prevalence of vancomycin resistant *Enterococci* is 30.1%, which is similar to the study of Jafari-Sales A et al. [(8)] as they found vancomycin resistant *Enterococci* 33.7%. Other studies also reported prevalence of VRE as 5.6%, 12% and 23%. [(6-7)] In our study, the maximum number of isolates were obtained from urine (66%), this is showing similarity with the study of Nautiyal S et al. [(9)] followed by pus (16.9%), other body fluids (7.5%), throat swab (7.5 %) & blood (1.8%) which is similar to the study of Maj Puneet Bhatt et al. [(10)] In our study *E. faecalis* (60.3%) formed the major isolate, followed by *E. faecium* (39.6%), similarly S. Sreeja et al. [(11)] reported *E. faecalis* (76%) and *E. faecium as* (24%). In the present study, 58.2% isolates were resistant to Ampicillin, which is similar to the study of Mathur et al. [(12)] who reported 66% isolates are resistant to Ampicillin. 64.1% isolates were resistant to Gentamicin and Piperacillin which showed a drastic increase in resistance of the commonly used drugs, this type of resistance pattern with

Gentamicin (77.7%) was also reported by Nautiyal S et al. & J. Parameswarappa et al. [(13)] In present study Linezolid and Teicoplanin showed the maximum susceptibility which is similar with the study of Chitnis S et al [(14)] as they found 100 per cent susceptibility of VRE to Linezolid. In present study 4 out of 32 isolates (12.5%) of *E. faecalis* & 12 out of 21 isolates (57.1%) of *E. faecium* were found to be resistant to vancomycin. In a study conducted by A Tripathi et al. [(15)] (61.02%) of *E. faecalis* and (38.98%) of the *E. faecium* isolates were resistant to vancomycin. Among sixteen(16) VRE, 05 isolates showed high level resistant to vancomycin (MIC > 256 mcg/ml) while some other isolates showed different MIC values as 1.5 mcg/ml, 3 mcg/ml, 4 mcg/ml, 8 mcg/ml and 16 mcg/ml etc which could be compare to the study conducted by Maj Puneet Bhatt et al. [(9)]

CONCLUSION

Prevention of *Enterococcal* infections in general and VRE in particular, needs active surveillance cultures and aggressive implementation of infection control measures. This study demonstrates the increased prevalence of multidrug resistant *Enterococci*, thus posing a serious therapeutic challenge. This situation warrants the implementation of an efficient infection control program and regular surveillance of antimicrobial resistance of *enterococci* in order to establish a rational antibiotic policy for the better management of *Enterococcal* infections.

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