Isolation and identification of resistance Staphylococcus aureus to vancomycin & Methicillin from outpatients clinic in AL-Shefa General Hospital in Basrah district

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Abstract

Research doing on isolation and identification of *S.aureus* resistance to vancomycin and Methicillin on 547 samples outpatient (adults and non –adults) clinic of Al Shefa General Hospital in Basrah district during the period from October, 2007 to March, 2008, their age was ranged from 5-65 years.

One urine sample was collected from each patient under investigation and cultured by the streaking method onto Enrichment media and Selective (differential) media because of the common urinary pathogens and other pathogens grow well on these cultures media.

For isolation species depending to genus staphylococcus especially *S.aureus* (COPS) and (CONS); the samples urine streaking on Mannitol-Salt agar (selective media), the biochemical reactions (identification tests) were doing for staphylococcus Spp. especially COPS, CONS and other bacterial species in dependence on Bergey's Manual of Determinative Bacteriology (Holt *et.al*., 1994).

Furthermore, two antimicrobial agents were used in this research : Vancomycin in (30 mg) and methicillin (5 mg) for antimicrobial susceptibility test to *S.aureus* bacterial isolates (COPS); (CONS).

Results showed that the highest isolation rate of infection with *S.aureus* in cases: VRSA,VISA and VSSA were correlated with age group above 50 years in proportion: 7.93% , 4.76% and 12.69% respectively. Also, the Methicillin Resistance Staphylococcus aureus (MRSA) was recovered 6.42% and 9.52% in age groups above 50 years. The MR Staphylococci (CONS) was isolated in this study in proportion 4.76%.

Besides, different species of bacterial isolates other than *S.aureus* were identified in 55 infected person with UTI by the common diagnosis method. They consisted of *E.coli* (38.18%) as the most common bacterium, *P.aeruginosa* (29.09%), *K. pneumoniae* (21.81%) and *E.faecalis* (10.90%).

Introduction

A urinary tract infection (UTI) is a condition where one or more structures in the urinary tract become infected after bacteria overcome its strong natural defenses [1]. In spite of these defenses, UTIs are the most common of all infections and can occur at any time in the life of an individual [2-3]. Almost 95% of cases of UTIs are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder (Known as the ascending route) [2]. Much less often, bacteria spread to the kidney from the bloodstream [4]. Normal urine is sterile [5]. It contains fluids, salts, and waste products, but it is free from bacteria, viruses and fungi [5]. An infection occurs when microorganisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply [6-7, 8] .

Staphylococci are Gram-Positive spherical or cocci bacteria that occur in microscopic clusters resembling grapes. Bacteriological culture of the nose and skin of normal humans invariable yields Staphylococci. In 1884, Rosenbach described the two pigmented colony types of Staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (Yellow) and *S. albus* (white), the latter species is now named *S.epidermidis* [9]. Taxonomically, the genus Staphylococcus is in the bacterial family Staphylococcaceae, which includes three lesser known genera, Gamella, Macrococcus and Salinicoccus [10-11]. The Staphylococci are spherical, non-motile, non- sporogenous bacteria, occurring singly, in pairs, in tetrads and in irregular clusters (from which they derive their genus name) [10] . They are facultative anaerobes that grow by aerobic respiration and by fermentation that yields principally lactic acid.

Many strains produce orange or yellow, white pigments which have traditionally been associated with but are not definitive of the genus or species. The Coagulase- positive strains produce a variety of toxins (acquired resistance to practically all antibiotics) and the genus as a whole is potentially pathogenic [10-13].

AROs are bacteria that resist the effects of antibiotics. Bacteria possess a remarkable number of genetic mechanisms for resistance to antibiotics [14-15].

MRSA: *Staphylococcus aureus* is a species of bacteria normally found in the nares and on the skin. It is commonly known as an apportunistic pathogen. This means that normally it does not cause disease, but may cause disease given the apportunity to invade the human host [16]. Furthermore, the emergence of vancomycin resistance in *S. aureus* has been anticipated [12].

I have done this research because, the fewer researches and information in Basrah University about this subject (aim study)

Materials and Methods
Study population: The population under study was among people attending to the outpatient clinic of Al-Shefa General Hospital. A total number of five hundred and seventy four people (547) were investigated, their age ranged from 5 to 65 years, the investigation period extended from October, 2007 to March, 2008.

Sample collection and method culture: Urine samples were collected in a sterile container from each people and transported to the laboratory for cultured by the streaking method on MacConkey and blood agar. Streaking samples on Blood agar for microbes activation then cultured on Mannitol –Salt agar by such method for the members of staphylococci groups, then incubated at 35-37 C^o for 24-48 hrs. after that sub cultured on Nutrient agar for each isolates [11,17].

The media used in this research were prepared following the instructions of the manufacturers and using sterile laminar flow cabinet experiments has done, which present in microbiological laboratory (Lab) for postgraduate in biology department and microbiological Lab in Al- Shefa General Hospital.

1 **In laboratory diagnosis** the General Urine Examination (GUE) have been done [18], to detect the color , turbidity , acidity , albumin content , Sugar content and microscopic examination. Furthermore, we made primary diagnostic tests include: Gram's stain, Oxidase test, Catalase test,

Oxidation / fermentation test and mortality tests [11,17,19]. Finally, the identification tests of *Staphylococcus Spp*. especially COPS, CONS and other bacteria were applied according to Bergey's Manual of Determinative Bacteriology, 994, [10].

Antimicrobial Susceptibility Test: The determination of bacterial susceptibility of different isolates of *S.aureus* to two antimicrobial agents (Methicillin and Vancomycin) in the present work was made by using Disc – plate method according to Weckbach and Langlois, 1976 [20], by putting 0.1 ml of a young culture 18 hrs. grown in 1.5ml from Nutrient broth (Oxoid) in Mueller –Hinton agar (MHA) plates and spreading onto surface of the medium with a sterile glass rod (L-Shape) then, the appropriate discs were placed on the agar (MHA) with flamed forceps and gently pressed down to ensure contact, plates were incubated at 37 C^o for 48hrs. Finally, the diameters of inhibition zones were measured by using a ruler and the sizes of the zones inhibition were then interpreted by referring to standard Table (1-a,b).

Table (1,a) Diameter of zones inhibition				
Antibiotic	Disc concentration	Diameter (Millimeters) of zones inhibition		
		Resistant	Intermediate	Susceptible
Vancomycin	30 mcg	9 or less	10-11	12 or more
Methicillin (Penicillinase resistant penicillin class)	5 mcg	< 10	10-13	>13

Bauer *et.al* ., 1966, [14].

Table (1,b) Size of zones of inhibition

Antibiotic	Concentration	Bacterial species	Diameter of zones	No. of isolates	Result
Vancomycin (VA)	30 mcg	COPS (S.aureus)	8-9mm 10-11mm 12-14mm	10 7 22	VRSA VISA VSSA
		CONS Staphylococcus spp	11mm	4	VICONS
Methicillin (ME)	5 mcg	COPS (S.aureus)	9mm 11-12mm 14-15mm	25 11 3	MRSA MISA MSSA
		CONS Staphylococcus spp	8-9mm	3	MRCONS

My results according to Bauer *et.al.,*(1966).

Results

Table (2) : Shows, the occurrence of UTI in patients depending on sex.(101 patients /547, 18.46 %) were proved to be infected with urinary tract infection (UTI). 32 patients were males (14.95%) and 69 females (20.72%).

The patients under study were divided into six age groups(5-14, 15-24,25-34,35-44,45-54 and 55-65), as shown in **Table (3)**. It is evident that the highest isolation rate of infection with *S. aureus* in any case; (VRSA, VISA and VSSA) occurred in ages above 50 years in all patients, which correspond to 2.14 % , 7.93% in patient (+ve VRSA) while, represents 2.14 % , 4.76% in patients (+ve VISA) and in (+ve VSSA) patients represented 4.28% and 12.69 % .

Data present in **(Table 4)** revealed that the highest isolation rate of infection with *S. aureus* (+ve MRSA) associated with patient in age groups 45-54 and 55-65 years which recovered 6.42%, 9.52% whereas, in patients (+ve MISA) tend to be frequently distributed only in age group 45-54;3.57%.Besides,in patients (+ve MSSA) tends to be frequently distributed in age group 55-65 years and correspond to 3.17%.

Concerning **Table (5)** it shows that, a high rate of infection with MRStaphylococci (CONS) correlated with patients in age group 55-65 years which correspond to 4.76%.

Table (6) shows , several bacterial species other than *S. aureus* were isolated from patient's UTI, they consisted of *E.coli* which was the most frequently isolated bacterial species 38.18% followed by *P. aeruginosa* 29.09% , *K. pneumonia* 21.81% and *E.faecalis* 10.90%.

The antimicrobial susceptibility for vancomycin & methicillin were ascertained when, the diameters of inhibition zones measured by a ruler and the sizes of the zones of inhibition were then interpreted by referring to standard Bauer *et.al*., 1966,[14]. **(Table 1-a,b) and (Fig. 1-3).**

Table (2) Distribution of UTI according to sex.

Sex	No. of tested Patient	No. of Patient UTI and percentage
Male	214	32(14.95)
Female	333	69 (20.72)
Total	547	101 (18.46)

Table (3) Distribution of Patients with Vacomycin Susceptibility according to age

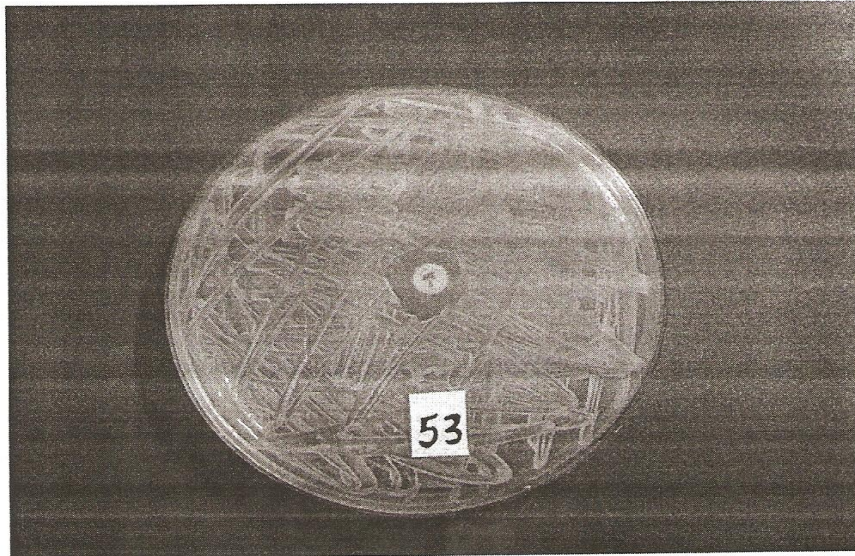| Age year	No. of tested patient	No. and (%) of patient +ve in VSSA	No. and (%) of patient +ve in VISA	No. and (%) of patient +ve in VRSA
5-14	48	0	0	0
15-24	81	1(1.23)	0	0
25-34	96	3 (3.12)	0	2 (2.08)
35-44	119	4(3.36)	1 (0.84)	0
45-54	140	6(4.28)	3 (2.14)	3 (2.14)
55-65	63	8 (12.69)	3 (4.76)	5 (7.93)
Total	547	22 (21.78)	7 (6.93)	10 (9.90)

Figure (1)
The susceptibility to methicillin:
Isolate number 53 susceptible to methicillin with inhibition zone correspond to 16 mm
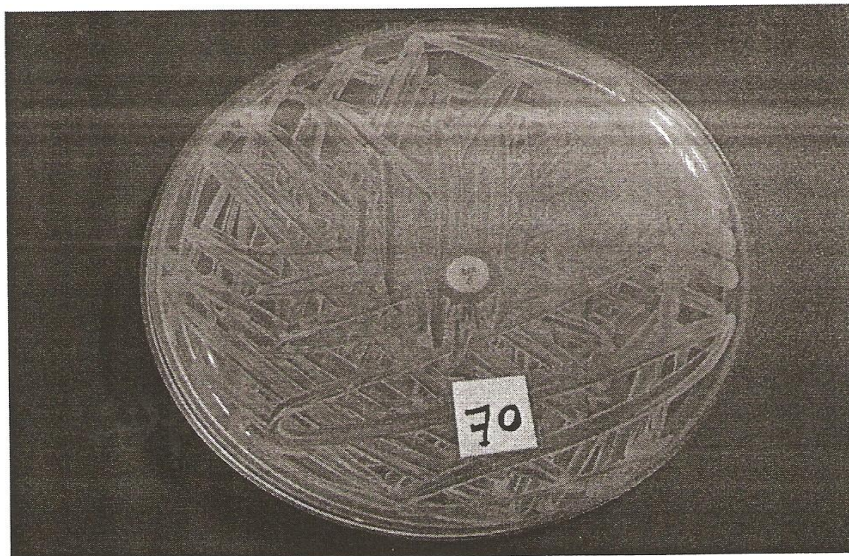

Figure (2)
The susceptibility to methicillin:
Isolate number 70 resistant to methicillin with inhibition zone correspond to 4 mm
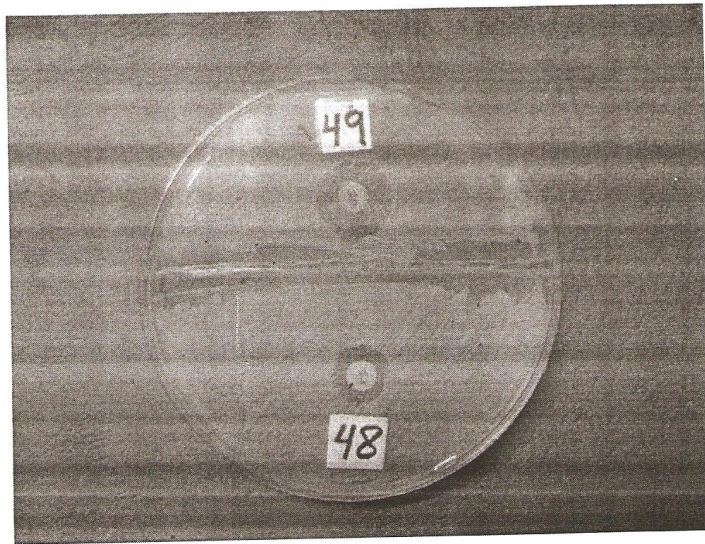
Figure (3)
The susceptibility to vancomycin:
Isolate number 49 susceptible to vancomycin, Isolate number 48 resistant to
vancomycin with inhibition zone correspond to 15 mm and 9 mm respectively

Table (4) Distribution of Patients with Methicillin susceptibility according to age

Age year	No. of tested patient	No. and (%) of patient +ve in MSSA	No. and (%) of patient +ve in MISA	No. and (%) of patient +ve in MRSA
5-14	48	0	0	0
15-24	81	0	0	0
25-34	96	0	1(1.04)	4 (4-16)
35-44	119	1 (0.84)	3 (2.52)	6 (5.04)
45-54	140	0	5 (3.57)	9 (6.42)
55-65	63	2 (3.17)	2 (3.17)	6 (9.52)
Total	547	3 (2.97)	11 (10.89)	25(24.75)

Table (5) Distribution of Patients with Methicillin resistance staphylococci (CONS)

Age year	No. of tested patient	No. and (%) of patient +ve MRS in VICONS
5-14	48	0
15-24	81	0
25-34	96	0
35-44	119	1 (0.84)
45-54	140	3 (2.14)
55-65	63	3 (4.76)
Total	547	7 (6.93)

Table (6) Frequency of bacterial isolates in patients UTI

Other bacteria	No. of isolates	Relative frequency (%)
Escherichia coli	21	38.18
Pseudomonas aeruginosa	16	29.09
Klebsiella pneumoniae	12	21.81
Entercoccus faecalis	6	10.90
Total	55	99.98

Discussion

Methicillin – Resistant *Staphylococcus aureus* (MRSA) has become a major epidemiological and clinical problem over the last decades [21-23]. These strains have spread worldwide, causing nosocomial and more recently community – based infections [24]. This has led to the overuse of glycopeptides, and to the emergence of Vancomycin –resistant *S.aureus* [25]. Isolates of Vancomycin resistant *S.aureus* have emerged in many parts of the world. These isolates appear to achieve clinically relevant levels of resistance to Vancomycin that leads to treatment failure. With VRSA-MRSA infection, the wrong of frequent use of Vancomycin, Methicillin causes the Staphylococcus bacteria to become resistant [26].

As strains of *S.aureus* with reduced susceptibility continue to emerge and evolve, perhaps to full resistance, there is a clinical need to fully characterize them and conduct well-designed research and epidemiological studies.

Concern over development of VRSA emanates from the newly widespread occurrence of Vancomycin resistant strains of enterococci (VRE) [27].

In this study, 101 patients (adults of non –adults) from 547 (18.46%) were proved to be infected with UTI. Males were 32 patients (14.95%) while, female's patients were 69(20.72%), Table (2). We know in female sex short urethra allowing bacteria quick access to the bladder, also, females urethra opening is near sources of bacteria from the anus and vagina these play a role in the migration of pathogens and lead to UTI [5, 28]. Anyhow, our results were consistent with workers [10, 24] observation.

Concerning Table (3), the highest isolation rate of *S.aureus* (VRSA, VISA and VSSA) tend to be frequently distributed in ages above 50 years , which correspond in patient's (+ve VRSA) to 2.14%, 7.93% while , in patient's (+ve VISA & +ve VSSA) were represented 2.14% , 4.76% , 4.28% and 12.69% respectively.

Hakim *et.al* [29] found that, out of 850 isolates (*S.aureus*), 250 were MRSA, of which 22% were resistant to 4mcg and 13.2% to 30mcg vancomycin.

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Also, 13% of the strains were intermediates (VISA), this result was somewhat in accordance with the results of present study.

The current study shows that, the highest isolation rate of infection with *S.aureus* (+ve) in MRSA correlated with patients in age groups 45-54 and 55-65 years which correspond to 6.42%, 9.52% respectively and the total isolation rate of infection by *S.aureus* (MRSA) was 24.75% in 25 cases , Table (4).

In study of Lin *et.al* [30] detected that, infections cases by *Staphylococcus aureus* (MRSA) particularly nosocomial infection in a Medical Center , 1990 correspond to 53 (66.3%). Those findings were, to some extent, in agreement with the results of current study only concerning with the isolation rate of MRSA.

On the other hand, in this study, the occurrence of infection with MR Staphylococci (CONS) correspond to (4.76%) in 7 cases which, associated with patients in age group 55-65 years, Table (5).

However, a few recent studies proposed that significance of some CONS species as uropathogens may have been somewhat under estimated [31], a result was somewhat in consistence with the results of present study.

Furthermore, the current search shows, presence of several bacterial species other than *S.aureus* in 55 infected people (patient's UTI), as appeared in Table (6).

They consisted of *E.coli* (38.18%) which was the most common bacteria, followed by *P.aeruginosa* (29.09%), *K. pneumoniae* (21.81%) and

E.faecalis (10.90%). However, the total of bacterial isolates in patient's UTI was 99.98%.

Nasseab [32] study, revealed, the most common bacteria caused NUTI was *E.coli* (26.47%) followed by *P.aeruginosa* (23.53%) and frequency of total bacterial isolates in patient's NUTI was 100%, this result was somewhat in accordance with the our findings.

In Conclusion: I believe that the current vancomycin staphylococci in hospital are alarming situation to the clinicians and the emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin threatens to return us to the era that preceded the development of antibiotics.

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LIST OF ABBREVIATIONS

Breviary	Name
1-AROs	Antibiotic Resistant Organisms
2-MRSA	Methicillin Resistant *Staphylococcus aureus*
3-VRSA	Vancomycin Resistant *Staphylococcus aureus*
4-COPS	Coagulase Positive Staphylococci
5-CONS	Coagulase Negative Staphylococci
6-VISA	Vancomycin Intermediate *Staphylococcus aureus*
7-VSSA	Vancomycin Susceptible *Staphylococcus aureus*
8-MISA	Methicillin Intermediate *Staphylococcus aureus*
9-MSSA	Methicillin Susceptible *Staphylococcus aureus*
10-MRCONS	Methicillin Resistant Coagulase Negative *Staphylococcus*
11-VICONS	Vancomycin Intermediate Coagulase Negative *Staphylococcus*
12-UTI	Urinary Tract Infection
13-NA	Nutrient Agar
14-MA	MacConkey Agar
15-BA	Blood agar
16-MSA	Mannitol Salt Agar

عزل وتشخيص جرثومة العنقوديات الذهبية *Staphylococcus aureus* المقاومة للفانكومايسين والمثلسين من المرضى المراجعين لمستشفى الشفاء العام في مدينة البصرة

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الخلاصة

عمل البحث على عزل وتشخيص *S.aureus* المقاومة للفانكومايسيين والمثلسين على 547 عينة من المـراجعين   (راشـدين وغير راشدين) للعيادة الاستشارية الخارجية في مستشفى الشفاء العام في مدينة البصرة للفترة من تشرين الأول 2007 إلى آذار 2008 وكانت معدلات أعمارهم تتراوح بين 5–65 سنة. حيث جمعت عينة إدرار واحدة من كل مريض قيد الفحـص وزعـت بطريقة التخطيط على الأوساط الزرعية المدعمة والانتقائية (التفريقية) لان اغلب ممرضات الجهاز البولي الشائعة والممرضــات الأخرى تنمو جيداً على هذه الأوساط الزرعية. ولعزل الأنواع التابعة لجــنس *Staphylococcus* وخاصــة نــوع *S.aureus* (COPS) و (CONS) فقد خططت عينات الإدرار على وسط Mannitol –Salt agar (الانتقــائي) ، وعملـت الاختبــارات البايوكيميائية (الاختبارات التشخيصية) لــ Spp. *Staphylococcus* وخاصة COPS و CONS والأنواع البكتيرية الأخـرى من المصدر المعتمد (Holt *etal*.,1994) Bergey's Manual of Determinative Bacteriology   إضــافة إلـى ذلـك، استخدم في هذا البحث نوعان من مضادات الأحياء المجهرية: الفانكومايسين بتركيز (30 mcg) والمثلسين بتركيــز (5 mcg) لاجراء اختبار الحساسية لعزلات *S.aureus* الجرثومية (COPS); (CONS) . أظهرت النتائج ان المعدلات الأعلى للإصابة بــ *S.aureus* مرتبطة بالاعمار فوق 50 سنة وفي حالات: VRSA و VISA و VSSA و بنسب 12.69%,4.76%,7.93% بالترتيب. كذلك غطت (MRSA) Methicillin – resistant Staphylococcus aureus 6.42% و9.52% في المجـاميع العمرية الأكبر من 50 سنة. وعزلت في هذه الدراسة (CONS) MRStaphylococci المقاومة للمثلسين بنسبة4.76% .
والى جانب ذلك شخصت الأنواع المختلفة للجراثيم من غير *S.aureus* في 55 شخص مصــاب بـــ UTI باسـتخدام طـرق التشخيص التقليدية، حيث كانت الجراثيم المعزلة *E.coli* بنسبة (38.18%) وهي أكثر الجراثيم شـيوعاً ثـم *P.aeruginosa* (29.9%)و*K.pneumoniae* (21.81%) و*E.faecalis* (10.90%).