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Original Research Article

Phenotypic and Genotypic Characterisation of Vancomycin Resistant Enterococci (VRE) in a Tertiary Care Hospital of Kanpur, India

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ABSTRACT

Background: Vancomycin resistant enterococci related infection is an emerging nosocomial problem in India. This study was aimed to detect phenotypic and genotypic basis of VRE in a tertiary care hospital of northern India.
Methods: AST and high level gentamicin & high level streptomycin resistance pattern of enterococci isolates was determined by Kirby-Bauer's disc diffusion method. VRE were detected by agar dilution method. Species differentiation was done according to Facklam & Collin's classification. Molecular testing was done for detection of VanA & VanB genes among VRE.
Results: Out of 50 enterococci, 6(12%) were VRE. HLG & HLS resistance was 60% & 46% respectively. Species differentiation showed 16% isolates as *E. faecium* & 84% as *E. faecalis*. 5 out of 6 VRE were *E. fecium*. Molecular testing revealed, out of 6 VRE, 3 contained VanA or VanB genes and remaining 3 contained genes other than VanA or VanB responsible for vancomycin resistance.

Key-words: VRE, HLG, HLS, VanA, VanB, hospital, Kanpur.

INTRODUCTION

Vancomycin resistant enterococci (VRE) is an emerging nosocomial problem in India. [1-4] It has led to difficulty in treatment of enterococci related urinary tract infection (UTI), bacteremia, peritonitis, surgical site infections etc. [5,6] Very few studies have been done on VRE in northern part of India, specially Lucknow. [1] The present study was aimed at detecting antimicrobial resistance pattern among enterococci isolates obtained from various clinical specimens in a tertiary care hospital of Kanpur with special emphasis on vancomycin resistance in enterococci and its genetic basis, report of which could be highly beneficial to medical community for hospital infection control and antibiotic policies in this region.

MATERIALS AND METHODS

A prospective cross sectional study was conducted in Clinical Microbiology Laboratory and Central Research Laboratory of Rama Medical College Hospital and Research Centre, Kanpur from November 2017 to May 2018. A total of 50 enterococci isolates isolated from various clinical specimens were included in the study. All enterococci isolates were identified according to standard microbiological technique. [7] AST of all enterococci isolates was done by Kirby-Bauer's disc diffusion method. [8] *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. [9,10] Vancomycin resistance was determined by agar dilution method and growth of enterococci on media containing concentration of vancomycin = or > 32mcg/ml was recorded as VRE. [11] Species differentiation for enterococci was done according to Facklam and Collin's classification. [12] For genotypic characteristaion of VRE isolates, DNA was isolated using qiagen minikit according to standard guidelines. [13] Then, by using primers; Forward 5'-GGGAAAACGACAATTGC-3' & Reverse 5'-GTACAATGCGGCCGTTA-3' for VanA and Forward 5'-ACGGAATGGGAAGCCGA-3' & Reverse 5'-TGCACCCGATTTCGTTC-3' for VanB respectively, DNA was amplified according to standard protocol. [1,14] PCR product was run in gel and required genes (VanA & VanB) were detected in gel doc and by sequencing reports. [15]

RESULTS

Out of total 50 enterococci, 6 (12%) were VRE by agar dilution method. 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 (88%), tetracycline (12%), ciprofloxacin (100%), vancomycin (14%), teicoplanin (14%), linezolid (2%), nitrofurantoin (32%), norfloxacin (56%), & erythromycin (52%).

Species identification by Facklam & Collin's classification showed that, 8 isolates were *E. faecium* and 42 were *E. faecalis.* Among 6 VRE, 5 were *E. faecium* and 1 was *E. faecalis.*

Molecular testing for detection of genes responsible for vancomycin resistance showed 3 VRE isolates contained VanA & VanB genes out of which 1 isolate contained both vanA & VanB genes and 2 other isolates contained only vanA genes. However, remaining 3 VRE isolates contained genes other than VanA or VanB. (Fig.1)

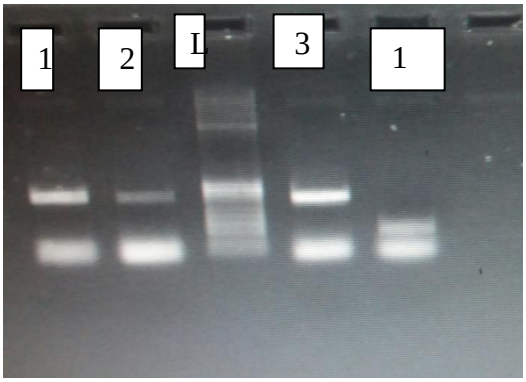

Fig.1 - showing VanA and VanB genes;(1,2,3=VanA, 1B=VanB, L=Ladder).

DISCUSSION

Geographical location and antibiotic policies in hospital have a great role in high prevalence of VRE, HLG & HLS resistance. In our study, AST pattern of enterococci to various antibiotics was similar to that of study done in Lucknow and Meerut. [1,16] High prevalence of HLG, HLS and VRE in our study might be due to irrational use of antibiotics as the antibiotics are easily available in medical shops over the counter in this region. However, our findings contrast with the findings of studies done in other neighbouring country (0% VRE in Nepal) and which might be due to different geographical locations, different antibiotic policies and method of testing in various hospitals. [6]

Among various species of VRE, *E. faecium* are more common than *E. faecalis.* [1] Our study showed, out of 50 enterococci, 8 were *E. faecium* and 42 were *E. faecalis.* Moreover, among 6 VRE, 5 were *E. faecium* and 1 isolate was *E. faecalis* which is similar to findings of study done in Lucknow. [1]

It has been found that, among enterococci, VanA & VanB genes are commonly responsible for vancomycin resistance. [17] Our study findings showed that, Out of 6 VRE isolates, 1 isolate contained both VanA and VanB genes & 2 others contained VanA genes. However, in 3 other VRE isolates, gene other than VanA or VanB were responsible for vancomycin resistance. VRE isolate in which both VanA & VanB genes were found was resistant to

even linezolid. The reason behind this might be due to treatment of this infection with linezolid in past also as bacterial infections frequently treated with linezolid may acquire both VanA and VanB genes responsible for vancomycin resistance. [17] Moreover, our findings have shown that genes other than VanA or VanB are also responsible for vancomycin resistance among enterococci in Kanpur that we couldn't detect, which is limitation of our study.

CONCLUSION

Prevalence of VRE is high in Kanpur. *E. faecium* is the common species among VRE. Along with VanA & VanB, other genes are also responsible for vancomycin resistance among enterococci in Kanpur. So, prudent use of vancomycin, regular surveillance detection of VRE and special strategies for infection control and antibiotic policies in hospital should be made to reduce the burden of VRE in this region.

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