



Original Research Article

Comparison of different phenotypic methods for detection of Vancomycin Resistant Enterococci (VRE) among clinical isolates

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ARTICLE INFO

Article history:

Received 05-06-2020

Accepted 15-06-2020

Available online 06-07-2020

Keywords:

VRE

Enterococci

Phenotypic methods

Vitek2

Estrip

Resistance

ABSTRACT

Aim: To compare the different phenotypic methods for detection of bacteriological profile on Vancomycin Resistant Enterococci (VRE) among clinical isolates

Design: Prospective study

Material and Methods: 480 Clinical samples sent for bacteriological examination and culture sensitivity were taken up in this study. Preliminary findings and identification of Enterococci species was carried out using Grams staining, Catalase test, Bile esculin test and growth in NaCl, followed by Antibiotic sensitivity testing in Muller Hilton Agar. The resistant strains were subjected to agar dilution method, Vancomycin e-strip method and Vitek-2 automated system for phenotypic detection of Vancomycin resistance. The results were observed and analysed.

Results: Among 480 clinical specimens analysed, 120 Enterococci species (25.0%) were isolated. Out of 120 isolates, 40 (33.33%) were resistant to Vancomycin and 80 (66.7%) were sensitive to Vancomycin by disc diffusion method. On further analysis, Vancomycin E-Strip and Vitek-2 showed almost similar results making them more reliable compared to agar dilution method.

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1. Introduction

Enterococcus spp. is classified under the Group A streptococcus, Gram-positive, non-sporing, anaerobic cocci. It is mostly arranged in pairs or in short chains. Some of the strains are highly tolerance to 6.5% NaCl and also heat resistant at 60°C.¹ This bacterium was categorized under opportunistic pathogens which can cause severe nosocomial infections. Hence, they are difficult to treat because of multiple drug resistance to the various classes of antibiotics.² There are more than 40 species of the genus Enterococcus have been identified but some may have clinical importance to cause major infections in humans. *Enterococcus faecalis* and *Enterococcus faecium* plays a 90% of the infections in humans.³⁻⁹ Vancomycin resistance was first identified in England 1986 by Uttley et al., and it was increased across the worldwide.³ Recently,

many studies have reported that Vancomycin Resistant Enterococci (VRE) can be transmitted from one person to another by their patient requirements or environmental sources.^{3,5,7,9} There are nearly nine types of glycopeptide resistance genes viz., vanA, vanB, vanC1, canC2, vanC3, vanD, vanE, vanG, vanL, vanM and vanN genes¹⁰. VanA and VanB are most common type of resistance was detected in Enterococci and other streptococci.¹¹⁻¹⁵ VanA is highly resistance to Vancomycin and Teicoplanin was detected by MIC. Different phenotypic methods are employed for detection of VRE by disc diffusion methods, Minimum Inhibitory concentration (MIC) like agar dilution methods,⁹ E-test,¹¹ an automated VITEK – 2 system.^{9,11,14,16} Our main objective is to compare the different phenotypic methods to detect Vancomycin Resistant Enterococci (VRE) strains among clinical isolates. Preventing misleading therapeutics to patients and also intended to create awareness among clinicians about

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increasing of Vancomycin Resistant Enterococci.

2. Materials and Methods

This prospective and laboratory-based study was conducted in the Department of

Microbiology, Mahatma Gandhi Medical College & Research Institute, Puducherry. This study was carried out during the period of June 2018 to May 2019. Different clinical samples like exudates, blood and urine samples were included for this study from the laboratory which came for routine bacteriological culture & sensitivity testing. These samples were inoculated into Blood agar and MacConkey agar for exudates and CLED agar is used for Urine samples. Antibiotics which was routinely tested for all Enterococcal isolates against Ampicillin (10 µg), Erythromycin (15 µg), Clindamycin (2 µg), Ciprofloxacin (5 µg), Linezolid (30 µg), Teicoplanin (30 µg), Nitrofurantoin (300 µg) and Vancomycin (30 µg) by the Kirby Bauer disk diffusion method using commercially available disks (HiMedia, Mumbai, India) were impregnated on Mueller-Hinton agar (MHA) as per CLSI guidelines. The results of antibiotics sensitivity testing were categorized based on the Enterococcus species (either *Enterococcus faecium* or *Enterococcus faecalis*) which are resistant to Vancomycin zone size ≤ 12 mm. These isolates were screened for Vancomycin Resistant Enterococci (VRE) using Minimum Inhibitory Concentration (MIC) were compared with different methods viz., Agar dilution method,² Commercially available Vancomycin E Strip (HiMedia Laboratories, India)^{9,11} and VITEK-2 automation system (BioMeriux, USA).^{11,13,14,16} For agar dilution method, Muller Hinton broth were incorporated with Vancomycin was prepared in four different concentrations viz., 4µg, 8µg, 16µg & 32µg per ml.^{2,9} Vancomycin E Strip and VITEK-2 automation system were performed and the interpretation of the results reported based on the manufacturers' instructions.

3. Results

The results were interpreted by the absence of growth in 4µg indicates that the strain is sensitive, followed by the growth was observed up to 16µg concentrations were reported as Intermediate sensitive and the strain is said to be resistant, when the growth was observed in ≥ 32 µg and it was identified as VRE. Simultaneously, Vancomycin E strip and VITEK-2 were simultaneously tested for these sensitive as well as resistant isolates. Quality control of the antibiotic disks was carried out using *Enterococcus faecalis* ATCC 29212.

Among 480 clinical specimens, 120 Enterococci species (25.0%) were isolated. Out of 120 isolates, 40 (33.33%) were resistant and 80 (66.7%) were sensitive to Vancomycin by disc diffusion method. In Agar dilution method, of the

eight resistant strains 4 (10.0%) were sensitive with a MIC value of ≤ 4 µg/ml and the other 4 (10.0%) showed a MIC value of 16µg/ml and remaining 32 (80.0%) strains had a MIC of ≥ 32 µg/ml concentration.

By using Vancomycin E strip 4 strains showed growth up to a dilution of 16µg/ml (10.0%) and 24 isolates showed growth with titres at 128µg/ml and 12 isolates at 256 µg/ml (90.0%).

In VITEK-2, 20 isolates had a MIC value of 128 µg/ml and 12 isolates had a value of 256 µg/ml and 8 resistant strains had a MIC value of ≤ 16 µg/ml.

Remaining 80 isolates were sensitive by disc diffusion method which also confirmed by MIC using these three methods. However, 32 strains were identified as VRE by agar dilution methods and one was sensitive and another was intermediate to Vancomycin. 36 isolates had shown resistance to Vancomycin by E test and VITEK-2 shows that all the 40 isolates had grown in the plate containing ≥ 256 µg/ml.

4. Discussion

Drug resistance is one of the major impacts on global issue. Various studies from India and overseas have reported drug resistance from gram negative as well as gram positive organisms.⁹ Recently, from India reported as first study by citing the resistance pattern of Enterococcus and Staphylococcus against Linezolid.⁵ In our study, we found that Linezolid was found sensitive for all enterococcal isolates including resistant strains. *Enterococcus faecalis* was predominant among the other enterococcal isolates.^{2,5}

Nearly, 86.7% VRE isolates was observed in *E. faecalis* than *E. faecium*.⁹ These two bacterial isolates showed highly resistance to Erythromycin, Clindamycin and Ampicillin antibiotics. Most of the studies have isolated the Enterococci from pus samples, but in this present study most them were from urine. High-level Gentamicin (HLG) antibiotics resistance were ranged from 12.6% to 100% among *E. faecalis*.⁷ In contrast, our study shows only 4 strains with HLG resistance among 120 isolates. In the present study VRE was detected by disk diffusion method though it sometimes makes trained personnel for interpreting zone size and cautious observation of various concentrations of Vancomycin in the plates.¹¹ All Enterococci species were screened for Vancomycin resistant strains by disc diffusion method and confirmed by three different methods for identification of VRE among clinical specimens. The E-test is a preferred method for confirming the resistance.^{11–14} 36 isolates had shown resistance to Vancomycin by E test and 95.0% sensitivity when compared to the agar dilution method. This may be due to the error in the preparation of Muller Hinton broth and in the dilutions. An automated system VITEK-2 shows 100.0% sensitivity and specificity when compared to the other methods. Surveillance for Vancomycin resistant

strains may be considered in endemic hospitals.⁸ Existence of resistance level was observed between community strains and hospital strains. Vancomycin sensitivity was reported after performing MIC detection in case of Staphylococcus. Our study findings shows that MIC detection by E strip and VITEK-2 is highly sensitive compared to the Agar dilution method. For multidrug resistance cases, MIC is an apt for antibiotic sensitivity testing as per CLSI protocol and should be reported accordingly. Fermentation of Furanose in *Enterococcus faecalis* highly helpful in rapid identification between VRE and Vancomycin-Susceptible *E. faecalis* (VSE) isolates.⁴ Phenotypic evaluation of resistance bacteria is considered to be an indirect evaluation of bacterial genetics.⁶ Several studies have been reported the performance of commercial and gold standard reference methods for the identification of VRE among clinical isolates.^{11–16} Increased antibiotic resistance occurs by exchanging of resistant genes between clinical and environmental bacteria. Molecular detection by targeting various resistant genes of VRE among clinical isolates is our limitation.

In this study we conclude that a good correlation was observed between the three different methods for detection of VRE strains by MIC test. Performance of MIC by agar dilution method observed less sensitivity when compared with the other two methods. Commercially available Vancomycin E strip and VITEK 2 is simple, fast and reliable method for confirmation of VRE strains which was reported by disc diffusion method. It is highly made awareness as well as helpful among physicians for treating VRE patients in critical situation.

5. Source of Funding

Authors are very grateful to the Honourable Chairman, Chancellor, Vice-Chancellor, Dean Research, Sri Balaji Vidyapeeth (Deemed-to-be-University), Dean (Academics) for providing facilities for this research project.

6. Conflict of Interest

None.

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Cite this article: Valentina Y , Umadevi S , Pramodhini S .
Comparison of different phenotypic methods for detection of Vancomycin Resistant Enterococci (VRE) among clinical isolates.
IP Int J Med Microbiol Trop Dis 2020;6(2):123-125.