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ORIGINAL ARTICLE

Detection of VanA, VanB and VanC Genes in Vancomycin Resistant Enterococci in Zagazig University Hospitals

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ABSTRACT

Background: *Enterococci have emerged recently as a nosocomial pathogen especially in immunocompromized individuals and in intensive care units.* ***Objectives:*** *This study was carried out to detect incidence of VRE to detect genotypes of vancomycin resistance Enteroccci by PCR and to do a comparison between the results MIC of VRE and PCR results.* ***Methodology:*** *This study was conducted from May 2013 to January 2014. It was conducted on 560 hospitalized patients in Zagazig University Hospitals including 270 males and 290 female. The specimens were collected from patients suffering from any infections. Examination of isolates by conventional methods and API 20 Strep system. Susceptibility patterns of VRE isolates to antibiotics were determined by both disc diffusion method and E test. The genotypic detection of vancomycin resistance among enterococcal isolates was done by using polymerase chain reaction (PCR).* ***Results:*** *This study showed that the overall incidence of vancomycin resistance among Enterococci was 35.2% (57 isolates). The study showed that 38 of isolates (66.7%) had VanA gene and their MIC were more than 64 µg/ml. Twelve (21.05%) of isolates had VanB gene and their MIC also were more than 64 µg/ml. Five of isolates (8.8%) had VanC gene. MIC of these 5 isolates were between 4 and 16 µg/ml. Our study showed that VanA – PCR had the highest sensitivity to detect resistant isolates in comparison to VanB – PCR and VanC – PCR.* ***Conclusion:*** *The incidece of VRE in Zagazig University Hospitals was significantly high. The commonest genotype of vancomycin resistance in Enterococci was the VanA genotype. Other genotypes seen were VanB and VanC (low level resistance to vancomycin). VanA – PCR had the highest sensitivity to detect resistant isolates in comparison to VanB – PCR and VanC – PCR.*

INTRODUCTION

Enteroccocci are bacteria that are normally present in the human intestines and in the female genital tract and are often found in the environment. These bacteria can sometimes cause infections. Vancomycin is an antibiotic that is used to treat some drug-resistant infections caused by *Enterococci*. In some instances, *Enterococci* have become resistant to this drug and thus are called vancomycin-resistant *Enterococci* (VRE). Most VRE infections occur in hospitals [1].

In the last decade, the role of *Enterococci* in serious clinical and nosocomial infections has been increasing. The spread of vancomycin-resistant *Enterococci* (VRE) has become an important clinical concern, and VRE are now accepted as an emerging problem in hospitals worldwide [2].

In *Enterocooci,* two principal phenotypes of acquired vancomycin resistance have been described, VanA and VanB. Strains with a VanA phenotype posses a high level resistance to both vancomycin and teicoplanin, whereas strains with a VanB phenotype posses only moderate to high levels of vancomycin resistance. There is a third phenotype called vanC which is constitutive [3].

The rapid identification of VRE in both colonized and infected patients is important for appropriate antimicrobial treatment and prevention of VRE [4].

METHODOLOGY

This work was done in Microbiology and Immunology Department, Faculty of Medicine, Zagazig University during the period from May 2013 to January 2014.

This study was conducted on 560 hospitalized patients in Zagazig University Hospitals including 270 males and 290 females with their ages ranging from 5 to 80 years. Their samples were collected from General Surgery Department, General Medicine Department, Intensive Care Units (ICU), Orthopedic and Burn Units.

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All specimens were collected from patients suffering from any infections. Charts of all patients were reviewed for patient age, underlying diseases, period of hospitalization, administration of antibiotics and surgical procedures.

1. Sample collection:

Samples were collected under complete aseptic conditions using sterile containers, swabs, suction catheters and syringes. These samples included ear swabs, endo-tracheal aspirates, urine samples, sputum samples, and blood samples.

a. Inoculation of the samples:

The collected samples were transported as rapid as possible to the laboratory. Samples were cultured on *Enterococcus* selective differential medium **(ESD)** and blood agar plates and incubated together with the blood culture bottles at 37ºC for 24 hours to 72 hours. Subcultures from blood culture bottles on ESD medium were done daily for 7 days before reporting blood culture bottles as negative [5]. Isolated colonies were identified by colonial morphology on ESD agar plates, Gram stain, Catalase test, and Sodium chloride test.

b. API 20 Strep system (Bio-Merieux.Marcy L Etoile.France) this was done for identification of the isolates according to the manufacturer's instructions.

2. Antibiotic susceptibility tests:

a. Disk diffusion test:

All isolated strains being identified as *Enterococci* were tested for antimicrobial susceptibility by disk diffusion method. The following antibiotic discs were applied to the surface of the plate at constant distances (vancomycin, ampicillin, gentamicin, amikacin, tetracycline, ciprofloxacin, imipenem, chloramphenicol, cephradine and cefotaxime), the plates were incubated at 37ºC for 24 hours. Diameters of inhibition zones were measured with a ruler on the undersurface of the petri dishes according to *Clinical and Laboratory Standards Institute* [6].

b. E test (BioMerieux. Marcy L Etoile. France):

Vancomycin MIC was determined at the point of intersection of the edge of symmetrical inhibition eclipse with the strip. The cut off MIC used for detection of VRE was ≥ 32 µg|Ml [6].

3. Polymerase Chain Reaction (PCR):

The genotypic detection of vancomycin resistance among *Enterococcal* isolates was done by using polymerase chain reaction (PCR) which was done on 57 isolates.

a. DNA extraction:

DNA extraction from isolated colonies using I-genomic BYF DNA Extraction Mini Kit (iNTRON Biotechnology, Inc).

b. DNA amplification:

PCR Master mix solution (*i*-Taq™) (iNTRON Biotechnology, Inc) Primer pair targeting the VanA gene (Forwad primer Van-A-F17 sequence:5'-GGG AAA ACG ACA ATT GC-3',Reverse primer, termed VanA-R17, sequence:5'-GTA CAA TGC GGC CGT TA-3') Primer pair targeting the VanB gene (Forwad primer, termed VanB-F 17, with the following sequence:5'-TAC CTA CCC TGT CTT TGT GAA GCC-3'. Reverse primer, termed VanB-R17, with the following sequence:5'-CTT TTT CCG GCT CGT TTT CCT GAT G-3') Primer pair targeting the VanC gene (Forwad primer, termed VanC-2-F17, with the following sequence:5'-GTT TTC TTT AAG CCT AAT GAA GCT G-3 Reverse primer, termed VanC-2-R17, with the following sequence 5'-GTC ACA AGC ACC GAC AGT CAA AG-3'). (Bioron, The ENZYME Company, Germany).

c. Detection of the amplified product:

By agrose gel electrophoresis, the gel was examined under ultraviolet. Molecular weight marker gave different bands ranging from 100-1000 bp. (Roche, Lewes, East Sussex). Negative control gives no bands. A single DNA band of 732 bp was recorded as being positive for the Van A gene, single DNA band of 263 bp was recorded as being positive for the Van B gene and single DNA band of 192 bp was recorded as being positive for the Van C gene.

RESULTS

This work was done on 560 collected samples from hospitalized patients in Zagazig University Hospitals during the period from May 2013 to January 2014. Out of 560 different specimens, 162 (28.9%) *Enterococcus* strains were isolated from different clinical specimens including urine, endotracheal aspirate, sputum, wound swabs, blood samples and ear swabs. (Table 1).

Table 1: The prevalence of *Enterococcal* isolates according to the clinical samples.

Clinical samples	*No. of samples*	*No. of enterococcal isolates*	*Percentage (%)*
Urinary catheters	200	95	47.5
Endotracheal aspirate	88	27	30.7
Sputum	62	14	22.6
wound Swabs	150	26	17.3
Blood samples	10	0	0.0
Ear swabs	50	0	0.0
Total	560	162	28.9

$X^2 = 69.13$ **P value** < 0.001**

This study shows that *E.faecalis* (37.7%) and *E.faecium* (44.4 %) were the most common isolated *Enterococcus* species. The number and percentage of isolated *Enterococci* are shown in Table (2).

Table 2: Number and percentage of isolated *Enterococcus* species.

Enterococcus *species*	***No.***	***%***
E.faecalis	61	37.7
E.faecium	72	44.4
E.avium	3	1.9
E.gallinarum	20	12.3
E. casseliflavus/E. flavescens	6	3.7
Total	162	100.0

$X^2 = 290.94$ **P value** < 0.001**

Out of 162 *Enterococcal* isolates, 57 isolates (35.2**%)** were resistant to vancomycin while 105 isolates (64.8%) were susceptible. The most effective drug against the tested isolates was imipenem to which 93.2% of isolates were susceptible (P<0.001), while the least susceptibility was reported with ciprofloxacin (73.5%) followed by cephalosporins including cephradine and cefotaxime (58.6% & 62.9% respectively) (P. value 0.002 & <0.001 respectively). (Table 3)

Table 3:- Antimicrobial susceptibility testing of *Enterococcal* isolates against different antibiotics by disc diffusion method.

Antibiotic	***Susceptible***		***Resistant***		***X2***	***P .value***
	No.	%	No.	%		
Vancomycin	105	64.8	57	35.2	28.44	<0.001**
Ampicillin	87	53.7	75	46.3	1.78	0.182
Gentamicin	102	62.9	60	37.04	21.78	<0.001**
Amikacin	129	79.6	33	20.4	113.78	<0.001**
Tetracycline	73	45.1	89	54.9	3.16	0.075
Ciprofloxacin	43	26.5	119	73.5	71.31	<0.001**
Imipenem	151	93.2	11	6.8	241.98	<0.001**
Chloramphenicol	120	74.1	42	25.9	75.11	<0.001**
Cephradine	67	41.4	95	58.6	9.68	0.002*
Cefotaxime	60	37.04	102	62.9	21.78	<0.001**

This study showed that that 50 (87.7%) patients had a previous history of antibiotic therapy, 30 (52.6%) had a history of ICU stay, 29 (50.9%) were hospitalized more than 20 days, 19 (33.3%) suffered from underlying diseases, 18 (31.6%) had device related infections and 10 (17.5%) had history of surgical procedures. (Table 4)

Table 4: Risk factors associated with isolation of VRE.

Risk factors	***Number of VRE (57)***	***Percentage (%)***
Prolonged hospitalization	29	50.9
ICU stay	30	52.6
Previous antibiotic therapy	50	87.7
Surgical procedures	10	17.5
Underlying diseases	19	33.3
Device related infections	18	31.6

$X^2 = 68.6$ **P value** < 0.001**

The present study showed that VRE were significantly more sensitive to imipenem (82.5%), while they were significantly more resistant to ciprofloxacin (85.9%). (Table 5)

Table 5: Antimicrobial susceptibility testing of 57 VRE isolates against different antibiotics by disc diffusion method.

Antibiotic	*Sensitive*		*Resistant*		*X2*	*P .value*
	No.	%	No.	%		
Ampicillin	30	52.6	27	47.4	0.32	0.574
Gentamycin	26	45.6	31	54.4	0.88	0.349
Amikacin	20	35.1	37	64.9	10.14	0.001*
Tetracycline	18	31.6	39	68.4	15.47	<0.001**
Ciprofloxacin	8	14.1	49	85.9	58.98	<0.001**
Imipenem	47	82.5	10	17.5	48.04	<0.001**
Chloramphenicol	40	70.2	17	29.8	18.56	<0.001**
Cephradine	16	28.1	41	71.9	21.93	<0.001**
Cefotaxime	18	31.6	39	68.4	15.47	<0.001**

The present study showed that there is a difference between the phenotypic characters of different VRE isolates by E test and the genotypic characters by PCR. It showed that 38 of isolates (66.7%) had Van A gene and their MIC were more than 64 µg/ml and 12 (21.05%) of isolates had VanB gene and their MIC also were more than 64 µg/ml. The *Enterococcus* isolates that have VanA and VanB genes were *E.faecium* and *E.faecalis* species. There is also 5 isolates (8.8%) had VanC gene. MIC of these 5 isolates were between 8 and 16 µg/ml. There were 2 isolates (3.5%) showing both VanA and VanC genes together which were *E.gallinaram*. (Table 6)

Table 6: The distribution of van A , Van B and Van C genes in VRE isolates.

Gene(s) detected	*No. of isolates and (%)*	Enterococcus *spp.*	*Vancomycin MIC*
VanA	38(66.7)	*E.faecium* *E.faecalis*	≥ 64
VanB	12(21.05)	*E.faecium* *E.faecalis*	≥ 64
VanC	4(7.01)	*E.gallinaram*	8 - 16
	1(1.8)	*E.avium*	8
VanA + VanC	2(3.5)	*E.gallinaram*	≥ 128

X^2= 38.51 **P value =** <0.001*

In a Comparison between E test and VanA - PCR for detecting vancomycin susceptibility profiles of *Enterococci*, we found that the ability of VanA - PCR to detect resistant isolates (sensitivity) was 66.7%, while the ability to exclude resistance (specificity) was 99.04%. Positive and negative predictive values of VanA - PCR in relation to E test were calculated to be 97.4% and 84.6% respectively and accuracy 87.7%. (Table 7)

Table 7: Comparison between E test and VanA - PCR for detecting vancomycin susceptibility profiles of *Enterococci*.

		E test		*Total*
		VRE	**VSE**	
VanA - PCR	**Positive**	38	1	39
	negative	19	104	123
Total		57	105	162

Comparing the results of E test and VanB - PCR for detecting vancomycin susceptibility profiles of *Enterococci*, we found that the ability of VanB - PCR to detect resistant isolates (sensitivity) was 21.05%, while the ability to exclude resistance (specificity) was 97.1%. Positive and negative predictive values of VanB - PCR in relation to E test were calculated to be 80% and 68% respectively and accuracy 70.4 %.(Table 8)

Table 8: Comparison between E test and VanB - PCR for detecting vancomycin susceptibility profiles of *Enterococci.*

		E test		*Total*
		VRE	**VSE**	
VanB - PCR	**Positive**	12	3	15
	negative	45	102	150
Total		57	105	162

In a Comparison between E test and VanC - PCR for detecting vancomycin susceptibility profiles of Enterococci, the ability of VanC - PCR to detect resistant isolates (sensitivity) was 8.8%, while the ability to exclude resistance (specificity) was 87.6%. Positive and negative predictive values of VanC - PCR in relation to E test were calculated to be 38.5% and 65.1% respectively and accuracy 63 %. (Table 9)

Table 9: Comparison between E test and VanC - PCR for detecting vancomycin susceptibility profiles of *Enterococci.*

		E test		*Total*
		VRE	**VSE**	
VanC - PCR	**Positive**	5	8	13
	negative	52	97	149
Total		57	105	162

DISCUSSION

Enterococci are part of the normal flora in humans and animals. [7] These opportunistic pathogens have recently emerged as nosocomial infectious agents, especially in patients with haemato-oncological disease and those in intensive care units [8].

Since the vancomycin resistance genes are transferable among different *Enterococcal* species or even among different genera, the inability to detect *Enterococci* promptly may cause delays in reporting VRE; this situation may lead to complex and costly containment efforts to eliminate VRE colonization and infection [9].

This study was carried out to detect VRE in Zagazig University Hospitals, to evaluate the susceptibility tests of the isolated strains, to study the vancomycin-resistance genotypes of these strains and to study the different phenotypic patterns of VRE in relation to the results of the PCR assay for the genes encoding vancomycin resistance.

This study was conducted on 560 patients from General Surgery Departments, Orthopedic Departments, Burn Unit, Oncology Unit and ICUs. It included 270 males and 290 females aging from 5 to 80 years.

Enterococci are one of the leading causes of nosocomial infections worldwide because of increasing resistance to a wide range of antibiotics [8]. In our study a 162 (28.9%) *Enterococcal* isolates were isolated from 560 different clinical samples which was high compared to other studies.

In a previous study in Zagazig University Hospitals, El-Shafei *et al.*[10] isolated *Enterococci* from 5.3% of collected clinical samples and Nawara *et al.*, (2003)[11] reported that *Enterococci* were isolated from 10.4% of isolated samples. Abbadi *et al.* [12] isolated it from 3.3% of cases in Suez Canal University Hospitals.

In our study, the isolation of Enterococci was significantly higher from urine samples (59.1%) followed by endotracheal aspirate (30.7%), sputum (22.6%) and wound swab samples (17.3%). On the other hand, no *Enterococci* were isolated from blood or ear swabs. These results were in accordance with many local studies [10, 11,12].

Enterococci are part of the normal intestinal flora of humans and animals but are also important pathogens responsible for serious infections. The genus *Enterococcus* includes more than 17 species, but only a few cause clinical infections in humans. *Enterococcus faecalis* and *Enterococcus faecium* are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates[13]. Other *Enterococcal* species known to cause human infection include *Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus and Enterococcus mundtii* [14].

Among the 162 *Enterococcal* isolates in this study, 72 (44.4%) isolates were *E. faecium*, 61 (37.7%) were *E.faecalis*, 20 (12.3%) were *E.gallinarum*, 6 (3.7%) were *E.casseliflavus* and 3 (1.9%) were *E.avium.*

These results are comparable to the distribution of *Enterococcal* species in other local [10] and international studies [8].

In the study of El-Shafei *et al.* [10], 70.7% of their *Enterococcal* isolates were *E.faecalis* and 29.3% were *E.faecium*. Mihajlović-Ukropina *et al.* [8] found that the most common species was *E.faecalis* (55.05%), followed by *E.faecium* (41.57%). These differences might be explained by the small number of isolates included in their studies.

Enterococci have emerged as important nosocomial pathogens in the last few decades and the major reason for this is the trend of increasing antimicrobial resistance seen in these organisms [15]. Resistance to a wide range of antibiotics is a great problem to clinicians because it has seriously affected the treatment of *Enterococcal* infections leaving limited therapeutic options. Keeping in mind that antimicrobial susceptibility of *Enterococci* is not predictable and that it differs by *Enterococcal* species and changes rapidly over time. Species identification and its susceptibility to antimicrobial drugs are important for clinicians for choosing the most effective drug and also useful for epidemiological investigations [8].

The present study, the most effective drug against the tested isolates was imipenem to which 93.2% of isolates were susceptible, while the least susceptibility was detected with ciprofloxacin (73.5%) followed by cephalosporins including cephradine and cefotaxime (58.6% & 62.9%). Also 89 (54.9%) of all *Enterococcal* isolates were resistant to tetracycline, 75 (46.3%) were resistant to ampicillin, 60 (37.04%) were resistant to gentamicin, 42 (25.9%) were resistant to chloramphenicol and 33 (20.4%) were resistant to amikacin. Our results agree with those of El-Shafei *et al*.[10], and Ira *et al*. [16].

In the study of El-Shafei *et al*. [10], the most effective drug against the tested isolates was imipenem to which 98.3% of isolates were susceptible, while the least susceptibility was reported with cephalosporins including cephradine and cefotaxime (93.1% of isolates were resistant).

In the present study, it was found that 57 (35.2%) of *Enterococcal* clinical isolates were resistant to vancomycin of which 20 (35.08%) were isolated from urine, 15 (26.3%) were isolated from wound swabs, 14 (24.6%) were isolated from endotracheal aspirate and 8 (14.04%) were isolated from sputum.

Lower percentages of VRE isolates had been reported, El-Shafei *et al*. [10] identified 11 (18.9%) out of 58 *Enterococcal* isolates were VRE, also Nawara *et al.*[11] reported that the overall percentage of VRE was 12%. Ira *et al*. [16] identified 34 (9.26%) VRE isolates out of 367 isolates of *Enterococcus* species. In contrast, in the study of Mihajlović-Ukropina *et al.* [8] a high percentage of *E. faecium* resistant to vancomycin (54.05%) was detected.

The VRE percentage among our isolates was high, probably reflecting the increased use of vancomycin in Zagazig University hospitals over the past few years. Broad spectrum antibiotics, such as third-generation cephalosporins and fluoroquinolons, also contributed to the selection of resistant isolates [17].

This fact highlights the importance of strict enforcement of antibiotic policies coupled with greater adherence to infection control measures to prevent emergence and spread of antibiotic resistant bacteria. El-Kholy *et al*.[18] reported that 80% of Egyptian patients were prescribed antibiotics without documented proof of infection and 30% of these patients received repeated courses without apparent reasons. Increase in VRE poses several challenges, including firstly the sole availability of expensive new antimicrobials for therapy of infections due to VRE, since most VRE are also resistant to multiple other economically acceptable drugs in developing countries, e.g., aminoglycosides or ampicillin, and secondly the possibility that the vancomycin resistance genes present in VRE may be transferred to other gram positive microorganisms such as *S. aureus* [19].

Enterococci are intrinsically resistant to many antimicrobial agents, including cephalosporins, low concentrations of aminoglycosides, and trimethoprim—sulfamethoxazole. Furthermore, the ability of *Enterococci* to acquire resistance to other agents like erythromycin, rifampin, chloramphenicol, ciprofloxacin, high concentrations of aminoglycosides, and vancomycin is well recognized [20].

In the present work, it was found that VRE were significantly more sensitive to imipenem, while they were significantly more resistant to ciprofloxacin. These results are similar to those previously reported by Ira *et al.* in India.[16] In accordance all VRE isolates in the studies of El-Hadidy *et al.*[21] and Nawara *et al.* [11] were sensitive to imipenem. Also in the study of El-Shafei *et al.*, [10] VRE were significantly more sensitive to imipenem while they were significantly more resistant to ciprofloxacin .

The PCR assay is a convenient and rapid method for determining glycopeptide resistance genotypes for *Enterococcus* spp. in the clinical microbiology laboratory. The assay provides a more specific and rapid alternative to classical phenotypic methods for the detection of low level glycopeptide resistance (MIC range, 4 to 8 mg/ml), as occurs with *vanC* associated resistance in *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* [7].

The commonest phenotypes seen among VRE strains is the VanA and VanB phenotypes in which high level inducible resistance to vancomycin was seen (MICs ≥64 μg/ml). VanA and VanB phenotypes are due to acquisition of new genetic elements and have been mostly reported in *E. faecalis* and *E. faecium* isolates whereas VanC phenotype is constitutive low level vancomycin resistance seen in motile species of *Enterococcus* like *E. gallinarum* and *E.casseliflavus* [22].

It had been reported that the VanA phenotype is of a serious concern because of the risk of transmission of this gene to other organisms. From an epidemiological point of view, the most dangerous VRE are VanA and VanB phenotypes as they represent resistence which is acquired and transferable [8].

Different studies in different countries showed variable results concerning the detection rates of VanA

and VanB phenotypes. In accordance with the present study, El-Shafei *et al*. [10] found that 90.9% of VRE isolates (8 *E.faecalis* and 2 *E. faecium* isolates) were of VanA genotype. El-Hadidy *et al.* [21] detected a more predominance of VanA genotype (85.7%) than VanB genotype (14.3%). In the study of Ira *et al* . [16] VanA genotype was seen in 87.5 % of all VRE isolates and 2 VRE isolates showed VanB genotype. On the other hand, **in the study of Surendra** *et al.*[23] all the isolates were positive for *VanB* resistance genotype and no *VanA* resistance genotype was detected.

Both the VanA and VanC genes were detected in 2 isolates of *E. gallinarum*. The occurrence of VanA and VanC genes in a single *Enterococcus* isolate was reported for the first time by Dutka *et al* [24]from the faeces of a patient under oral therapy with vancomycin .

There have been a few reports of *E. gallinarum* harboring both the VanA and VanC gene. The possession of both VanA and VanC gene clusters will alter the resistance phenotype of an VRE isolate [25.] In the present study, the *E. gallinarum* isolate which possessed both the VanA and VanC genes showed a VanA phenotype with high level resistance to vancomycin.

Also in this study, the detection of VanA genotype in one vancomycin susceptible isolates (VSE), VanB genotype in 3 VSE and VanC genotype in 8 VSE might suggest that these genes were present in these isolates but were non functional [26.]

The present work showed that sensitivity of VanA - PCR to detect resistant isolates was 66.7%, while sensitivity of VanB - PCR to detect resistant isolates was 21.05%, and sensitivity of VanC - PCR to detect resistant isolates was 8.8%. These results reflect the high sensitivity of VanA – PCR to detect resistant isolates in comparison to VanB – PCR and VanC – PCR.

In conclusion, the molecular methods are significant tools in detecting the genotypes of vancomycin resistance *Enteroccci. This is* because of the difficulties of phenotypical characterization of vancomycin resistance in *Enterococcal* clinical isolates. This is important for clinicians to start treatment and for implementation of infection control measures.

REFERENCES

1. Siegel J.D., Rhinehart E., Jackson M., et al. Management of multidrug-resistant organisms in health care settings. Am J Infect Control; 35(10 Suppl 2), 2006; S165-S193.
2. Palladino, Ian D. K., Anna M. C., et al. Real-time PCR for the rapid detection of vanA and vanB genes. Diagnostic Microbiology and Infectious Disease.Volume 45, Issue 1, 2003; Pages 81–84.
3. Patel J.R.U., Kohner P., Hopkins M. K., et al. Multiplex PCR detection of vanA, vanB, vanC-1, and vanC-2/3 genes in Enterococci. Journal of Clinical Microbiology, vol. 35, no. 3, 1997 ; pp. 703–707.
4. Centers for Disease Control and Prevention. Recommendations for preventing the spread of vancomycin resistance: recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). MMWR Morb Mortal Wkly Rep; 44(RR-12), 1995; 1-13.
5. Lee A., Mirrett S., Reller L.B., et al. Detection of bloodstream infections in adults: how many blood cultures are needed? J.Clin. Microbiol, 2007 ; 45 (11): 3546–8.
6. Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing. Twentieth Informational Supplement. M100S20. (CLSI, 2010)
7. Huh J. Y., Lee W. G. and Jin H. Y. Molecular characterization of vancomycin-resistant Enterococci from clinical and surveillance specimens. Infect Control Hosp Epidemiol 27, 2006 ; 1076–1080.
8. Mihajlović-Ukropina M., Medić D., Jelesić Z. et al. Prevalence of different Enterococcal species isolated from blood and their susceptibility to antimicrobial drugs in Vojvodina, Serbia, 2011-2013; African Journal of Microbiology Research: Vol. 8(8), 2014 ; pp. 819-824.
9. Woodford N., Johnson A. P. and Morrison D.Current perspectives on glycopeptide resistance. Clin Microbiol Rev 1995 ;8:585–615.
10. El-Shafei M.A., Shaheen A.A., Mohamed L.A., et al. Bacteriological and molecular study on Enterococci clinically isolated from Zagazig University Hospitals. Thesis, Zagazig University, Faculyt of Medicine. 2008.
11. Nawara A.M.A., Gawish A., Samor S., et al. Glycopeptide resistant Enterococci, an emerging clinical proplem in Zagazig University Hospitals. Thesis, Zagazig University, Faculty of Medicine, 2003.
12. Abbadi S.H., El- Hadida G.S., Gomaa N.I. et al. Species identification and pattern of antibiotic resistance of Enterococci isolated from cases of nosocomial infections in Suez Canal University Hospitals. The 15th Conf. Of Egy. Soci. Med. Microbiol. 2007.
13. Vagnerova I., Sauer P., Kolar M., et al. Sources and pathways of spread ofvancomycin-resistant Enterococci in hemato-oncological patients.Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub150, 2006 ; 117–120.
14. de Perio M.A., Yarnold P.R., Warren J., et al. Risk factors and outcomes associated with non-Enterococcus faecalis, non-Enterococcus faecium Enterococcal bacteremia. Infect Control Hosp Epidemiol 2006 ; 27(1):28-33.

15. Arias C.A. and Murray B.E. Antibiotic resistant bugs in the 21st 1. Century - A clinical super-challenge. N Engl J Med; 2009 ; 360 : 439-43.
16. Ira P., Sujatha S., and Subhash C.P. Phenotypic & genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. Indian J Med Res 2013; 138(4): 549–556.
17. Sood S., Malhotra M., Das B.K., et al. Enterococcal infection and antimicrobial resistance. Indian J. Med. Res 2008; 128:111-121.
18. Arther M., and Courvalin P. Genetics and mechanisms of glycopeptide resistance in *Enterococci*. Antimicrob.Agents chemother; 1991; 37:1563-1571.Abelle-Horn M., Vogel U., Klare I., et al. Molecular epidemiology of hospital acquired vancomycin-resistant Enterococci. J. Clin. Microbiol 2006 ; 44: 4009 – 4013.
19. Alejandra C.C., Paula S.G. and Marisa M.R. Molecular epidemiology of vancomycin-resistant Enterococcus faecium in Argentina. International Journal of Infectious Diseases; 2007; 11: 69-75.
20. El-Hadidy M.B.; Risk M.S.; El shafei M.M., et al. revalence of nosocomial infection with vancomycin resistant Enterococci (VRE) in Mansoura University Hospitals (MUHS). Thesis, Mansoura University, Faculty of Medicine; 2000.
21. Cetinkaya Y., Falk P. , and Mayhall G.C. Vancomycin resistant Enterococci. Clin. Microbiol. Rev.; 2000 ; 13:686–707.
22. Surendra K., Leanne H., Gillian L., et al. Prevalence and risk factors for VRE colonisation ina tertiary hospital in Melbourne, Australia: a crosssectional study, 2012; 1:31.
23. Dutka-Malen S., Blaimont B., Wauters G., et al. Emergence of high-level resistance to glycopeptides in Enterococcus gallinarum and Enterococcus casseliflavus. Antimicrob Agents Chemother.; 1994 ; 38:50.
24. Camargo ILBC, Barth AL, Pilger K, et al. Enterococcus gallinarum carrying the vanA gene cluster: first report in Brazil. Braz J Med Biol Res; 2004 ; 37: 1669-71.
25. Arther M., and Courvalin P.Genetics and mechanisms of glycopeptide resistance in Enterococci. Antimicrob.Agents chemother; 1991; 37:1563-1571.