"Molecular Characterization of Vancomycin resistant enterococci isolated from urine samples at a tertiary care centre in Kanpur"

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Abstract:

Introduction: The emergence of Vancomycin resistant enterococci (VRE) poses a major public health problem since it was first reported. Enterococci are important human pathogens that cause many infections including nosocomial infections. Around, 80 - 90% of infections are commonly caused by E. faecalis followed by E. faecium with a contribution of about 10 - 15% along with emergence of multi-drug resistance (MDR) resistance towards many antibiotics including to high level aminoglycosides.

Aim and objective: To study the Molecular Characterization of Vancomycin resistant enterococci isolated from urine samples at a tertiary care centre in Kanpur.

Material and Methods: It was a cross sectional study which was carried out in the Department of Microbiology and Central Research Lab of RMCH &RC Mandhana, Kanpur for a period of 1 year i.e ,January 2021 to December 2022. A total of 135 Enterococci isolates were collected from clinical samples of urine was obtained which were then processed to the Clinical Microbiology Laboratory of Rama Medical College Hospital and Research Centre, Kanpur. The Isolated Enterococcal spp were further processed for the Microscopic examination, Biochemical's test and species differentiation according to the CLSI guidelines. The Identification of VRE was done by the E-test strip method and the MIC determination of vancomycin using agar dilution method. Molecular characterization of all VRE isolates was performed for the detection of resistance gene by using the DNA extraction Qiagen Kit.

Results: Out of the 135 Enteroccoccal isolates, 15 isolates were E. faecium and 120 were E. faecalis. Among 10 VRE, 6 were E. faecium and 4 was E. faecalis Out of the total 135 Enterococcal spp 10 was found to be VRE by agar dilution method. The prevalence of VRE in our study was 7.4%.AST pattern showed that, resistance to HLG was 60% and 46% to HLS. Similarly, resistance pattern shown by Enterococcal spp to various antibiotics was as follows; penicillin (86%), tetracycline (14%), ciprofloxacin (100%), vancomycin (14%), teicoplanin (14%), linezolid (12%), nitrofurantoin (32%), norfloxacin (54%), & erythromycin (50%).. Molecular testing for the detection of genes responsible for vancomycin resistance showed that 6 VRE isolates contained VanA & VanB genes out of which 1 isolate contained both vanA & VanB genes and 5 other isolates contained only vanA genes. However, remaining 4 VRE isolates contained genes other than VanA or VanB.

Conclusion: The prevalence of VRE and drug resistant Enterococci are on rise in Uttar pradesh. Enterococcal isolates showed resistance to one or more of the commonly prescribed drugs in different or the same drug lines. There is an increase in cases of Multidrug resistant (MDR) enterococci observed. The increasing emergence of resistance to Daptomycin and Linezolid is an alarm for searching new ways for the treatment and control of VRE infections in hospital to be done urgently on priority.

Keywords: VRE, MDR, molecular characterisation, DNA.

Introduction

The emergence and spread of vancomycin-resistant Enterococcus (VRE) in health care settings has added risks and complexities in patient management due to the misuse of antibiotics. VRE can cause a variety of health care-associated infections, particularly bacteraemia and urinary tract infections. Most enterococcal infections are caused by two species, E. faecalis or E. faecium.

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VRE belonging to the species Enterococcus faecium, was first encountered in clinical isolates in England and France in 1986 [1], followed the next year by isolation of VRE faecalis in the United States [2-4]. Infection caused by VRE leads to poor outcome, and it remains a challenge with 60–70% mortality rate [5].

Intensive use of broad spectrum antibiotics has been responsible for the emergence of enterococci as important nosocomial pathogens [6]. Although the frequency of isolation of VRE is currently not very high in India [6-8] ,this may just be the beginning of the problem, in contrast to the USA and Europe, where VRE appeared in the late 1980s. Treatment options and effective antimicrobial agents for VRE are often limited [7]. Further, detection of genes responsible for

vancomycin resistance among enterococci could be helpful for confirmatory diagnosis of VRE as epidemiological purpose and for empirical therapy against VRE. Currently, eight phenotypic variants of acquired glycopeptide resistance in enterococci have been described (VanA, VanB, VanD, VanE, VanG, VanL, VanM, and VanN), with one type of intrinsic resistance (VanC) being unique to E. gallinarum and E. casseliflavus [8-10]. VRE colonisation can lead to infections which prolong hospital stay, increase the cost of care and increase morbidity and mortality [11-12]. The identification of modifiable risk factors may assist in identifying targets for intervention to reduce the incidence of VRE colonisation.

Material and Methods

It was a cross sectional study which was carried out in the Department of Microbiology and Central Research Lab of RMCH & RC Mandhana, Kanpur for a period of 1 year i.e., January 2021 to December 2022. A total of 135 enterococci isolates were collected from clinical sample of urine which were further processed to the Clinical Microbiology Laboratory. The Isolated enterococci were further processed for the Microscopic examination, Biochemicals test, species differentiation and AST of all enterococci isolates was done by KirbyBauer's disc diffusion method [13] according to the CLSI guidelines. E. faecalis ATCC 29212 was used as control. Zone diameter of = or < 6 mm for HLG (120 mcg) and HLS (300 mcg) were reported as HLG and HLS resistant. [14]

Vancomycin resistance was determined by agar dilution method and The Identification of VRE was done by the E-test strip method and the MIC determination of vancomycin using agar dilution method growth of enterococci on media containing concentration of vancomycin = or > 32mcg/ml was recorded as VRE. [13]

Molecular characterization of all VRE isolates was performed for the detection of VanA and VanB genes by using the DNA extraction Qiagen Kit according to the standard guidelines [15].

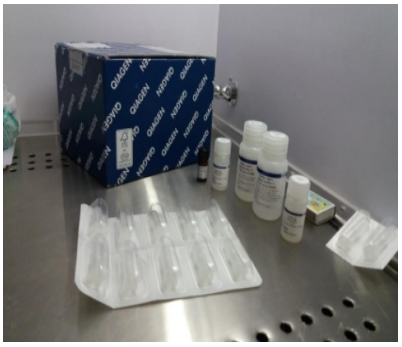

Figure No. 1: DNA extraction kit.

The primers used for the Detection of vanA and vanB genes:

Table no. 1 The Primers sequence used for the detection of VanA and VanB genes

Gene	Primer sequence
VanA	Forward 5'-GGGAAAACGACAATTGC-3' Reverse 5'-GTACAATGCGGCCGTTA-3'
VanB	Forward 5'-ACGGAATGGGAAGCCGA-3' Reverse 5'-TGCACCCGATTTCGTTC-3'

The DNA was amplified according to standard protocol. [16] PCR product was run in gel and required genes (VanA & VanB) were detected in gel doc and by sequencing reports.

Results

The present study was undertaken to detect the antimicrobial resistance pattern among enterococci isolates and also the detection of gene vanA and vanB obtained from urine clinical samples in a tertiary care hospital of Kanpur, report of which was highly beneficial to medical community for hospital infection control and antibiotic policies in this region. Out of the total 135 enterococci, 10 was found to be VRE by agar dilution method. The prevalence of VRE in our study was 7.4%.AST pattern showed that, resistance to HLG was 60% and 46% to HLS. Similarly, resistance pattern shown by enterococci to various antibiotics was as follows; penicillin (86%), tetracycline (14%), ciprofloxacin (100%), vancomycin (14%), teicoplanin (14%), linezolid (12%), nitrofurantoin (32%), norfloxacin (54%), & erythromycin (50%).

Out of the 135 Enteroccoccal isolates, 15 isolates were E. faecium and 120 were E. faecalis. Among 10 VRE, 6 were E. faecium and 4 was E. faecalis. Molecular testing for the detection of genes responsible for vancomycin resistance showed that 6 VRE isolates contained VanA & VanB genes out of which 1 isolate contained both vanA & VanB genes and 5 other isolates contained only vanA genes. However, remaining 4 VRE isolates contained genes other than VanA or VanB, which means some other gene might be responsible for its resistance.

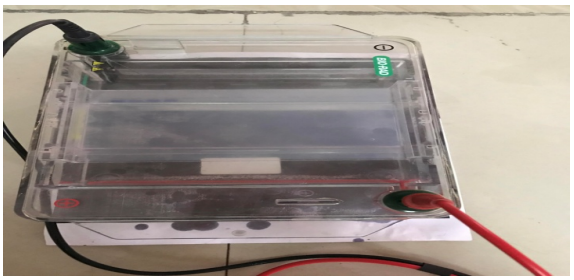

Figure No 1: Gel Electrophoresis for the DNA Extraction

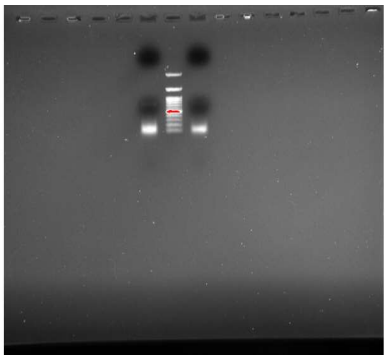

Figure No 2: Presence of Van A gene

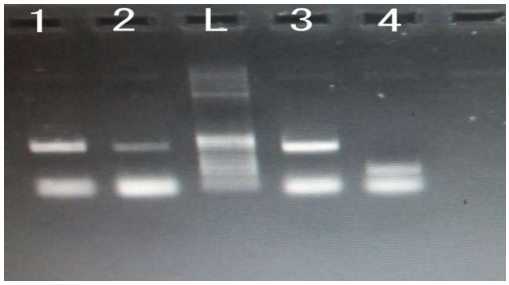

Figure No 3: L is the Ladder in the centre. 1, 2, 3=VanA positive, 4= VanB positive.

Discussion

In the present study out of the total 135 Enterococci, 10 were found to be VRE by agar dilution method. The prevalence of VRE in our study was found to be 7.4%. This was in support with the study by Olawale K et al.,[17] where prevalence of enterococci was 5.9% but in contrast with the study by Ferede ZT et al.,[18] Similarly, resistance pattern shown by enterococci to various antibiotics was as follows; penicillin (86%), tetracycline (14%), ciprofloxacin (100%), vancomycin (14%), teicoplanin (14%), linezolid (12%), nitrofurantoin (32%), norfloxacin (54%), & erythromycin (50%). This was in accordance with the study by Praharaj I et al. where least resistance was shown towards Linezolid , Teicoplanin and Vancomycin [19]. AST pattern showed that, resistance to HLG was 60% and 46% to HLS, which was parallel to the study by other authors [20-21]. The samples obtained in our study were urine samples which was in support with the study by other author [19,22] Out of the 135 isolates, 15 isolates were E. faecium and 120 were E. faecalis. This was in support with the study by [19]. Among 10 VRE, 6 were E. faecium and 4 was E. faecalis. Molecular testing for the detection of genes responsible for vancomycin resistance showed that 6 VRE isolates contained VanA & VanB genes, this was in accordance with the other studies [19,23]. Among the isolates 1 isolate contained both vanA & VanB genes which was in similar to the other study where both the vanA and vanB gene was isolates[24] and 5 other isolates contained only vanA genes[23] However, remaining 4 VRE isolates contained genes other than VanA or VanB, which means some other gene might be responsible for its resistance. This finding was in support with the other studies where some other gene might be responsible for resistance other than vanA and vanB gene like vanC,vanN , vanG, vanM and many other[25-27]..

Conclusion

The misuse of the Antibiotics should be checked and the strict enforcement programs and hospital surveillance along with regular antibiotic policies for the detection of VRE should be followed.

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