

Isolation of the vancomycin resistant enterococci from different sources**Khwan R. Al-Husseiny Thaar Ali Hussain Yahya A. Abass****Medical Dep- Al-Nassyria Technical Institute****Abstract**

A total of sixty five isolates of enterococci were isolated; thirty six isolates from clinical samples (stool, urine, vaginal swabs, intra-abdominal and pelvic wounds and blood cultures) and twenty nine isolates from hospital environment (operating theaters, bed wards, floor, medical instruments, and sewage).

The results revealed that 14 isolates (21.53%) were vancomycin-resistant enterococci including 8 isolates from clinical samples and 6 isolates from hospital environment. Moreover, 8 isolates (12.30%) showed intermediate resistance, and remaining (43) isolates are sensitive to vancomycin

Introduction:

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, *Enterococcus faecalis* and *Enterococcus faecium*, account for most clinical infections in humans[1]. With increasing antibiotic resistance, enterococci are recognized as feared nosocomial pathogens that can be challenging to treat [2].

Enterococcus species are hardy, facultative anaerobic organisms that can survive and grow in many environments. In the laboratory, enterococci are distinguished by their morphologic appearance on Gram stain and culture (gram-positive cocci that grow in chains) and their ability to : hydrolyze esculin in the presence of bile, grow in 6.5% sodium chloride, demonstrate pyrrolidonyl arylamidase and leucine aminopeptidase, and react with group D antiserum. They were formerly known as group D streptococci until assigned their own genus [3].

Vancomycin-resistant enterococci (VRE), first reported in Europe in 1988, are emerging as a global threat to public health, the incidence of VRE infection and colonization among hospitalized patients has increased rapidly in the last years [4]. From 1989, the year VRE was first identified in the United States, through 1993, the proportion of enterococcal isolates resistant to vancomycin reported to the National Nosocomial Infections Surveillance System (NNISS) increased 20-fold [5]. Infection with VRE may be associated with increased mortality, and no effective antimicrobial therapy is available for many VRE [6]. Enterococci are important nosocomial pathogens [7]. Their emergence in the past two decades is in many respects attributable to their resistance to many commonly used antimicrobial agents [8].

The aim of this study is to detect the prevalence of vancomycin resistant enterococci in clinical causes and in environment of hospital.

Materials and Methods:

The study was carried out from January to June 2008 at A-Nassyria general hospital .A total of sixty five of enterococci (36 isolates from clinical samples and 29 isolates from hospital environment) were isolated. The clinical isolates were collected from stool, urine, vaginal swabs, intra-abdominal and pelvic wounds and blood cultures. The environmental samples were collected from operating theaters, bed wards, floor, medical instruments, and sewage (table 1). Presumptive enterococci were identified on the basis of Gram stain, catalase reaction, grow in presence of bile and hydrolyze esculin (bile esculin-positive) and they grow in 6.5% NaCl [9]. Minimum inhibitory concentrations (MIC) of enterococci against vancomycin antibiotic was determined by standard broth dilution method, Mueller-Hinton broth, pH 7.2-7.4 was used as the test medium and inoculated with the turbidity adjusted suspension of isolate to achieve a final inoculum [10].The National Committee for Clinical Laboratory Standard (NCCLS) guidelines were followed ,which define enterococci for the MIC of vancomycin ≤ 16 $\mu\text{g/ml}$ to be susceptible, while isolates for which the MIC is 16 to 64 $\mu\text{g/ml}$ are intermediate resistance and those for which the MIC is ≥ 128 $\mu\text{g/ml}$ are resistant [11,12].

Results:

From 65 isolates of enterococci, 22 isolates (33.84%) were appeared vancomycin resistant (resistant and intermediate; 12 isolates from clinical sources and 10 isolates from hospital environment).

Fourteen isolates (21.53%) were vancomycin resistant enterococci, which MIC are ≥ 128 $\mu\text{g/ml}$. On the other hand, 8 (12.30%) isolates were showed an intermediate vancomycin resistant (VIRE) with MIC 16 to 64 $\mu\text{g/ml}$. Other isolates 43 (66.15%) were sensitive to vancomycin with MIC ≤ 16 $\mu\text{g/ml}$ (table 2).

In comparison for the data of MIC with disc diffusion method, the results showed that all of the 8 vancomycin intermediate isolates appear sensitivity to vancomycin by disc method.

Table 1: The sources and frequencies of isolates

1-Clinical samples	No.	%
Stool	12	18.46
Urine	8	12.30
Vaginal swabs	4	6.15
Intra-abdominal & pelvic wounds	10	15.38
Blood culture	2	3.07
2-Hospital environment samples		
Operating theatres	8	12.30
Bed wards	4	6.15
Floor	5	7.69
Medical instruments	2	3.07
Sewage	10	15.38
Total	65	100

Table 2: The MIC of vancomycin against enterococci from different samples

samples susceptibility	St*	Ur	IAW	VS	BC	OP	BW	Fl	MI	SW	Total	
											No	%
Resistance MIC \geq 128 $\mu\text{g/ml}$	3	2	2	1	-	2	1	1	-	2	14	21.53
Intermediate resistance MIC 16-64 $\mu\text{g/ml}$	2	1	1	-	-	1	1	1	-	1	8	12.30
Sensitive MIC \leq 16 $\mu\text{g/ml}$	7	5	7	3	2	5	2	3	2	7	43	66.15
Total	12	8	10	4	2	8	4	5	2	10	65	100

*St: Stool specimens; Ur: Urine; IAW: Intra-abdominal and pelvic wounds; VS: Vaginal swabs; BC: Blood cultures; OP: Operating theatres; BW: Bed wards; Fl: Floor; MI: Medical instruments; SW: Sewage; IMR: Intermediate resistance.

Discussion:

During the last years a number of studies have reported the presence of VRE in the environment. Although, only limited information is available regarding the possible connection between human VRE infections and potential environmental and animal reservoirs [4, 13]. The epidemiology of VRE has not been completely elucidated; we know that certain patients are at increased risk for VRE colonization or infection. These include critically sick patients, those with severe underlying disease or immunosuppressant, patients in the oncology or transplant wards, those with intra-abdominal or cardiothoracic surgical procedures, patients with indwelling urinary or central venous catheters, and those with prolonged hospitalization or receiving broad-spectrum antimicrobial and vancomycin therapy [5,14].

Resistance to vancomycin (MIC \geq 128 $\mu\text{g/ml}$) was found in 14 (21.53%) isolates, and 8 (12.30%) isolates as intermediate vancomycin resistant enterococci, this in agreement with other studies [4,15,16], which reported that VRE isolates herein described are showing the overuse of antimicrobials.

The study showed that six isolates of VRE were isolated from the hospital environment, while eight were isolated from the clinical samples. It was initially feared that enterococci would acquire the Van gene coding for vancomycin resistance from other species, this finding suggests that hospitals may be the sources of VRE and other factors may exert a selective pressure [4].

Since the VanA and VanB resistance genes are transferable, antibiotics resistance might be carried to other microorganisms like methicillin- resistant *Staphylococcus aureus*, as was reported by Tenover *et al.* [17], therefore, these strains could be very difficult to treat with the antimicrobial drugs currently available. Isolation of enterococci resistant to multiple antibiotics has become increasingly common in the hospital setting. Extreme precautions should be taken to avoid the spread of these strains. Education, permanent vigilance, and appropriate use of antibiotics are the foundations to minimize this problem [18,19].

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الخلاصة

عزلت ٦٥ عزلة من المكورات المعوية (36 عزلة من مصادر سريرية شملت عينات البراز، الادرار، مسحات المهبل، جروح البطن - الحوض وزروع الدم بالاضافة الى ٢٩ عزلة ماخوذة من بيئة المستشفى وشملت صالات العمليات، ردهات المرضى، الارضية، الادوات الطبية ومجاري المستشفى).

اظهرت النتائج ان ١٤ عزلة (21.53%) من المكورات المعوية مقاومة للفانكوميسين، ثمان عزلات من مصادر سريرية وست عزلات من بيئة المستشفى، بالاضافة الى ذلك اظهرت النتائج ثمان عزلات (٣٠، ٢٠%) كانت متوسطة المقاومة للفانكوميسين، وباقي العزلات (٣٠%) كانت حساسة للفانكوميسين.