Isolation and Identification of Vancomycin-Resistant *Enterococcus Faecalis*

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Abstract

One hundred thirty - five clinical specimens of urine, blood, teeth root canal and burns were obtained from patients in hospitals of Baghdad. The specimens were cultured on Pfizer Selective *Enterococcus* agar to purify Enterococci isolates. 20 *E. faecalis* isolates were identified biochemically by growing in 10Cº, 45Cº, 6.5% NaCl, at pH 9.6 and confirmed by VITEK. Determination of Vancomycin-Resistant *E. faecalis* isolates were done by the minimum inhibitory concentrations [MICs] using agar dilution method. Seventeen *E. faecalis* isolates were determined as Vancomycin-Resistant and Intermediate Resistant.

Keywords: Pfizer Selective *Enterococcus* agar, VITEK, Vancomycin-Resistant *E. faecalis*.

عزل وتشخيص بكتريا المكورات المعوية البرازية المقاومه لمضاد الفانكومايسين

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الخلاصة:

مائة وخمس وثلاثين عينة سريرية من الادرار والدم وقنوات جذر الأسنان واصابات الحروق تم الحصول عليها من المرضى في مستشفيات بغداد. وقد تم زرع العينات المأخوذه على الوسط الزرعي الصلب فايزر الاختياري لبكتريا المكورات المعويه لغرض تنقيه عزلات *E. faecalis*. بكتريا المكورات المعويه البرازية تم تشخيصها من خلال تنميتها عند درجة حرارة 10 و 45 مئوية، تنميتها في وسط مغذي يحتوي 6.5 بالمئة من كلوريد الصوديوم وايضا تنميتها في الاس الهيدروجيني القاعدي 9.6 بالاضافة الى ذلك تم تشخيصها بواسطة الفايتك. عشرون عزلة من المكورات المعوية البرازيه تم تشخيصها. عزلات المكورات المعوية البرازية المقاومة لمضاد الفانكومايسين تم تحديدها بطريقة التركيز المثبط الادنى بواسطة عملية التخفيف الزرعي. سبعة عشر عزلة من المكورات المعويه البرازية تم تحديدها كعزلات مقاومة أو متوسطه المقاومة لمضاد الفانكومايسين.

Introduction

Enterococci organisms are Gram positive cocci, spherical or ovoid in shape [0.6-2.5µm], usually occurring in pairs or short chains in broth culture [1]. They are diverse and versatile group of bacteria with several intrinsic characteristics that allow them to survive and grow under a variety of conditions and a remarkable metabolic adaptability in order to fulfill diverse roles as commensals and as opportunistic pathogens [2]. They are able to cause a variety of infections in humans and are now recognized among the major etiological agents of nosocomial infections associated with limited

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therapeutic options, due to their ability to acquire resistance to most of the clinically relevant antimicrobial agents [3].

Among the Enterococcal species described *E. faecalis* represented one of the most important causes of nosocomial infections, since it is responsible for 80 - 90% of human Enterococcal infections [4-6], and cause a wide range of diseases such as; bacteremia, surgical wound infection, endocarditis, urinary tract infections, intra-abdominal and central nervous system infections [7].

The general interest for *E. faecalis* and treatment of Enterococcal infections has increased due to the appearance of antibiotic multi resistant strains [8]. One of the major reasons why these organisms have survived in the hospital environment is their intrinsic resistance to several commonly used antibiotics, perhaps more important, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons [9]. Frequently identified risk factors for Vancomycin-Resistant *E. faecalis* colonization and infection include prolonged hospital stays, exposure to intensive care units, transplants, hematologic malignancies, and exposure to antibiotics [10].

Orally administered vancomycin was a widely used treatment for *Clostridium difficile* colitis. Consumption of huge quantities of glycopeptides was also occurring in an entirely different population; specifically, avoparcin [another glycopeptide drug] was being used as a growth promoter in food animals. This use of a glycopeptide at sub therapeutic concentrations in animals may have played a role in the development of acquired vancomycin resistance in Enterococci [11]. Therefore this study was undertaken with an aim to isolate and identify vancomycin resistant *Enterococcus faecalis* from clinical specimens.

Materials and Methods

Between September 2013 and December 2013, one hundred thirty - five clinical specimens were collected from urine, blood, teeth root canal and burns of patients' suffering from urinary tract infection, bacteremia, endodontic infections and burns infections, respectively Table-1. They were obtained from Al-Kindy Teaching hospital in Baghdad, the Central Health Laboratories and Educational Laboratories/Medical City in Baghdad.

The collected specimens were streaked on Pfizer Selective *Enterococcus*; and incubated at 37°C for 24 hr.

Isolates were identified to the genus level based on the standard biochemical and microbiological methods such as: morphologic appearance after staining by Gram stain, catalase test, testing their ability to hydrolyze Esculin in the presence of bile, growth in the presence of 6.5% NaCl at 45°C and pH 9.6.

VITEK was employed for *E. faecalis* isolates confirmation. The isolates cultured on Pfizer selective *Enterococcus* agar then incubated at 37 ºC for 24hr. the isolated colonies were loaded in the VITEK gram positive kit.

Table 1- Number and Nomenclature of bacterial isolates.

specimens	No. of specimens	*E. faecalis* isolate symbol
Urine	15	U
Blood	60	B
Root canal	50	R
Burns	10	W.i

Determination of minimum inhibitory concentrations [MICs] of Vancomycin.

Minimum inhibitory concentration [MIC] is the lowest concentration that inhibits the visible growth of bacteria. The MIC was determined to all *E. faecalis* isolates for the Vancomycin antibiotic. This test was achieved according to Morello *et al*. [12].

The value of MIC of each VRE isolate for vancomycin included the two-fold agar dilution susceptibility. Susceptibility test results were assessed after 24-48 hr incubation at 37°C. The MIC values were based on break point recommended by CLSI [13], for the estimation of response. For Vancomycin, 1-4µg was sensitive, more than 4µg isolate was considered as an intermediate resistant and ≥ 32µg considered as resistant.

Results and Discussion

Isolation and Identification of *E. faecalis*

Twenty isolates of the genus *Enterococcus* were isolated from 135 clinical specimens have the ability to grow on Pfizer selective *Enterococcus*. The highest numbers of isolates were distributed among urine specimens and the lowest one was observed among wound infection specimens. Pfizer selective *Enterococcus* considered the selective medium for the isolation and identification of *Enterococcus* which has the ability to discriminate Enterococci from specimens containing multiple microbial components, since it contains sodium azide and sodium citrate which have great inhibitory effect associated bacterial flora [14, 15].

All the isolates were examined microscopically; the isolates were identified as Gram positive cocci. Cells were spherical or ovoid, arranged singly, in pairs or in short chains, non-spore former [16, 17].

Increasing the selectivity medium was done by incubation at 45ºC [18]. All the bacterial isolates were related to the genus *Enterococcus* gave negative results for catalase test, they have ability to grow in 6.5% NaCl and at pH 9.6 with incubation at 10 and 45ºC table-2.

Table 2-Biochemical tests results of *E. faecalis* identification**.**

Test	20 isolates
Esculine Hydrolysis	+
Catalase	-
Oxidase	-
Growth at [10 and 45]ºC	+
Growth at 6.5% NaCl	+
Growth at pH 9.6	+

VITEK were employed to confirm the presence of *E. faecalis* isolates, regarding samples' type and isolated *Enterococcus* spp., there were a 20 isolates identified, they were demonstrated in table-3.

Table 3-Numbers and percentages of E. *faecalis* isolates from clinical specimens**.**

Sample type	No. of samples	*E. faecalis*	% of *E. faecalis* from each source
Urine	15	7	46.6%
Blood	60	5	8.3%
Root canal	50	7	14%
Burns	10	1	10%
Total	135	20	78.9%

E. faecalis isolates from urine in this study was 46.6%, Alebouyeh *et al.* [19] showed that the percentage of *E. faecalis* isolates from urine was 75%. Al-jmor [20] found that the percentage of *E. faecalis* isolates from urine was 20.6%.

For blood specimens, the percentage of *E. faecalis* isolates were 8.3%, this result was compatible with the results by Tellis and Muralidharan [2]; they showed that percentage of *E. faecalis* isolated from blood was 18%. While other studies reported by Al-Jarousha *et al.* [21], AL-khafaji *et al.* [22] and Mira *et al.* [23] revealed that the percentage of *E. faecalis* isolated from blood were 3%, 0% and 55.05%, respectively.

The percentage of *E. faecalis* isolated from root canal specimens was 14%, this result was higher than the result of local study obtained by Mahmoudpour *et al.* [24], who showed the percentage of *E. faecalis* isolated from root canal was 10%, another studiy by Zoletti *et al.* [25] and Preethee *et al.* [26], showed that the percentage of *E. faecalis* isolated from root canal was 80% and 46.87%, respectively.

The percentage of *E. faecalis* isolated from wounds was 10%, this result was compatible with the

result of Giacometti *et al.* [27] and Al-Jarousha *et al.* [21], who indicated that the percentage of *E. faecalis* isolated from wounds was 5.6%, 1.9%, respectively.

The differences between isolation percentages may be related to the number of specimens, the differences in the source of isolates, hospitals included in each study, their geographical regions and differences in the identification methods.

Determination of Vancomycin susceptibility

Vancomycin susceptibility was determined by the minimum inhibitory concentration [MIC] for all *E. faecalis* isolates, according to Clinical and Laboratory Standards Institute [CLSI], if the MIC ≤ 4 µg / ml then the isolate is sensitive, MIC 8–16 µg/mL the isolate have intermediate resistance and if the MIC ≥ 32 µg/ml the isolate is resistant to vancomycin.

The MICs result of vancomycin for *E. faecalis* isolates were indicted in the table-4.

Table 4-The Minimum Inhibitory Concentrations [MICs] of Vancomycin for *E. faecalis* isolates.

Id	isolates	specimen	MIC[µg/ml]	susceptibility
1	1U	urine	32	R
2	2U	urine	4	S
3	3U	urine	64	R
4	4U	urine	32	R
5	5U	urine	64	R
6	6U	urine	64	R
7	7U	urine	32	R
8	3B	Blood	64	R
9	4B	Blood	4	S
10	6B	Blood	16	IR
11	7B	Blood	128	R
12	8B	Blood	16	IR
13	1w.i	Wound	4	S
14	1R	Root canal	16	IR
15	2R	Root canal	32	R
16	3R	Root canal	64	R
17	4R	Root canal	128	R
18	5R	Root canal	32	R
19	6R	Root canal	64	R
20	7R	Root canal	16	IR

S: Sensitive, **IR:** Intermediate Resistant, **R:** Resistant.

Results of Vancomycin sensitivity test obtained by this study showed that from 20 isolates, 13 isolates [65%] were resistant to Vancomycin, 4 isolates [20%] were intermediate resistant and 3 isolates [15%] were sensitive table-5.

Table 5-Vancomycin susceptibility percentages of *E. faecalis* isolates from each source**.**

specimens	VSEF	VIEF	VREF
urine	1	——	6
Blood	1	2	2
Root canal	——	2	5
Burns	1	——	——
Total	3	4	13

VSEF: Vancomycin Sensitive *E. faecalis*,
VIEF: Vancomycin Intermediate *E. faecalis*
VREF: Vancomycin Resistant *E. faecalis*.

In a study reported by Fatholahzadeh *et al.* [28] stated that 38% of *E. facalis* isolates were resistant to vancomycin. While Camargo *et al.* [29] demostrated that 20.8% of *E. faecalis* isolates were resistant to vancomycin and 79.1% of isolates were sensitive. The results of this study was close to the results

of Chabuck *et al.* [30], Al-jmor [20] and Praharaj *et al.*[31], who showed that the percentages of vancomycin resistant were 71.43%, 50% and 90.6%, respectively.

The spread of antimicrobial resistant among Enterococcal species in Iraq has presented a serious challenge for medical community, unfortunately; treatment failures in Enterococcal infections are on the rise because of the lack of adequate information regarding antimicrobial resistance especially glycopeptide resistance among endemic Enterococci. Such information is required for appropriate treatment of patients with Enterococcal infections, which rank among the third common cause of bacteremia and the second frequent cause of UTI [9, 32].

A major reason for the survival of *Enterococcus* in hospital environment is their intrinsic resistance to several commonly used antibiotics and, perhaps more important, their ability to acquire resistance to all currently available antibiotics, either by mutation or through the transfer of plasmids and transposons, Five phenotypes of vancomycin resistance; termed van A ،van B, van C, van D, and van E are known. The van A and van B phenotypes are clinically significant as these phenotypes can be induced by vancomycin use.

VanA and VanB, encoded by two distinct gene clusters, the *vanA* and *vanB* clusters, respectively, which are carried on transposons *Tn1546* and *Tn1547*, respectively [30-34]. Therefore; it's easily to transfer the high resistance to other organisms especially vancomycin resistant, as long as; the sources of resistance hereditary were carried on plasmids and transposons.

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