Antibiotic resistance markers in *Enterococcus* sp. isolates

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Abstract

Total of 34 of *Enterococcus* include 15(44%) of *Enterococcus faecium* and 19 (56%) of *Enterococcus faecalis*, samples have been isolated during 2016-2017 from patients hospitalized in the National Institute for cardiovascular disease the samples have been taken from different clinical sources (Urine sample, Blood, Wound, Tracheal secretions and stool culture) the highest rate of isolates 15 (44%) from urine and least from stool and wound was 2(6%) meanwhile 7(12%) from tracheal secretion and 4(12%)from blood. The phenotypic analysis revealed the 4(12.5%) of the *Enterococcus* sp. This study aimed to compare the rate of infection with *Enterococcus* sp. between the hospitalized patient and detecting the *Enterococcus faecalis* and *Enterococcus faecium* by PCR (Polymerase chain reaction) amplification. Strains were vancomycin –resistant, 25% showed resistance to ampicillin, 12.5% to tetracycline, 91.7% to erythromycin, 87.5% to ciprofloxacin and 54.2% to rifampicin. The genotypic support of vancomycin resistance was represented by the *vanA* gene present in 20.6% of the *E. faecium* strains and 14.7% of *E. faecalis*.

Keywords: *Antibiotic resistance, Enterococcus, Isolates.*

مقاومة المضادات الحيوية في عزلات *Enterococcus* sp.

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الخلاصة

إجمالي 34 من المكورات المعوية تشمل 15(44%) من ***Enterococcus faecium*** و19 (56%) من ***Enterococcus faecalis***، تم عزل العينات خلال الفترة 2016-2017 من المرضى في المستشفى في المعهد الوطني لأمراض القلب والأوعية الدموية. مأخوذة من مصادر سريرية مختلفة (البول ، الدم ، الجروح ، إفرازات القصبة الهوائية وزراعة البراز) أعلى نسبة عزلات 15 (44٪) من عينات البول وأقلها من عينات البراز والجروح كانت 2 (6٪) بينما كانت 7 (12٪) من إفراز القصبة الهوائية و 4 (12٪) من الدم. هدفت هذه الدراسة إلى مقارنة معدل الإصابة بالمكورات المعوية ***Enterococcus sp*** بين المرضى في المستشفى والكشف عن ***Enterococcus faecalis*** و ***Enterococcus faecium*** باستخدام تقنية **PCR** (تفاعل البلمرة المتسلسل). أظهر الفحص المظهري ان 4 (12.5٪) من سلالات ***Enterococcus sp.*** كانت مقاومة للفانكومايسين ، 25٪ أظهرت مقاومة للأمبيسيلين ، 12.5٪ للتتراسيكلين ، 91.7٪ للإريثروميسين ، 87.5٪ للسيبروفلوكساسين و 54.2٪ للريفامبيسين. وقد تم تمثيل الدعم الوراثي لمقاومة الفانكومايسين بواسطة جين ***van A*** الموجود في 20.6٪ من سلالات ***E. faecium*** وفي 14.7٪ من ***E. faecalis*** .

الكلمات المفتاحية : *مقاومة المضادات الحيوية ، المكورات المعوية ، العزلات .*

1. Introduction

Antibiotic-resistant bacteria have emerged, with the excessive use of antimicrobials for infectious disease therapy, and infections caused by a lot of bacteria are a global problem. [1]. *Enterococcus* sp. considered one of part of the normal flora of biliary tract, gastrointestinal tract, anterior urethra and female genital tract in humans. These are important universal causes of nosocomial infection disease and causes of nosocomial infection disease. [2] There are two sources of infections with *Enterococcus* sp. are proposed: firstly, infections may be caused by *Enterococcus* species of patient's flora; secondly, infections may be caused by *Enterococcus* sp. acquired from hospital

environments. There are two common *Enterococcus* sp. isolated from hospital-acquired infections are *E. faecium* and *E. faecalis*. *Enterococcus* strains resistant to different antibiotics are a great global problem, especially species isolated from nosocomial infections. [2،3] *Enterococcus faecalis* is responsible for 80% - 90% and *Enterococcus faecium* for the remaining human enterococcal infections. [4،5]. There are 7066 infectious cases reported in 2005, that caused by *Enterococcus* sp. in the UK, which approximately 28% were antibiotic resistant. [6] Vancomycin-resistant *Enterococcus* (VRE) is established as a significant nosocomial pathogen since it was first reported 20 years ago. [7]

Glycopeptide resistance is encoded by the *van* operon and can be divided into many types, of which the most frequently are *vanA* and *vanB* genotypes. [8]

2. Material and method

2.1. Clinical samples collection

A total of 34 samples were collected from hospitalized patients in National Institute during the period 2016-2017 isolates were obtained from the following sources: 15 Urine 15, 4 blood, 2 wound, 7 tracheal secretion and 2 stool the isolates were identified by means of routine tests and identification was confirmed via API 20 Strep system, SLIDEX Strep to Plus kit and PCR (Polymerase chain reaction).

2.2. Antimicrobial susceptibility

The antibiotic susceptibility testing was carried out for all the isolates on Muller-Hinton agar method using disk diffusion method (CLSI,2017) to measure zone of inhibition against standard concentration for the following antibiotics (Table-1).

Table (1): The antibiotic discs used in this study

Classes	Antibiotics	Symbol	Consecration µg
Penicillins	Ampicillin	AMP	10
Glycopeptide	Vancomycin	V	30
Tetracycline	Tetracycline	TE	30
Macrolides	Erythromycin	E	15
Fluoroquinolones	Ciprofloxacin	CIP	5
Ansamycins	Rifampicin	RD	5

3. Molecular detection

3.1. DNA extraction

In this purpose, 1-5 colonies of bacterial cultures were suspended in Eppendorf tubes containing 20µl solution of 0.05M NaOH (sodium hydroxide) and 0.25% SDS (sodium dodecyl sulfate) and heated on a thermo block at 95°C for 15 min. During this time, the detergent disrupted cell membranes and allowed the alkali to contact and denature both chromosomal and plasmid DNA and tearing a part of the cell membrane by SDS. The following step was the addition of 180µl of TE buffer (TRIS+EDTA) 1X and centrifuged at 13000 rpm for 3 minutes. The finally step, the supernatant was taken and put in new Eppendorf tube 1.5 or 2.0 mL.

3.2. PCR assay

All PCR reactions were performed using the Thermal Cycler machine Corbet. Genomic DNA was used as a template for the PCR screening of resistance vancomycin which encoded by *vanA*, *vanB* and *vanC* for alginate. The PCR reactions were initiated with 1 cycle at 95°C for 5min, followed by 30 cycles at

95°C for 1min, 51°C for 30 sec, 72°C for 1 min and a final elongation step at 72°C for 10 min (Table -2). The amplification products were visualized by electrophoresis on a 1% agarose gel, stained with the specific weight marker (3000pb, Ladder Thermo Scientific).

Table (2): PCR conditions used to amplify *Van* gene

Gene	Amplification program					
	Initial denaturation	**No. of cycles**	**Denaturation in each cycle**	**Annealing**	**Primer extension**	**Final extension**
vanA	**95°C , 5 min.**	**30**	**95°C, 1 min.**	**51°C, 30 sec.**	**72°C, 1 min.**	**72°C, 10 min.**
vanB	**95°C , 5 min.**	**30**	**95°C, 30 sec.**	**53°C, 30 sec.**	**72°C, 30 sec.**	**72°C, 10 min.**
vanC	**95°C , 5 min.**	**30**	**95°C, 30 sec.**	**53°C, 30 sec.**	**72°C, 30 sec.**	**72°C, 10 min.**

4. Results and discussion

This study was conducted on a total of (n=34) strains isolated during 2016 - 2017 from patients hospitalized in the National Institute for Cardiovascular Diseases; *Enterococcus faecium* (n=15) and *Enterococcus faecalis* (n=19). The selected strains were isolated from many different clinical sources most of them being from urine, blood culture, wound secretions, tracheal secretions and stool culture. All *Enterococcus* isolates were identified to species level by using API 20 Strep. Among hospitalized patients, *E. faecalis* was the predominant identified species 19 strain (56 %) followed by *E. faecium* 15 strain (44%). Regarding the distribution of strains according to sources, it was found that 15(44%) of the strains came from urine culture, 7 (20%) of tracheal secretion, 4(12%) of blood culture, 4(12%) from wound secretion, 2 (6%) from unknown source and 2 (6%) of stool culture .

Enterococcus sp. (*E. faecalis* & *E. faecium*) were tested for their antibiotics susceptibility toward six antibiotics, 13% of the strains were resistant to vancomycin, 52% of the strains showed resistance to rifampicin, 91% of the strain were resistant to erythromycin, 25% of the strains analyzed showed resistance to ampicillin, 87% were resistant to ciprofloxacin and 13% of the strain showed resistance to tetracycline (Figure-1).

Most common enterococcus infections are caused by *E. faecalis* (80%) but the epidemiological of these infections is variable. *E. faecium* currently accounts for up to 20% of enterococcus infections. In Europe, epidemiological data showed a large variation in vancomycin resistance in different countries, VER rates ranging from <2% (Finland, the Netherlands) to 20% (Ireland, Greece, Portugal). [9]

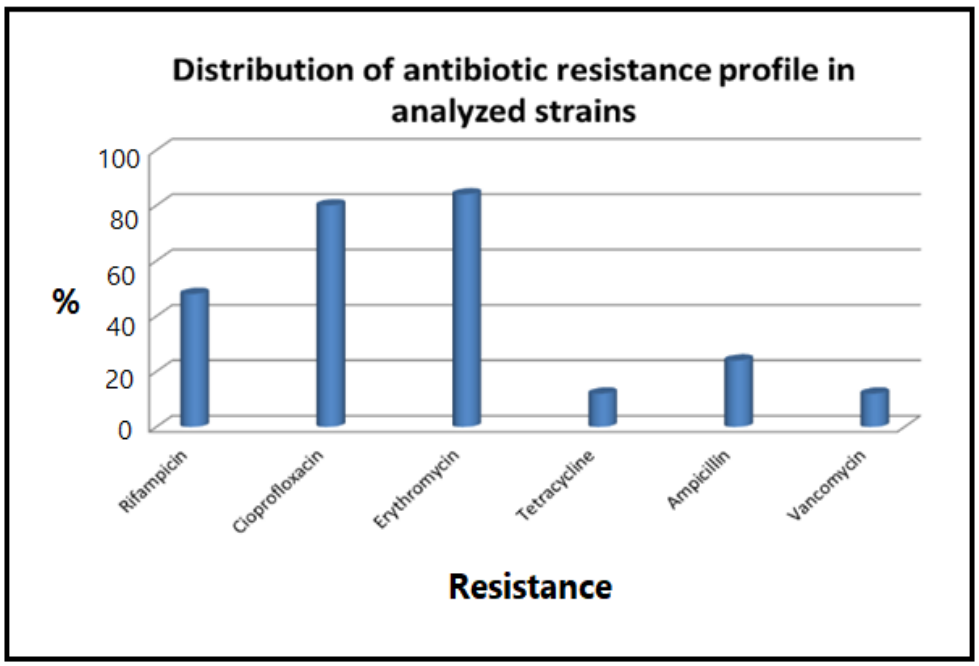

Figure (1): Graphic representation of the antibiotic resistance profile in the analyzed strains.

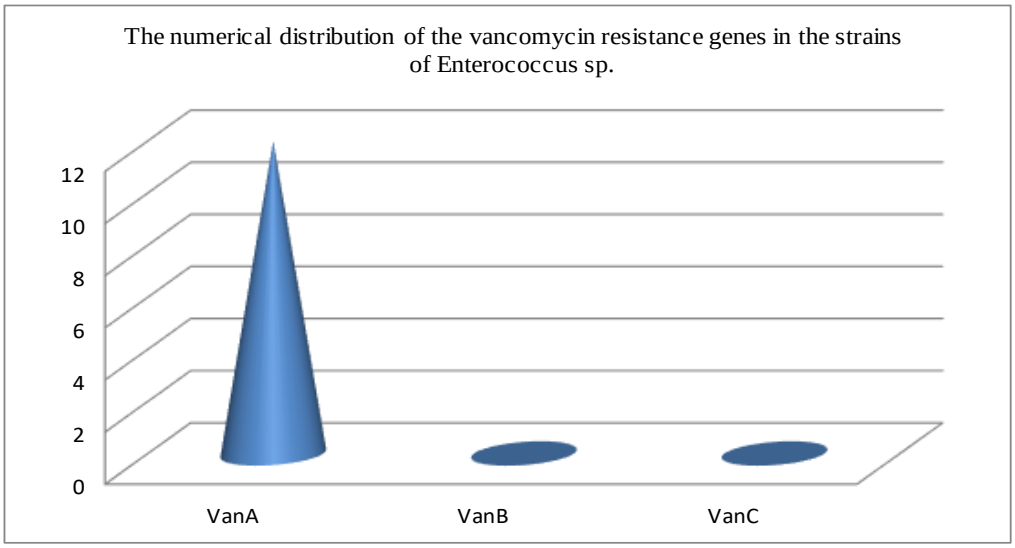

Figure (2): Diagram with the numerical distribution of the Van genes identified in the analyzed strains

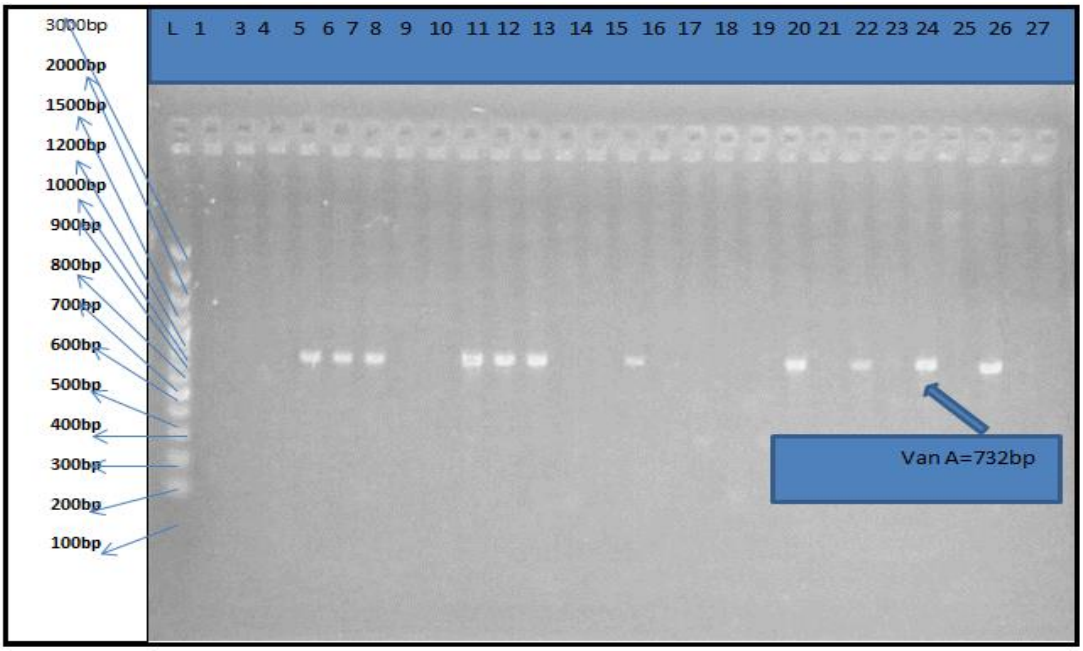

Figure (3): Electrophoresis gel for (*VanA*, *VanB* and *VanC*). Well L- Marker (Thermo Scientific) –3000pb– strain no.1-27 (wells - are empty samples); positive strain for *VanA* gene; no. 4=748A; 5=910; 6=995; 9=66; 10=103; 11=258; 14=748B; 19=505; 21=1841; 23=152; 25=533.

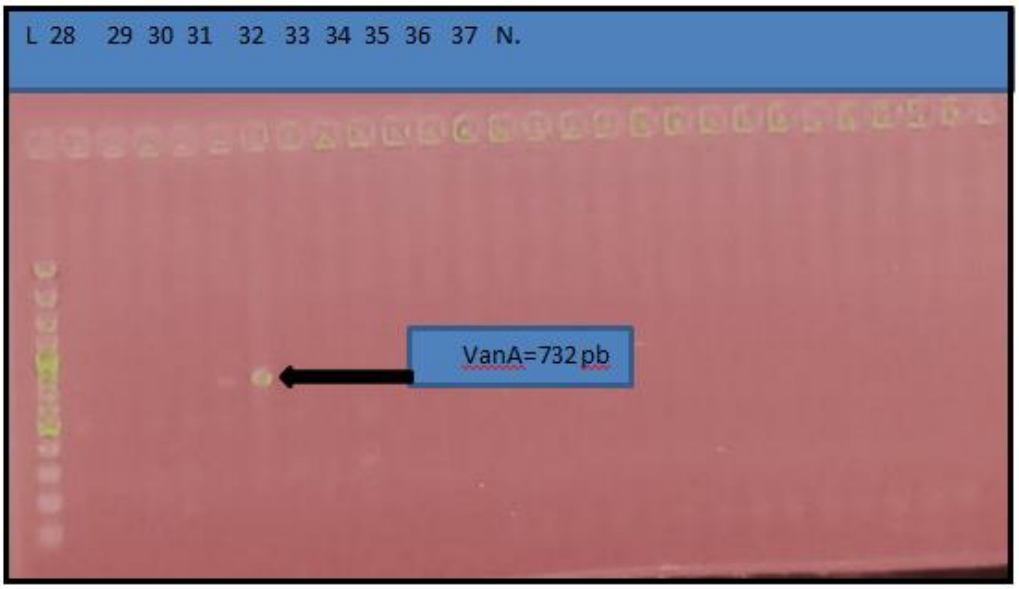

Figure (4): Electrophoresis gel for (*VanA*, *VanB* and *VanC*). Well L- Marker (Thermo Scientific) –3000 pb– strain no. 28-36; N: negative control. Positive strain for *VanA* gene ; no. 33=504.

For all isolates initial PCR results were confirmed; 12 were positive for *vanA* and all were negative for *vanB* and *vanC*. The phenotypic vancomycin resistance study demonstrated the presence of the *VanA* gene at 35.3% of the strains. The *VanA* gene is the antimicrobial resistance gene most commonly associated with vancomycin immune enterococci responsible for some of the most serious infections contracted by patients during hospitalization. Although vancomycin resistance genes are currently described, *VanA* is the most commonly expressed marker of resistance, followed by the *VanB* gene conferring vancomycin resistance and Sensitivity to other glycopeptides as teicoplanin. [10]

Vancomycin-resistant enterococci are usually resistant to other antimicrobial agents, are easily transmitted in the hospital environment and can disseminate vancomycin resistance factors to other Gram-positive bacteria. [10]

Despite this, large outbreaks affecting several hundred patients occurred in several hospitals in 2005, and this led the French authorities to recommend in 2005 and 2006 the notification of all cases of infection/colonization due to vancomycin resistance. In addition, they recommended strict rules and control measures to isolate infections. [10]

References

1. World Health Organization (WHO), (2014). Antimicrobial Resistance: Global Report on Surveillance 2014. Available online: www.who.int/drugresistance.

2. Magi G, Capretti R, Paoletti C, Pietrella M, Ferrante L, Biavasco F, et al. (2003).

3. Heidari H, Emaneini M, Dabiri H, Jabalameli F (2016). Virulence factors, antimicrobial resistance pattern and molecular analysis of Enterococcal strains isolated from burn patients. *Microb Pathog.* 90:93-7.

4. Talebi M, Rahimi F, Katouli M, Kühn I, Möllby R, Eshraghi S, et al. (2007). Prevalence and antimicrobial resistance of enterococcal species in sewage treatment plants in Iran. *Water Air Soil Pollut* . 185(1-4):111-9.

5. Laukova A, Kandricakova A, Scerbova J., Strompfova V. (2015). Enterococci isolated from farm ostriches and their relation to enterocins. *Folia microbiol.* 1-7.

6. Fisher, K. & Philips, C. (2009). The ecology, epidemiology and virulence of *Enterococcus*. Microbiology, 155, 1749-57.

7. Kandricakova A, Laukova A, Strompfova V. (2015). Characteristic and susceptibility to enterocins of enterococci in pheasants possessing virulence factor genes. Pol J Vet Sci. 18(3):507-14.

8. Gurtler V, Grando D, Mayall BC, Wang J, Ghaly-Derias S. (2012). A novel method for simultaneous *Enterococcus* species identification/typing and *van* genotyping by high resolution melt analysis. J Microbiol Methods. 90(3):167–81. doi: 10.1016/j.mimet. 05.002.

9. Berdeu Ion, (2015). Optimization of antibiotic monitoring of microbial resistance in septic-purulent infections at the level of medical institution. Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing 27th ed.,2017. West Valley

Road,Pennsyvania,USA. Presence of a *vanA*-carrying pheromone response plasmid (pBRG1) in a clinical isolate of *Enterococcus faecium*. *Antimicrob Agents Chemother*. 47(5):1571-6.

10. Werner G. (2012). Surveillance of antimicrobial resistance amongEnterococcus faeciumandEnterococcus faecalisisolated from human (clinical/commensal), food animal, meat and environmental samples. Enterococcus and safety. Edited by: Semedo-Lemsaddek T, Barreto-Crespo MT, Tenreiro R., Nova Science Publishers Inc, Hauppage, N.Y.