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PREVALENCE OF VANCOMYCIN RESISTANT ENTEROCOCCI AMONG PATIENTS WITH NOSOCOMIAL INFECTIONS IN INTENSIVE CARE UNIT

By

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ABSTRACT

Background: Enterococcus has increasingly become a major nosocomial pathogen worldwide. It has been well documented that intensive care units (ICUs) are the major reservoirs of vancomycin resistant enterococci (VRE) in the health care setting. It is a matter of concern due to its ability to transfer vancomycin resistant gene to other organisms.

Objective: Evaluation of the incidence of VRE as a cause of nosocomial infection in ICU patients, to analyze their antibiotic resistance profile, and to search for resistance genes of VanA and VanB in VRE isolates from these patients.

Patients and methods: Four hundred ICU patients with nosocomial infections were studied during the period between Jan, 2016 and Feb, 2017 at Misr University for Science and Technology Hospital.

Results: Enterococci were isolated from 12% of patients, of whom 66.7% E.faecalis and 33.3% E. facium. 41.7% isolates exhibited resistance to vancomycin. Among these isolates, 60% of strains were E. faecium, while 40% were E. faecalis. Polymerase chain reaction (PCR) detection of Van A and Van B resistance genes showed that Van A gene was detected in 85% of the VRE isolates, while Van B gene not found in any of the VRE isolates.

Conclusion: VRE and Van A gene were important as a cause of nosocomial infections in ICU patients. All VRE were multidrug resistant and few treatment options were available. Effective infection control measures against VRE are required.

Key Words: Nosocomial Infections, Enterococcal Species, Vancomycin Resistance, Intensive Care Units, Antibiotic Susceptibility, Genotypes.

INTRODUCTION

Enterococci are part of the normal flora of human and animals. They have emerged as important nosocomial pathogens over the last years, ranking only second to staphylococci as a leading cause of nosocomial infections *(O" Driscoll and Crank, 2015).*

Enterococci species are facultative anaerobic organisms that can survive temperature of 60°C for short periods; also they can grow in high salt concentrations. They are gram +ve cocci, growing in short chains, characterized by their ability to hydrolyze esculin in the presence of bile, their growth in 6.5% sodium chloride, and their reaction with

group D antiserum *(Byappanahalli et al., 2012).*

Currently, 54 different species of enterococci have been described, the most prevalent species that cultured from human (more than 90% of clinical isolates) are enterococcal faecalis (E. faecalis) and enterococcal faecium (E. faecium) *(O" Driscoll and Crank, 2015).*

Enterococci are capable of causing various serious diseases such as endocarditis, bacteremia, urinary tract infection (UTI), wound infection, catheter-related infection, and intra-abdominal and pelvic infections. Meningitis, pleural space infection, and skin and soft tissue infections have also been reported *(Arias and Murray, 2015).*

Resistance of enterococci to multiple antibiotics has become increasingly common in hospital sittings. Several genes isolated from resistant enterococci (agg, gelE, ace, cyills, esp, Cpd and fsrB) encode virulence factors such as production of gelatinase and hemolysin, adherence to caco 2 and hep-2 cells, and capacity for biofilm formation *(Biswas et al., 2016).*

Enterococci have both intrinsic and acquired resistance to antibiotics; therefore, they are considered important nosocomial pathogens. They have remarkable genome plasticity and utilize plasmids, transposons and insertion sequences to efficiently attain and transfere mobile resistance elements, facilitating dissemination of resistance genes *(Cattoir and Leclercq, 2013).*

Vancomycin resistant enterococci (VRE) are important hospital pathogens especially in patients admitted to ICUs. The acquisition of vancomycin resistance by enterococci has seriously affected the treatment and infection control of these organisms. VRE are frequently resistant to all antibiotics that are effective in treatment of vancomycin susceptible enterococci *(O" Driscoll and Crank, 2015).*

The genes that encode intrinsic or acquired vancomycin resistance results in peptide to which vancomycin cannot bind, therefore cell wall synthesis is still possible *(Biswas et al., 2016).*

Glycopeptide resistant genotypes in enterococci include Van A, Van B, Van C/C2/C3, Van D, Van E, Van G, Van L, Van M and Van N *(Padmasini et al., 2014).* The Van A and Van B phenotypes are clinically significant; Van A and Van B genes are present on transposon Tn 1546. These genes can be potentially introduced to conjugative plasmids transferred within enterococcal strains, as well as to other organisms such as staphylococci, and can increase the potential risk of vancomycin-resistant staphylococci in the community, therefore, these 2 genes causes high grade resistance to vancomycin *(Cattoir and Leclercq, 2013).* While other genes cause low grade resistance to vancomycin and are located on chromosome *(Biswas et al., 2016).*

The aim of the study was to estimate the incidence of VRE as a cause of nosocomial infections in ICU patients, analyze their antibiotic resistance profile, and search for resistance genes of Van A and Van B in VRE isolated from those patients.

PATIENTS AND METHODS

The study was conducted at Misr University for Science and Technology Hospital during the period between January, 2016 and February, 2017.

Four hundred ICU patients were included in the study (214 males and 186 females) Patients subjected for the study were admitted to ICU for more than 48 hours with different complaints and presentations and they develop clinical evidence of infection.

Specimens have taken included blood, urine, cerebrospinal fluid (CSF), wound exudate, central venous (CV) line, ascetic fluid and pleural fluid effusion.

Identification of enterococci was done depending on colony morphology, gram staining, catalase test, bile esculin hydrolysis test, arabinose fermentation test, ability to grow on nutrient broth containing 6.5% sodium chloride.

Cultivation of isolates on Hicrome E. faecium agar (oxoid, UK) was done to identify the enterococcal species. E. faecium produced green colonies along with yellow coloration of the media, while E. faecalis produced blue colonies on the agar media.

Antibiotic susceptibility to ampicillin (10 ug), penicillin (10 units), tetracycline (30 ug), vancomycin (5 ug), ciprofloxacin (5 ug), levofloxacin (5 ug), gatifloxacin (5 ug) gentamycin (120 ug), nitrofurantoin (30 ug), teicoplanin (30ug) and linezolid (30 ug) was done using Kirby Bauer disk diffusion method. Muller Hinton agar (Merck, Germany) plates were incubated for 24 hours, and inhibition zones were measured using a metric ruler as recommended by Clinical Laboratory Standard Institute (2016). E. faecalis strain ATCC 29212 was used as control strain. Multidrug resistance (MDR) was defined as resistance to 3 or more different classes of antibiotics.

The vancomycin minimal inhibitory concentration (MIC) was determined by E test method (Himedia). Isolates with a minimal inhibitory concentration $\geq$ 32 ug/ml were considered to be resistant isolates.

Polymerase chain reaction (PCR) was performed on VRE isolates for Van A and Van B to determine the prevalence of Van A and Van B glycopeptide resistance genotypes among VRE.

The DNA of clinical isolates was extracted using a QIA amp DNA mini kit (Hilden, Germany). Van A gene primer sequences were: forward primer A1, GGG AAAACGACAATTGC, and reverse primer A2, GTACAATGCGGCCGTTA. Van B gene primer sequences were: forward primer B1, ATGGGAAGCCGATAGTC , and reverse primer B2, GATTTCG TTCCTCGACC *(Dutka-Malen et al., 1995)*. PCR was carried out using Gene Amp PCR System 9700 (Applied Biosystems, USA) with the parameters 94°C for 4 minutes, 30 cycles at 94°C for 1 minute, 58°C for 45 seconds, and 72°C for 1 minute, and a final cycle at 72°C for 10 minutes. The amplified product 732 bp long was positive for VanA gene, while the amplified product 635 bp was positive for VanB gene. Amplification products were analyzed on 1.5 agarose gels stained with ethidium bromide and visualized by UV illumination. Number and percentage of resistant strains were calculated.

RESULTS

The study was carried out on 400 ICU patients, amongst them 214 (53.5%) were males and 186 (46.5%) were females with a mean age 43.1 ± 23 year (ranging from 22 to 74 year).

Out of 400 ICU patients, enterococci were isolated from 48 (12%) patients, of whom 25 (52.1%) were males and 23 (47.9%) were females.

In total, 32 (66.7%) E. faecalis and 16 (33.3%) E. faecium were isolated from different clinical specimens, 20 (41.7%) from blood, followed by 12 (25%) from wounds, 8 (16.7%) from urine and 8 (16.7%) from sputum.

Among the 48 enterococcal isolates, 20 (41.7%) exhibited resistance to vancomycin (MIC >32ug/ml), 8 (40%) of them from blood, 8 (40%) from wounds and 4 (20%) from sputum. Among these isolates, 12 (60%) strains were E. faecium, while 8 (40%) were E. faecalis.

Table (1): Antimicrobial resistance patterns of enterococcal isolates

Antibiotics	No. of resistant strains	% of resistance
Penicillin	30	62.5
Ampicillin	32	66.67
Rifampicin	33	68.75
Chloramphenicol	42	87.5
Tetracycline	41	85.42
Minocycline	40	83.33
Gentamycin	28	58.33
Erythromycin	42	87.50
Ciprofloxacin	25	52.08
Gatofloxacin	21	43.75
Levofloxacin	18	37.5
Vancomycin	20	41.7
Teicoplanin	11	22.92
Linezolid	1	2.08

The resistance rates of enterococcal isolates to tested antibiotics were as follow: for penicillin (30 strains, 62.50%), ampicillin (32 strains, 66.67%), rifampicin (33 strains, 68.75%), chloramphenicol (42 strains, 87.50%), tetracycline (41 strains, 85.42%), minocycline (40 strains, 83.33%), gentamycin (28 strains, 58.33%), erythromycin (42strains, 87.50%), ciprofloxacin (25 strains, 52.08%), gatofloxacin (21 strains, 43.75%), levofloxacin (18 strains, 37.50%), vancomycin (20 strains, 41.7%), teicoplanin (11 strain,22.92%) and linezolid (1 strain, 2.08%). The teicoplanin resistant strains were found in 8 E.faecium and 3 E.faecalis VRE isolates, while linezolid resistant strain were found in 1 E .faecium VRE isolate (Table 1). AII VRE isolates were multi drug resistant with resistance to 3 or more classes of antibiotics.

PCR detection of Van A and Van B resistance genes showed that Van A gene was detected in 17 (85%) of the VRE isolates, (11 E. faecium isolates and 6 E. faecalis), while Van B gene not found in any of the VRE isolates.

Lane 1 contained the DNA ladder (50 bp) and lane 2 contained negative control. Lanes 3, 5, 6, 8 and 10 contained the 732 bp VanA gene. However, lanes 4, 7, and 9 contained negative samples for the VanA gene **(Figure 1)**.

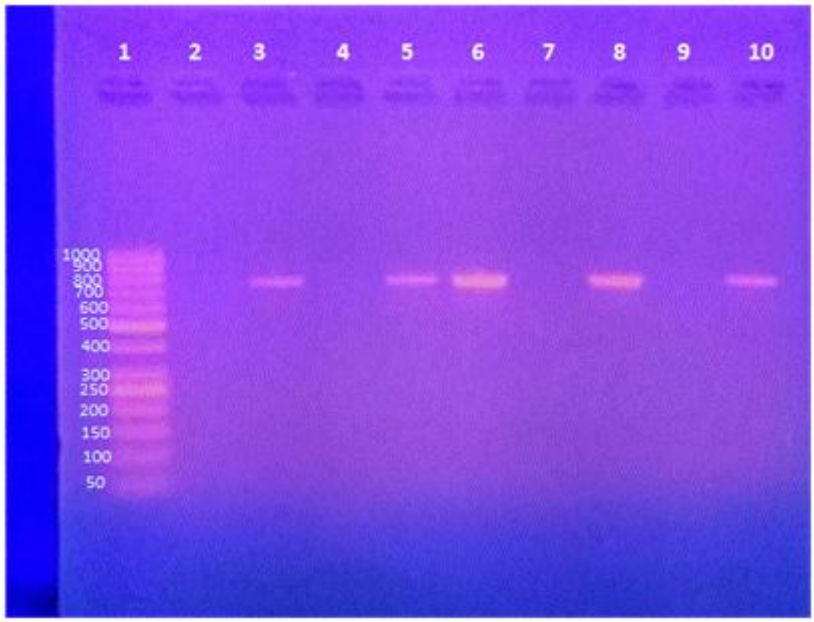

Figure (1): Agarose gel electrophoresis for the amplicons of the VanA gene

DISCUSSION

Enterococci have become important hospital acquired pathogens. Vancomycin is a glycopeptide used as an alternative choice to penicillin –aminoglycoside combination for treatment of enterococcal infections *(Padmasini et al., 2014).*

The spread of resistance to vancomycin has been reported worldwide .It has been well documented that ICUs are the major reservoirs of VRE in the health care setting *(Lind and Hyden, 2010).* The emergence of vancomycin resistant enterococci as an important nosocomial pathogen is due to its propensity for colonization of the gastrointestinal tract, persistence in hospital environment, genome plasticity, mobile genetic elements and increased mortality *(O" Driscoll and Crank, 2015).*

In the present study, enterococci were isolated from 12% of ICU studied patients, 41.7% of the enterococcus isolates showed vancomycin resistance. This finding indicates a high presence of vancomycin resistance in enterococcal isolates, and it is near to a study conducted in Ireland which revealed an incidence of VRE in 45.8% *(McDermott et al., 2018).* A lower isolation rates of VRE was reported in Iran (37.7% - *Kafil and Asgharzadeh, 2014),* Saudi Arabia (17.3% - *Alotaibi and Bukhari, 2017)* and USA (10.6%- *Ziakas et al., 2013).* In Europe, variable rates were reported, ranging from less than 1% in France, Spain and Sweden, to greater than 20% in

Greece, Ireland and Portugal *(O" Driscoll and Crank, 2015).* These differences might be due to difference in the study population and study design, also the differences in the published rates of VRE may reflect the differences in the infection control practices and antibiotic policies.

In the present study, the higher number of isolates were obtained from blood (41.6%), followed by wound swab (25%), and lastly urine (16.7%) and sputum (16.7%). Another study conducted by *Salem-Bekhit et al. (2012)* reported that maximum numbers of isolates were obtained from blood, followed by urine, and wound swab. On the other hand, *Sreeja et al. (2012)* found that maximum number of isolates were obtained from pus (43%) followed by urine (31%).

Most of the enterococcal isolates in this study were E.faecalis (66.7), followed by E. faecium (33.3%). This species distribution was near to the studies reported by *Salem-Bekhit et al. (2012)* 69.2% and 11.3%, *Sreeja et al. (2012)* 76% and 24% and *Alotaibi & Bukhari (2017)* 72.4% and 22.8%, respectively.

In this study, Van A gene was the dominant resistance gene (85%) in isolated VRE. This result was similar to that reported by *Salem-Bekhit et al. (2012)* and *Daghighi et al. (2014)* who found VanA gene in 87.8% and 89.3% of the VRE isolates, respectively. Also, this result was near to that reported by *Amberpet et al. (2016)* who found VanA gene in all 83 VRE isolates, while *Phukan et al., (2016)* found Van A gene in 56.25% of VRE isolates. The high prevalence of this gene could be due to excessive use of vancomycin in these countries. On the other hand, Van B gene was not found in our study. This result was compatible with that reported by *Daghighi et al. (2014)* and *Amberpet et al. (2016)* who did not find Van B gene in any of the VRE isolates. However, *Karki et al., (2012)* found Van B gene in17.5% of VRE isolates, but none of them had Van A. Their explanation was that Van B gene in VRE has been endemic in the region. Unlike Van A gene, Van B is clustered and occupies a large area on the chromosome and the possibility of its transmission is less, while Van A is associated with clinical strains and is more existed in the patients that recieved vancomycin for a long period *(Moosavian et al., 2018).*

In the current study, a higher prevalence of Van A gene was found in E. faecium 91.66%, while in E. faecalis, Van A was present in 75%. This result was similar to that obtained by *Hashem et al. (2015). O" Driscoll and Crank (2015)* reported that E. faecalis is more pathogenic than E. faecium, but the latter exhibits more resistance, composing the majority of VRE infections. All vancomycin resistant isolates showed also multi drug resistance. This finding was reported also by *Hashem et al. (2015)* who reported that vancomycin resistant isolates were also showing multi drug resistance. In the present study, we found a very good sensitivity for Linezolid as a treatment option for VRE. This finding was also reported by *Padmasini et al. (2014)* and *Phukan et al. (2016).*

CONCLUSION

VRE were important as a cause of nosocomial infections in ICU patients, we found a high resistance to vancomycin in enterococcal isolates, associated with

higher incidence of Van A gene. With this progressive increase in the VRE rate and Van A gene, more effective measures are needed. Effective control of VRE will require a better understanding of the interaction between enterococci, hospital environment, and human. Better contact isolation in hospitals, improved surveillance, good antibiotic policy, and searching for additional new drugs is mandatory.

REFERENCES

1. **Alotaibi F and Bukhari E (2017):** Emergency of vancomycin resistant enterococci at a teaching hospital, Saudi Arabia. Chinese Med J., 130(3): 340–46.
2. **Amberpet R, Sistin S, Parija S and Thabah M (2016):** Screening for intestinal colonization with Vancomycin Resistant Isolates Enterococci and associated risk factors among patients admitted to adult Intensive Care Unit of a Large Teaching Hospital. J. Clin Diagn Res., 10(9): 06-09.
3. **Arias CA and Murray BE (2015):** Enterococcus Species. In: Bennett J E, Dolin R and Blaser M J, editors. Mandell, Douglas, and Bennett Principles and Practice of Infectious Diseases. Pbl. Philadelphia, Saunders: 2328-339.
4. **Biswas PP, Dey S, Sen A and Adhikari L (2016):** Molecular characterization of virulence genes in vancomycin resistant and sensitive enterococci. J Global Infect Dis., 8(1):16-24.
5. **Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR and Harwood VJ (2012):** Enterococci in the Environment. Microbiol Mol Biol Rev., 76(4):685-706.
6. **Cattoir V and Leclercq R (2013):** Twenty – five years of shared life with vancomycin resistant enterococci. J Antimicrob Chemother., 68(4):73-84.
7. **Clinical and Laboratory Standards Institute (2016):** Performance Standards for Antimicrobial Susceptibility Testing, 26th ed., Pbl. Wayne PA: Clinical and Laboratory Standards Institute (An informational supplement, M100S).
8. **Daghighi Z, Tajbaksh S, Goudarzi H and Karimi A (2014):** Molecular detection of Van A and Van B genes in vancomycin resistant enterococcus isolated by polymerase chain reaction from the intestines of children admitted to the intensive care units. Arch Ped Infect Dis., 2(4): e18414-18.
9. **Dutka-Malen S, Evers S and Courvalin P (1995):** Detection of glycopeptide resistance genotype and identification to the species level of clinically relevant enterococci by PCR. J Clin Microb., 1: 24-27.
10. **Hashem YA, Yassin AS and Amin M (2015):** Molecular characterization of enterococcus SPP clinical isolates from Cairo, Egypt. Ind J Med Microbiol., 33(5): 80 – 86.
11. **Kafil SK and Asgharzadeh M (2014):** Vancomycin resistant enterococcus faecium and enterococcus faecalis isolated from education hospital of Iran. Maedica (Buchar)., 9(4):323-27.
12. **Karki S, Houston L, Land G, Bass P, Borrell S and Cheng A (2012):** Prevalence and risk factors for VRE colonization in a tertiary hospital in Melbourne, Australia. Antimicrob Resis Infect Control., 1(1): 31-35.
13. **Lind MY and Hyden MK (2010):** MRSA and VRE: Recognition and prevention in ICUs. Crit Care Med., 38(8 suppl: S): 335 – 44.
14. **McDermott H, Skally M, Humphreys H and Fitzgerald-Hughes D (2018):** Vancomycin Resistant Enterococci in the Intensive Care Units in a Nonoutbreak Sitting., 39(1):40-45.
15. **Moosavian M, Ghadri H and Samli Z (2018):** Molecular detection of VanA and VanB genes among vancomycin resistant enterococci in ICU - hospitalized patients in Ahvaz in Southwest of Iran. Infect Drug Resist., 11 :2269-75
16. **O" Driscoll T and Crank C W (2015):** Vancomycin resistant enterococcal infections. Infect Drug Resist., 8: 217-30.

17. **Padmasini E, Padmaraj R and Ramesh SS (2014):** Detection of vancomycin resistant enterococci with Van A genotype in clinical isolates from a tertiary care center. Ind J Med Microb., 32(1): 89–90.

18. **Phukan C, Lahkar M, Ranotkar S and Saikia K (2016):** Emergence of Van A gene among VRE in a tertiary care hospital of North –East India. Indian J Med Res., 143(3): 357-61.

19. **Salem–Bekhit MM, Moussa IM, Muharram MM and Hefni HM (2012):** Prevalence and antimicrobial resistance pattern of multidrug resistant enterococci isolated from clinical specimens. Indian J Med Microbiol., 30: 44–51.

20. **Sreeja S, Babu PR, and Prathab AG (2012):** The prevalence and characterization of the enterococcus species from venoms clinical samples in a tertiary care hospital. J Clin Diagn Res., 6: 1486–88.

21. **Ziakas PD, Thapa R and Mylonakis E (2013):** Trends and Significance of VRE Colonization in the ICU: A Meta-Analysis of Published Studies. PLoS one., 8(9): e 75658 70.

المكورات المعوية المقاومة لعقار الفانكوميسين فى مرضى الرعاية المركزة

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خلفية البحث: تمثل الميكروبات المعوية (enterococci) فى الوقت الحالى أهمية كبرى كمسبب لعدوى المستشفيات، وخاصة فى وحدات الرعاية المركزة، ويزداد الإهتمام بها لما لها من قدرة على نقل الجينات المسببة لمقاومة عقار الفانكوميسين لأنواع اخرى من البكتريا.

الهدف من البحث: تقييم مدى الإصابة بهذا الميكروب داخل وحدات الرعاية المركزة، وكذلك مدى مقاومته للمضادات الحيوية، ومدى انتشار الجينات المسببة لمناعة هذا الميكروب لعقار الفانكوميسين.

المرضى وطرق البحث: أجريت الدراسة على ٤٠٠ من مرضى الرعاية المركزة المصابين بعدوى المستشفيات فى الفترة من يناير ٢٠١٦ إلى فبراير ٢٠١٧ فى مستشفى جامعة مصر للعلوم والتكنولوجيا.

نتائج البحث: تم عزل هذا الميكروب المعوى من ١٢٪ من المرضى، كان منهم ٦٦,٧٪ من النوع (E. faecalis) و ٣٣,٣٪ من النوع (E. faecium). وقد وجد أن ٤١,٧٪ من هذه المكورات المعوية أظهرت مقاومة لعقار الفانكوميسين، كان منهم ٦٠٪ من النوع (E.faecium) و ٤٠٪ من النوع (E. faecalis). توصلت الدراسة لوجود الجين المتسبب Van A فى ٨٥٪ من الميكروب المعوى المقاوم للفانكوميسين، بينما لم يوجد الجين الآخر VanB.

الإستنتاج: المكورات المعوية المقاومة لعقار الفانكوميسين وكذلك الجين VanA سبب هام لعدوى المستشفيات داخل وحدات الرعاية المركزة. وتتميز هذه المكورات بمقاومتها الواسعة لسائر المضادات الحيوية لذلك فخيارات العلاج تعتبر محدودة. وتوصى الدراسة بتطبيق وسائل مكافحة العدوى بصورة اكثر فعالية للحد من تزايد الإصابة بهذه المكورات داخل وحدات الرعاية المركزة.