



Isolation and Identification of Vancomycin-resistant *Staphylococcus aureus*

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Abstract

Susceptibility of thirty seven clinical isolates of *Staphylococcus aureus* to various antibiotics was tested. 100 % of tested isolates were resistant to ampicillin, while the lowest resistance recorded to amikacin 8.10 %. Four of *S. aureus* isolates showed resistant to vancomycin. Minimum inhibitory concentration (MIC) of isolates 33 and 56 for vancomycin was $\geq 32 \mu\text{g/ml}$.

Keywords: *Staphylococcus aureus*, Vancomycin-resistant *staphylococcus aureus*, Antibiotic sensitivity test, MIC test. Vitek,

عزل وتشخيص المكورات العنقودية الذهبية (*Saphylococcus aureus*) المقاومة للفاونكوميسين

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الخلاصة

أختبرت حساسية 37 عزلة مرضية من *S.aureus* الى مختلف المضادات. 100 % من العزلات المختبرة كانت مقاومة للأمبسيلين ، بينما اقلها مقاومة للأميكاسين سجلت 8.10% . أربعة من عزلات *S.aureus* أظهرت مقاومة للفاونكوميسين. ألتركز المثبط الادنى للعزلة 33 و56 للفاونكوميسين كانت $\geq 32 \mu\text{g/ml}$.

Introduction

The Staphylococci are a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteraemia. In spite of large scale efforts to control their spread, they persist as a major cause of both hospital and community acquired infections worldwide. The two major opportunistic pathogens of this genus are *Staphylococcus aureus* and *Staphylococcus epidermidis* [1]. Because of the spread of multi-drug resistant Gram-positive bacteria as well as methicilin resistant *S. aureus* (MRSA), glycopeptides antibiotics, vancomycin and teicoplanin were used to treat severe staphylococcal infections[2].The first clinical Vancomycin-resistance *S.aureus* (MIC $\geq 32 \mu\text{g/mL}$) was reported from Michigan, USA in 2002 [3].

Aims of study: Investigation the distribution of Vancomycin resistance Staphylococci among patients and carriers (workers in the hospitals and restaurants) in the community. Studying of the susceptibility of Vancomycin resistance Staphylococci to other antimicrobial agents. Studying the effect of vancomycin on bacterial autolysis assay. Detection VanA gene by PCR.

Material and Methods

Isolation of bacteria

Fifty-six clinical swabs specimens (from September to November 2012) were taken from patients at Al-Kindy Teaching Hospital, Baghdad, Iraq. All collected samples were inoculated on mannitol salt agar and blood agar, incubated at 37°C for 24 hours[4]. Thirty-six specimens (67 %) were identified as *S.aureus* and eighteen specimens (33%) were identified as coagulase negative staphylococci.

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Identification of isolates

All collected samples was identified according to the morphological features on culture medium and biochemical test.[5]. VITEK2 were employed for the result confirmation. The identification of *S. aureus* was performed by direct inoculating of the clinical samples on mannitol salt agar (MSA) at 37°C for 24 hr., the positive result will convert the media from red to yellow color (because of its component: the mannitol sugar and the phenol red as an indicator). ABC streaking method on brain-heart infusion (BHI) agar was performed to purify and to make pure colonies, then re-inoculated onto MSA to confirm the results. All isolates that gave a positive result in mannitol fermentation. Then loaded vitek kit by samples (suspension cells in 5 ml normal saline) and put it in VITEK. Read the result after (9 hr).

Susceptibility to antibiotics

Antibiotic susceptibility was determined by disk diffusion on Mueller-Hinton agar based on Clinical and Laboratory Standards Institute guidelines [6] The antibiotics used for disc diffusion assays included vancomycin(30 µg/ml),methicillin(5 µg/ml),amikacin(30 µg/ml),erythromycin(15 µg/ml), amoxicillin (30 µg/ml),cephotaxim(30 µg/ml),tetracycline(10 µg/ml) and ampicillin(10 µg/ml).They were purchased from (Bioanalyse and Himedia) and were consistently tested for efficacy against the isolates.

Minimum Inhibitory Concentration Determination (MIC)

The MIC was determined by broth macro-dilution assay. A set of test tubes with different concentrations of antibiotics with the same volume were prepared. Tubes were inoculated with the test microorganism of 10^8 CFU/ml (0.5McFarland standard). After incubation of *S.aureus*, susceptibility test was done for *S. aureus* (4 isolates), tubes were examined for changes in turbidity as an indicator of growth. The first test tube that appeared clear was considered as MIC. This test was achieved according to Morello *et al.* (2006) [7] as the following :

- 1- Sterile tubes of Mueller-Hinton broth, each tube contained 2ml of sterile Mueller-Hinton broth.
- 2- A serial of two-fold dilutions of antibiotics were prepared by adding of 2ml of antibiotic stock solution (2000 µg/ml) to the first tube of Mueller-Hinton broth, mixed the contents, then 2ml transferred from this tube into a second tube, mixed the contents of the second tube and transferred of 2ml to a third tube. The dilution process was continued until reach the last tube. After the contents of the last tube mixed well, discarded 2ml of broth, so that the final volume in all tubes was 2ml.
- 3- From the Nutrient agar plate culture of bacterial isolate the suspension of organism was prepared in 5ml of normal saline that equivalent to McFarland 0.5 (10^8 CFU/ml)standard.
- 4- With a sterile pipette, 0.1ml of the bacterial suspension was transferred to the each of the serial of antibiotic broth tubes.
- 5- Each tube was shaken gently to mix the tube contents and placed in the incubator at 35°C for 18-24 hours.
- 6- The experiment was included the following control tubes:
 - A tube contained sterile broth (Sterility control).
 - A tube contained broth and bacterial isolate (Growth control).
 - A tube contained antibiotic and sterile broth.
- 7- After the incubation the tubes were examined for the presence or absence of turbidity, the lowest concentration that inhibits the visible growth of bacteria was determined as MIC.

Results and discussion

Fifty-six clinical swabs(nasal,burn,wound) specimens were taken from patients at Al-Kindy Teaching Hospital, Baghdad, Iraq. Thirty-seven specimens (66 %) were identified as *S.aureus* and nineteen specimens (34%) were identified as coagulase negative staphylococci.

Staphylococcus aureus isolates were identified depending on micro-scopical properties as well as biochemical tests as in table-1.

Table 1- biochemical and microscopical tests and their results for 37 *S.aureus*.

Tests	Results	Percentage %
Mannitol fermentation	Yellow colonies	100% positive
Gram stain	Cocci(grape-like clusters)	100% positive
Catalase	Bubbles formation	100% positive
Oxidase	Negative	100% negative
Coagulase	Clots	100% positive

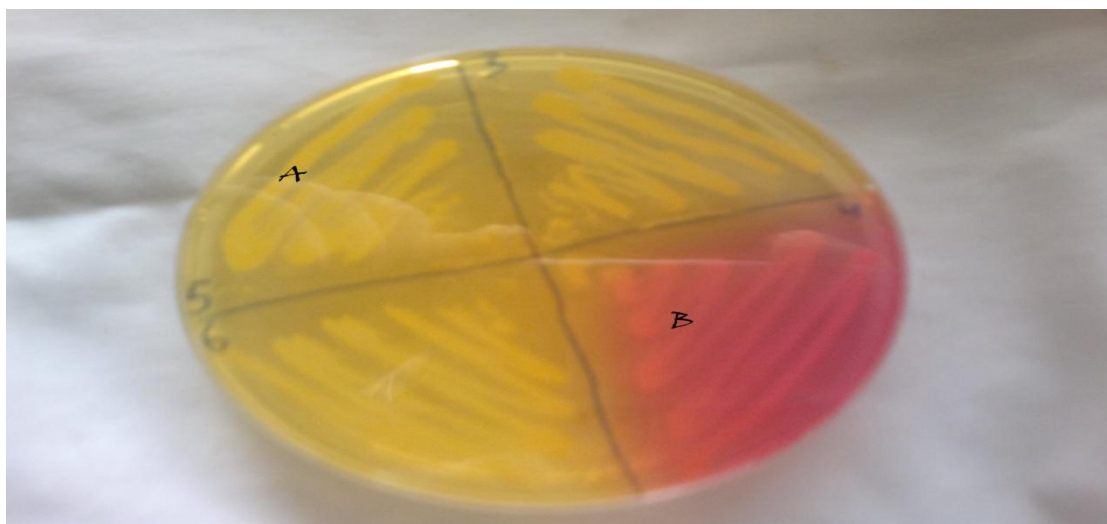


Figure 1-Staphylococcus ssp. On manitol salt agar (A) *S.aureus* (B) CONs.

VITEK2 were employed for the result confirmation. The identification of *S. aureus* was performed by direct inoculating of the clinical samples on mannitol salt agar (MSA) at 37C° for 24 hr., the positive result will convert the medium from red to yellow color. ABC streaking method on brain-heart infusion (BHI) agar was performed to purify and to make pure colonies, then re-inoculated onto MSA to confirm the results. All isolates that gave a positive result in mannitol fermentation were loaded vitek kit by samples (suspension cells in 5 ml normal saline) and put it in VITEK. Read the result after (9 hr).

After the identification of *S. aureus*, susceptibility test was done for all *S. aureus* (56 isolates) by Disk diffusion method to examine 8 different antibiotics with the using minimum inhibitory concentration (MIC) broth dilution method to determine the susceptibility of all isolates of staphylococci to vancomycin as recommended by CLSI (2011) [8]. Given that, the disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, all of which give similar size zones of inhibition. MIC tests should be performed to determine the susceptibility of staphylococcal isolates to vancomycin [8].

From the results of the present study, various levels of susceptibilities to different antibiotics among isolates were observed. The results are summarized in Figure-2.

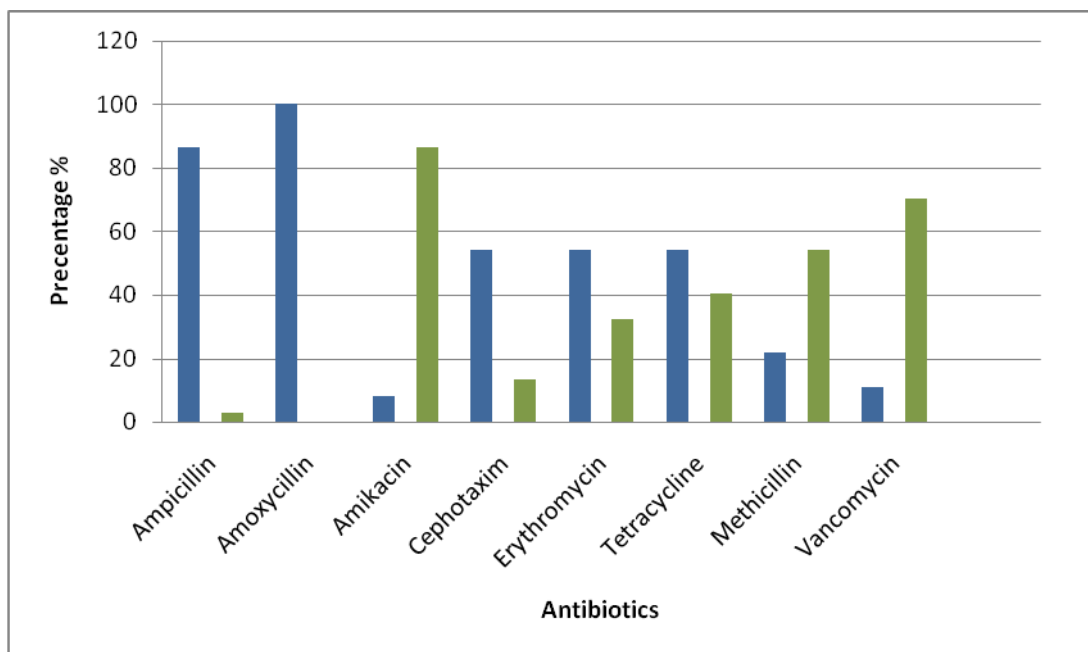


Figure 2- Antibiotic sensitivity of *S.aureus* isolates.

Figure-2 showed that *S.aureus* isolates were resistant to amoxicillin(100%),ampicillin(86.4%), amikacin(8.1%),erythromycin(54%),tetracycline (54%), cephotaxim (54%),methicillin(21.6%), and vancomycin (10.8%). In addition to that the incidence of the vancomycin-resistant *S.aureus* strain (VRSA), vancomycin intermediately susceptible *S. aureus*(VISA) and vancomycin susceptible *S. aureus*(VSSA) was (10.8%), (10.8%) and (72.9%) respectively. The highest level of sensitivity was observed with amikacin (86.4%) followed by vancomycin (72.9%). The lowest was observed with ampicillin (2.7 %).

Among the 37 clinical isolates of *S.aureus*, 8 (21%) were identified as methicillin resistant *S. aureus* (MRSA) and 4(10.8%) vancomycin resistant *S.aureus* (VRSA) by disc diffusion method.

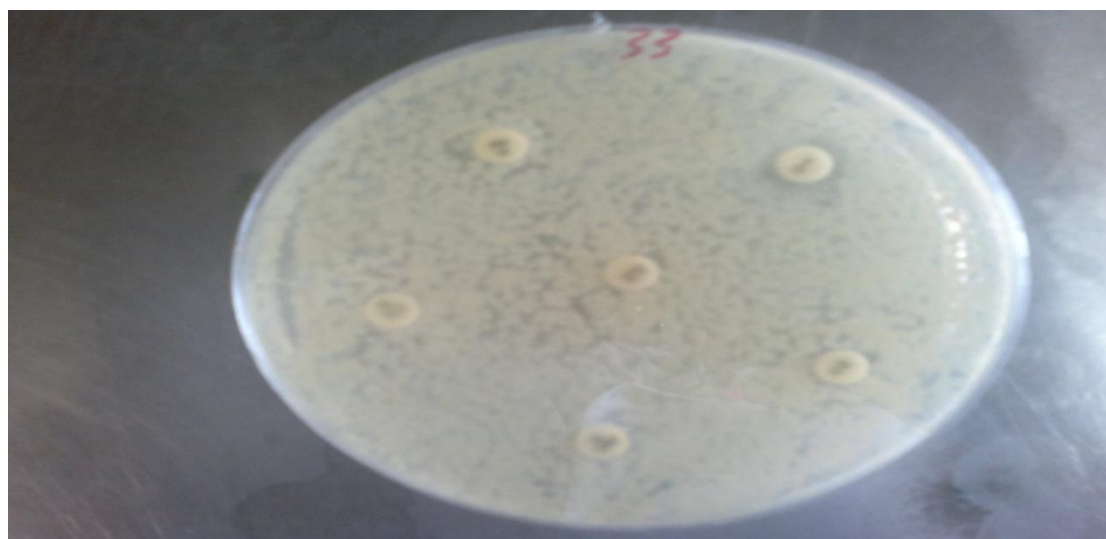


Figure 3- Vancomycin-resistant *S.aureus* by disc diffusion.

The MIC for 2 of 56 isolates for vancomycin was 32 and 64 $\mu\text{g/ml}$ indicating that these two isolates were vancomycin resistance. The two isolates showed resistance to a minimum of six other antibiotics including vancomycin and methicillin showed in table-2.

Table 2- Multi-drug Resistant Isolates of *S.aureus*

Isolate No.	AK	AMC	AMP	CTX	E	MET	VAN	TE
<i>S.aureus</i> 33	R	R	R	R	R	R	R	R
<i>S.aureus</i> 56	R	R	R	R	R	R	R	R

AK: amikacin, AMC: amoxicillin, AMP: ampicillin, CTX: cephotaxim, E: erythromycin, TE: tetracycline, MET: methicillin, VAN: vancomycin.

Since first being reported in 1997, the threat of vancomycin resistance in *S. aureus* has been the topic of intensive research and discussion. Although vancomycin resistance in *S. aureus* remains extremely rare, there is widespread concern that vancomycin-resistant *S. aureus* poses, by far, the greatest risk to patients, given the virulence of the organism[9]. The presence of van A genes in VRSA suggests that the resistance determinate was acquired from a vancomycin resistant *Enterococcus*[10]. In fact, experimental transfer of the van A genes from enterococci to *S.aureus* has been shown previously[11]. Vancomycin-resistant *S. aureus* tend to be multidrug resistant against a large number of currently available antimicrobial agents, compromising treatment options and increasing the likelihood of inadequate antimicrobial therapy and increase in morbidity and mortality[12].

Vancomycin resistance has been perceived as a fearsome threat to the already challenging therapy of MRSA and MDR-MRSA [13,14]. The emergence and the dissemination of resistance can be controlled by a heightened awareness of the issues, by encouraging proper personal hygiene, provision of adequate effective sewage disposal systems to prevent dissemination of the multidrug resistant bacteria from the gut, surveillance of the local bacterial population, early intervention, rigorous cross infection control measures and by the judicious use of current antimicrobial agents [15,16].

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