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Isolation, identification and treatment of Vancomycin-Resistant *Staphylococcus epidermidis*

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Abstract

Out of Hundred clinical samples, taken from different sources include burn, blood , wound and nasal swabs infections ; 90 isolates developed growth on mannitol salt agar. Among these, 40 (44.4%) were Coagulase positive (*Staphylococcus aureus*) isolates and 50 (55.5%)belong to coagulase negative staphylococci, in which the last *Staphylococcus epidermidis* isolates were 30(60%).Antibiotic susceptibility of *Staphylococcus epidermidis* isolates to 12 antibiotics were determined using disc diffusion method . The results revealed that high resistance to Penecillin G10 and Amoxiclav (Amoxicillin- clavulanic acid) (100%) and the high sensitivity to Imipenim (95%). The pattern of minimum inhibitory concentration of *S.epidermidis* isolates to vancomycin , was determined using MIC method; The results revealed that 12 *S.epidermidis* isolates (40 %) were vancomycin resistant, the MIC of them between (256- 32 µg\ml) , 4 *S.epidermidis* isolates (13.3%) were intermediate resistance , the MIC to 3 of them were 16 µg\ml and the last was 8 µg\ml.

Keywords: *Staphylococcus epidermidis*, vancomycin resistant, VRSE , VISE, VSSE.

عزل وتشخيص ومعالجه بكتريا المكورات العنقودية البشروية المقاومة لمضاد الفانكومايسين

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الخلاصة:

من مجموع مئة عينة سريرية جمعت من مصادر مختلفة شملت الحروق والدم والجروح ومسحات انفية، تمكنت 90 عزلة من النمو على اكار المنيتول الملحي ،ومن بينها 40(44.4 %) عزلة عائدة للبكتريا المنتجة للانزيم المخثر للبلازما و 50 (55.5%)عزلة عائدة للبكتريا الغير منتجه للانزيم المخثر للبلازما والتي بلغت حوالي 30(60%)عزلة عائدة للمكورات العنقودية البشروية، تم تحديد اختبار الحساسية لعزلات المكورات العنقودية البشروية لاثنى عشر من مضادات الحياة باستخدام طريقة الانتشار بالاقراص ،اظهرت النتائج ان المقاومة كانت100% لمضاد البنسلين والاموكسيكلاف ،وبينت حساسيتها الى مضاد الامبينيم بنسبة 95%. وتم تحديد اختبار التركيز المثبط الادنى لعزلات المكورات العنقودية البشروية لمضاد الفانكومايسين، واظهرت النتائج ان 12(40%) عزلة مقاومة للفانكومايسين وكانت قيم التركيز المثبط الادنى تتراوح بين(256–32 مايكروغرام /مل) ، 4عزلات (13.3) كانت متوسطة المقاومة للفانكومايسين وبلغت قيم التركيز المثبط الادنى لثلاث عزلات منها 16مايكروغرام /مل والاخيرة منها 8مايكروغرام /مل .

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Introduction

The genus *Staphylococcus* consists of gram-positive cocci, (0.5 to 1.5µm) in diameter, usually in irregular clusters, with in which pairs and tetrads are commonly seen [1]. They are nonmotile and nonsporing . Members of this genus are facultatively anaerobic. Colonies are round, convex, mucoid, and adherent to the agar. They are chemoorganotrophic, requiring nutritionally rich media [2]. Staphylococci are common commensals and opportunistic pathogens mainly found on the skin and in the nose of humans and also in domestic and companion animals [3].The Staphylococci are a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteremia. In spite of large-scale efforts to control their spread, they persist as a major cause of both hospital and community acquired infections worldwide [4].

General characterization of *S. epidermidis* is unable to produce coagulase, mannitol non fermentor, pigment of colony is white, opaque and smooth colony on blood agar, susceptible to novobiocin [5,6], *S.epidermidis* is one of primary opportunistic pathogens associated with indwelling medical devices and ability to form biofilm on surface of implanted medical device [7].

The biofilm that is formed at the interface between bone and the prosthesis shows resistance to both the host defense mechanisms and antimicrobial therapy [8].

Resistance to vancomycin among coagulase-negative staphylococci was first reported more than 20 years ago. However, the first report of a clinically significant isolate was in 1987 [9]. Vancomycin resistance staphylococci (VRS) is important as it indicates that these organisms can become endemic in a health care setting **[**10**] .** *S. epidermidis* has been found to be the commonest CoNS species associated with glycopeptide resistance [11].This study aims to isolation and identification and treatment of vancomycin resistant *Staphylococcus epidermidis* in Baghdad community.

Materials and Methods

Specimen collection

One hundred clinical specimens referring to Flow Catheter,Burn,blood and wounds swabs,were collected from patients attending to Baghdad teaching Hospital,Laboratery teaching of Madienat AL-Teb and AL-Kindy teaching Hospital for the period from August 2013 to December 2013. The specimens were collected by the attending physician and health officer using sterile applicator stick with cotton swabs moistened with normal saline and test tubes were used to collect the sample as inTable-1.

Table 1-Number of bacterial specimens.

Source of isolates	No. of specimens
Blood	30
Nasal swabs	25
Wounds swabs	15
Burns swabs	10
Catheters	20
Total	100

Isolation of staphylococci from different clinical sample by specific way depending on routine laboratory techniques. All specimens were streaked on mannitol salt agar for detecting on mannitol fermentation and Blood Agar for detecting hemolysis type. Thereafter, all plates were incubated aerobically for 24 h at 37°C.

The *S. epidermidis* isolates were identified depending on the morphological features on culture media and biochemical tests according to Bergey's Manual and API Staph system [6]. Was used as finally step of diagnosis

Antimicrobial susceptibility test

This was test performed by modified Kirby-Bauer method[12] Susceptibility test were determined for all *S.epidermidis* isolates to 12 different (Amoxiclav, Ceftazidime, Ciprofloxacin, Clindamycin, Erythromycin, Gentamycin, Imipenim, Penicillin G, Tetracyclin Refampin, Methicillin and Vancomycin) antibiotics by disc diffusion method recommended by National Committee for clinical laboratory standards (NCCLs) [20].

Determination of minimum inhibitory concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the lowest concentration that inhibits the visible growth of bacteria. The MIC was determined to antibiotic Vancomycin. This test was achieved

according to Morello [12]. The value of minimum inhibitory concentration (MIC) of each VRSE isolate for vancomycin included the two-fold 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 NCCLS [13], for estimation of the response. For Vancomycin (1-4µg) is sensitive, more than 4µg isolate is considered as intermediate resistant and (≥ 32µg) considered as resistant.

Results and discussion

Isolation and Identification of *Staphylococcus epidermidis*

Nineteen isolates (90%) from 100 sample had the ability to grow on the Mannitol salt agar which considered selective and differential media for genus *Staphylococcus* [5]. *Staphylococcus* appeared as round, smooth, raised, mucoid and glistening**,** *S.epidermidis* were non mannitol-fermenter and appeared as pink and small colonies and no colour change was on the medium. The higher numbers of isolates were distributed among nasal carrier specimens and the lower one were distributed among burns specimens. Microscopic examination was applied to all 90 isolates after staining by
Gram stain to detect their response to stain, the cells appeared as Gram positive cocci mostly arranged in grape-like irregular clusters. [14]. The prevalence of *Staphylococcus* varied among collected specimens, depending on the sources of isolation and type of clinical specimens as shown in Figure-1.

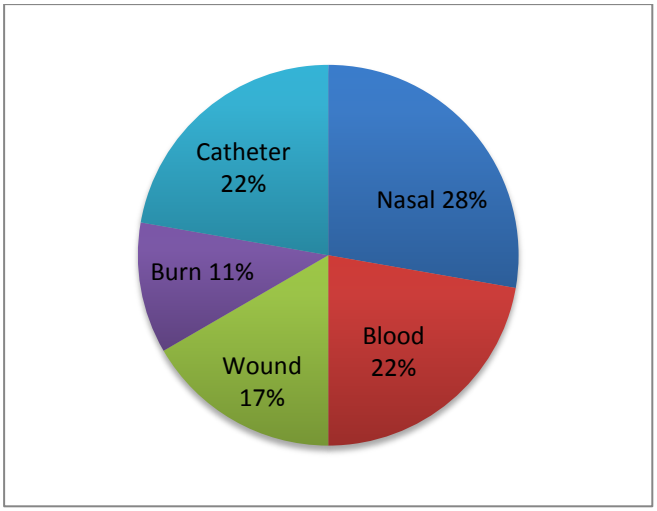

Figure 1- Distribution of *Staphylococcus* isolates according to the source of isolation

This study agreed with AL-Azzawi [17] which indicate the nasal cavity were larger source for isolation of staphylococci ,nasal cavity considered the major reservoir for staphylococci . Coagulase test used for distinguish between group of Staphylococci that produce coagulase enzyme coagulase positive Staphylococci(CoPS) and other group that does not produce it (CoNS), the CoPS were 40(44.4%) isolates and the CoNS were 50(55.5) isolates .
For more purification, culturing of isolates on Blood Agar Media[5], White opaque and smooth colony indicate Culture of *S. epidermidis* isolates as shown in Figure-2.

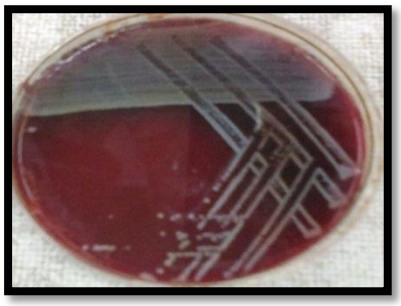

Figure 2- colonies of *S.epidermidis* on Blood Agar Medium

White colony was observed on skim milk agar as a result of of *S. epidermidis* isolates on this medium as shown in Figure-3.

Table 2-The biochemical and tests of 30 *S.epidermidis* isolates.

Biochemical test	Results
Mannitol salt agar	100% Pink colonies
Blood agar	100% White colonise
Milk agar (pigmentation)	100% White colony
Catalase	100% Babbles (+)
Oxidase	100% No Purple color (-)
Coagulase	100% (-)

(+): positive; (-): Negative

Finally the Analytical Profile Index API Staph system was used for precise and accurate identification of the isolates at generic and species and typing of *S. epidermidis* isolates as in Figure-3, which previously identified by conventional biochemical tests ,the obtained results from API Staph system were in agreement with those obtained from biochemical identification.

Figure 3- Results of API Staph. of *S. epidermidis*

The CoPS among 90(100%) staphylococcal isolates were 40(44.4%) and CoNS were 50(55.5%) in which the *S.epidermidis* were 30(60%) isolates.
The study agreed with study of Abd-Elateef [16] which indicate the nasal cavity rich source for isolation of *S.epidermidis* isolates as shown in Table-3. Kloos and Bannerman [15] indicated blood in case of bacterimea infection contain high prevlance of S.*epidermidis* were (50%), *S. epidermidis* is an important cause of bacteremia and has been correlated with the increase in the use of prosthetic and indwelling devices and the growing number of immunocompromised patients in hospitals.

Table 3- percentage of isolation *S.epidermidis* from source of isolation

Specimen type	No. of samples	Staphyllococci No.(%)	*S.epidermidis* No.(%)
Nasal swab	25	25(27.7%)	11(36.6%)
Blood	30	20(22.2%)	10(36.6%)
Wound	15	15(16.6%)	4(13.3%)
Burn	10	10(11.1%)	2(6.6%)
Catheter	20	20(22.2%)	3(10%)
Total	100	90(100%)	30(100%)

The results of presence *S.epidermidis* in wound were (13.3%) ,catheter(10%) and burn (6.6%) same results of Eftekhar and Mirmohammad [18] were formed from wound(14%) then from catheter(8%) but Perveen *et al*., [19] which indicate the least number of isolates from blood (9.71%) .

Antimicrobial susceptibility test

The results showed that all *S.epidermidis* isolates resistant to Penecillin G10, Amoxiclav (Amoxicillin- clavulanic acid) 100%,the resistance to Methicillin were 93%, Erythromycin were 90%, ceftazidime , Gentamycin and Clindamycin were 70%, Tetracyclin were 75% , Ciproflaxacin were 60%, Rifampin 30%. *S.epidermidis* isolates showed sensitivity to Imipenim 95% and intermediate resistant 5%, while 90% of isolates were sensitive to vancomycin as shown in Figure-4.

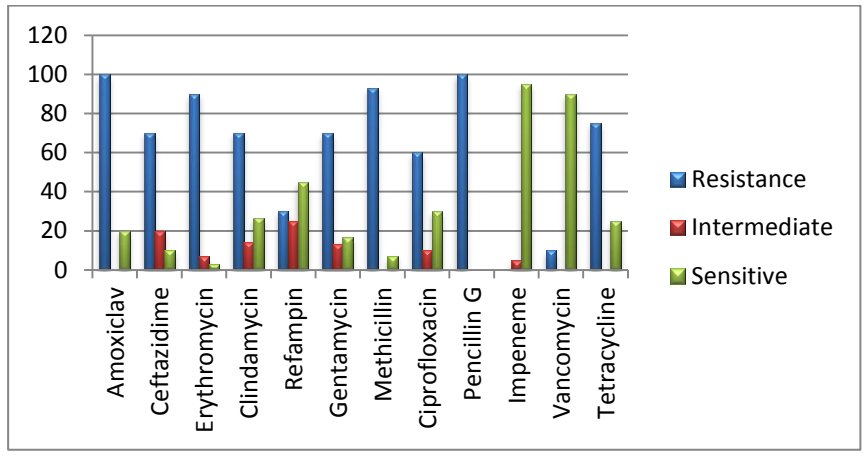

Figure 4- percentage of Antibiotic susceptibility for *S.epiermidis* isolates.

Determination of minimum inhibitory concentration (MIC)

Vancomycin susceptibility was determined by the minimum inhibitory concentration (MIC) for all *S.epidermidis* isolates, according to Clinical and Laboratory Standards Institute [20], 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 *S.epidermidis* isolates were indicted in the Table-4.

Table 4- The Minimum Inhibitory Concentrations (MICs) of Vancomycin for *S.epidermidis* isolates.

No.of*S.epidermidis* isolate	MIC mg/ml		Isolation source
8	64-256	R	Blood
3	32	R	Catheter
1	32	R	Burn
2	16	IR	Blood
2	8-16	IR	Wound
1	4	S	Burn
1	4	S	Catheter
2	4	S	Wound
10	2-4	S	Nasal

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

Results of Vancomycin sensitivity test Obtained by this study shown that from 30 isolates, 12 isolates (40%) were resistant to Vancomycin, 4 isolates (13.3%) were intermediate resistant and 14 isolates (46.6%) were sensitive Table-4.

According to CLSI [20] doesnot rely on the disc diffusion method to determine vancomycin-resistance isolate of *S.epidermidis*,therefore ;MIC required for detection on VRSE, distribution of VRSE, VISE and VSSE .

On the other hand, the CoNS population is clearly shifting toward greater resistance to glycopeptides, probably as a result of pressure due to the increase in use of these molecules [10]. 42% of CoNS isolates were intermediately resistant (MIC 6.25 µg /ml) to vancomycin. Natoli *et al.*, [11] reported a

prevalence of 5.4% of CoNS with reduced susceptibility to glycopeptides,the isolates in this study showed resistance to vancomycin, the commonly used antimicrobial agents that were tested against them; a finding that is quite alarming and different from other studies reported in other literatures that refer to sensitivity towards vancomycin depend on result of sensitivity test (disk method) .

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