ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Van A Gene for Intestinal Bacteria *Enterococcus faecium* (*E. faecium*) Resistant to Vancomycin

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Abstract

Out of 100 enterococcal strains clinical samples, a total of 59 (59%) *Enterococcus faecium* (*E. faecium*), 32 (32%) *E. faecalis,* 3 (3%) each of *E. casseliflavus*, and *E. avium,* and 1 (1%) each of *E. gallinarum*, *E. flavescens*, and *E. raffinosus* isolates were recovered, from burn, wound, and urine samples in Al-Hilla teaching hospital were identified to species level with a VITEK-2 system. For detection vancomycin resistance, all of *enterococcus* spp. isolates were tested by using multiplex PCR for (*Van*A, *Van*B, and *Van* C) genes. Results showed that only 5 isolates were resistant to vancomycin; 2 (*Van*A) genes were from *E. faecium,* 2 (*Van*B) genes were from *E. faecalis,* 1 (*Van*C) gene was from *E. gallinarum*.

Keywords: *VITEK-2, PCR.*

Introduction

Enterococci are some of the most diverse organisms found to infect hospitalized patients. The epidemiology of gastrointestinal infections has evolved since the emergence of these pathogens and has seen the rise of *Enterococcus faecium* as a nosocomial pathogen with severe clinical consequences. The effect of antibiotics on the microbiota of the gastrointestinal tract and subsequent changes in the regulation of the immune system of the intestinal tract may favour colonization by multidrug-resistant intestinal microbes [1].

Enterococci are usual populations of the intestinal tract in humans and numerous animals, counting food-producing and companion animals. They can easily contaminate the food and the environment, entering the food chain. In addition, *Enterococcus* is a significant opportunistic pathogen, particularly the species *E. faecalis* and *E. faecium*, causing a extensive range of infections.

This microorganism not only comprises inherent resistance mechanisms to many antimicrobial agents, but also has the ability to gain novel mechanisms of antimicrobial resistance [2]. The mechanisms of antibiotic resistance in enterococci are through alteration and horizontal gene transfer by plasmids and transposons [3].

Glycopeptide resistance is mediated via various vancomycin resistance (*Van*) gene operons viz. *Van A, Van B, Van C, Van D, Van E, Van G, Van L, Van M* and *Van N*. Out of these *Van A* is greatest shared followed by *Van B* and *Van C* is accountable for the basic resistance current in *E. gallinarum* and *E. casseliflavus* [4].

Materials and Methods

Bacterial Isolates

One hundred enterococcus isolates were obtained from clinical samples in Al-Hilla/Iraq during the period from January 2018 to 30 March 2018. Clinical samples were collected from Al-Hilla teaching hospital in Al-Hilla city, in addition to some private clinic. Clinical isolates were as follows: wound, burn, and urine. These bacterial isolates were identified as *enterococcus spp.* based on their morphology, Gram-staining, catalase properties. Vitek 2 system was performed to identify species level of enterococcus isolates.

Detection *Van* Genes in the Present Study

Well-characterized glycopeptides resistant enterococci belonging to phenotypes Van A, Van B, and Van C were studied. These were *E. faecium* (*Van A*), *E. faecalis* (*Van B*), *E.*

gallinarum (*VanC-1*), *E. casseliflavus* (*VanC-2*), and *E. flavescens* (*VanC-3*). *Van* genes were detected by multiplex PCR. DNA was purified from bacterial cells by using the wizard mini preps DNA Kit (Gene aid-USA).

Results and Discussion

A total of 100 enterococcal strains clinical samples, a total of 59 (59%) *Enterococcus faecium* (*E. faecium*), 32 (32%) *E. faecalis,* 3 (3%) each of *E. casseliflavus*, and *E. avium,* and 1 (1%) each of *E. gallinarum*, *E. flavescens*, and *E. raffinosus* isolates were recovered, from burn, wound, and urine samples in Al-Hilla teaching hospital. Multiplex PCR was used in the present study for detection of *van* genes (A, B, C) for vancomycin resistant.

Results showed that only 5 isolates were resistant to vancomycin; 2 (*Van* A) genes were from *E. faecium,* 2 (*Van* B) genes were from *E. faecalis,* 1 (*Van*C) gene was from *E. gallinarum* (Figure 1).

Enterococcus spp. loud *vanA* are extremely resistant to glycopeptides and are the leading vancomycin resistant *Enterococcus* (VRE) alternatives of *E. faecium* and *E. faecalis* internationally. Resistance is facilitated by replacing the rise-affinity fatal D-Ala-D-Ala peptide on NAM subunits by D-Ala-D-Lac. This amino acid replacement reasons a 1,000-fold reduction in the affinity of the pentapeptide for vancomycin [5].

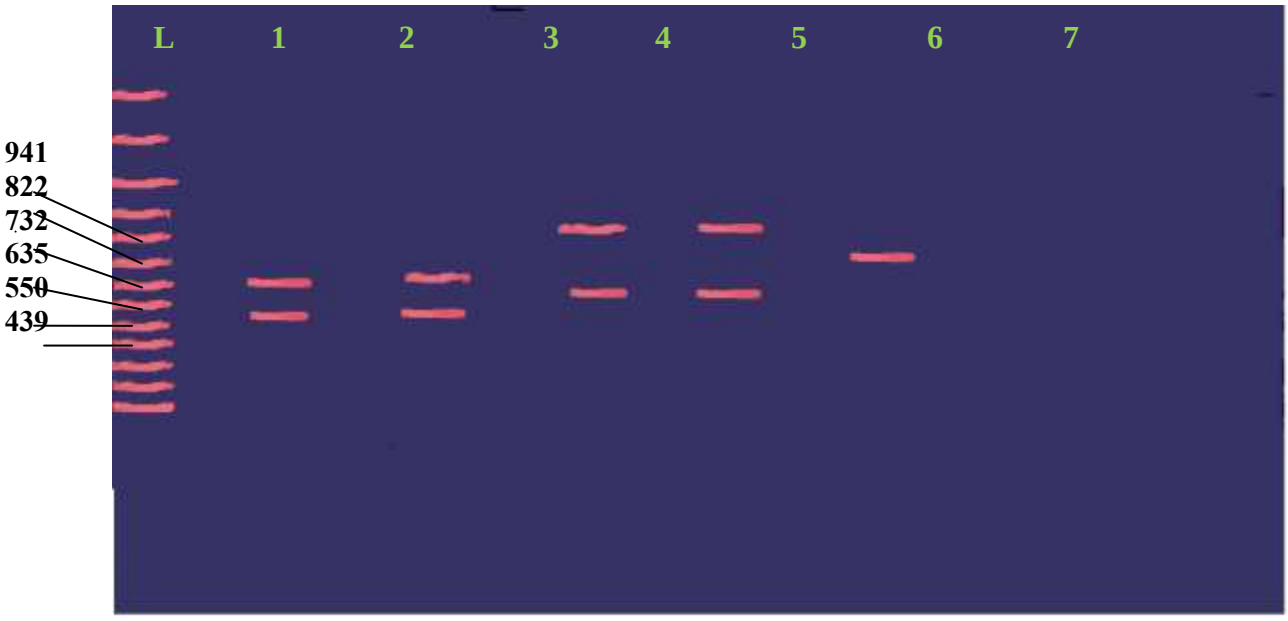

Figure 1: PCR analysis of DNA from glycopeptide resistant and susceptible enterococci
Lanes 1, 2: Van A-type *E. faecium* (732bp), *E. faecium* species-specific *ddl* gene (550bp).
Lanes 3, 4: Van B-type *E. faecalis* (635bp), *E. faecalis* species-specific *ddl* gene (941bp).
Lane 5: Van C-type *E. gallinarum* (822bp).
Lane 6: Van C-type *E. casseliflavus* (439bp).
Lane 7: Van C-type *E. flavescens* (439bp).

Sequesters carrying *van B* are fewer predominant than *van A*-carrying strains but can be originate throughout the world and are usually identified in Australia, where the common of *E. faecium* VRE isolates transport *van B* [6]. As through to *van A*, resistance in *van B* is mediated by changing D-Ala-D-Ala to D-Ala-D-Lac [5]. Though, *van B* gives diverse resistance to vancomycin, reaching from modest- to elevated resistance (MIC range, 4 to >256 µg/ml) [5]. Vancomycin is one of the little antibiotics that can be applied to treat infections subsequent from Gram-positive multidrug-resistant organisms (MDRO), like methicillin resistant *S. aureus* (MRSA); so, spread of vancomycin resistance from enterococci to MRSA is of main worry. Horizontal gene transfer has been exposed to be a mechanism of transmission among enterococci, and *in vitro* studies have established that transmits between *Enterococcus* and *S. aureus* can happen [7]. 14 situations of vancomycin-resistant *S. aureus* (VRSA) have been notify in the United States; yet, the likely recurrent communication among VRE and MRSA (co cultured, with the two organisms recovered

from the similar source) and infrequent occurrence of VRSA isolation propose that *in vivo* transfer of vancomycin resistance between these species happens at an very little incidence [8]. Horizontal gene transmission of the *van* operon between *Enterococcus* spp. and extra organisms too seems to arise at a actual squat rate .In a study that done coupling trials between *Enterococcus* species and Gram-positive gut flora (*Lactococcus* spp. and *Bifidobacterium* spp.), no transfer of *vanA* between types was detected [9]. Remarkably, the writers perceived that interspecies transmission inside the Enterococcus genus, e.g., *E. faecium* to *E. faecalis*, ensued at an abundant minor regularity ($1/10^8$ per donor/recipient) than intra species transmission ($1/10^6$ per donor /recipient). This can describe the advanced dominance of *van A* within *E. faecium* isolates, as show occurs between *E. faecium* isolates, but not between *E. faecium* and other *Enterococcus* species, at extraordinary frequencies. Infection surveillance and antimicrobial direction databases that goal to diminish achievement (i.e., colonization and/or infection) of VRE and MRSA infections may help in reducing the potential transmission of glycopeptides resistance to *S. aureus* in lessening coinfection per VRE and MRSA [10].

References

1. Arias CA, Murray BE (2012) The rise of the Enterococcus: beyond vancomycin resistance. Nat. Rev. Microbial., 10: 266-278.
2. Torres, C, Alonso CA, Ruiz-Ripa L, León-Sampedro R, Campo RD, Coque TM (2018) Antimicrobial Resistance in Enterococcus spp. of animal origin. Microbial Spectr.
3. Courvalin P (2006) Vancomycin resistance in Gram positive cocci. Clin Infect Dis., 42: 25-34.
4. Sujatha S, Praharaj I (2012) Glycopeptide resistance in Gram positive cocci: A review. Interdiscip Perspect Infect Dis., 781-679.
5. O'Driscoll T, Crank CW (2015) Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist, 8: 217-230.
6. Coombs GW, Pearson JC, Daley DA, Le T, Robinson OJ, Gottlieb T, Howden BP, Johnson PD, Bennett CM, Stinear TP, Turnidge JD (2014) Australian Group on Antimicrobial Resistance. Molecular epidemiology of enterococcal bacteremia in Australia. J Clin Microbial., 52: 897-905.
7. De Niederhäusern, S Bondi, M Messi, P Iseppi, R Sabia, C Manicardi G, Anacarso I (2011) Vancomycin-resistance transferability from Van A enterococci to Staphylococcus aureus. Curr. Microbial., 62: 1363-1367.
8. Walters MS, Eggers P, Albrecht V, Travis T, Lonsway D, Hovan G, Taylor D, Rasheed K, Limbago B, Kallen A (2015) Vancomycin-Resistant Staphylococcus aureus-Delaware, MMWR Morb Mortal Wkly Rep., 64: 1056.
9. Werner G, Freitas AR, Coque TM, Sollid JE, Lester C, Hammerum AM, Garcia-Migura L, Jensen LB, Francia MV, Witte W, Williams RJ, Sundsfjord A (2011) Host range of enterococcal vanA plasmids among Gram-positive intestinal bacteria. J. Antimicrobial. Chemother, 66: 273-282.
10. Faron ML, Ledeboer NA, Buchan BW (2016) Resistance Mechanisms, Epidemiology, and Approaches to Screening for Vancomycin-Resistant Enterococcus in the Health Care Setting. JCM., 54: 10.

