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PREVALENCE OF β-LACTAMASE-PRODUCING AND NON-PRODUCING STAPHYLOCOCCUS AUREUS ASSOCIATED WITH PATIENTS IN INTENSIVE CARE UNITS

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SUMMARY: A total of 125 samples were collected from intensive care units (ICUs) of two main hospitals in Basrah: 74 clinical samples including Skin, blood, eye, nose, wounds, and urine and 51 inanimate samples including bed, wall, instruments, and addresses. A total of 334 isolates of bacterial types were isolated from various sources, including the following number of isolates and their percentages: Staphylococcus aureus 45 (13.47%), Staphylococcus epidermidis 31 (9.28%), Staphylococcus saprophyticus 18 (5.38%), Staphylococcus xylosus 11 (3.29%), Staphylococcus capitis 7 (2.09%), Streptococcus pyogenes 28 (8.38%), Viridans streptococci 35 (10.47%), Streptococcus pneumoniae 12 (3.59%), Pseudomonas aeruginosa 41 (12.27%), Escherichia coli 19 (5.68%), Klebsiella spp 20 (5.98%), Proteus spp 10 (2.99%), Enterobacter 9 (2.69%), Propionibacterium acnes 24 (7.18%), Acinetobacter spp 9 (2.69%), and Corynebacterium spp 15 (4.49%). A total of 31 isolates of S. aureus (68.89%) were β-lactamase producers, while 14 isolates (31.11%) were β-lactamase non-producers. The prevalence of multidrug resistance of S. aureus against eight antibiotics was carried out in the present study. The resistance against three antibiotics had the biggest percentage (25.8%) for β-lactamase-producing S. aureus with resistance of eight antibiotics, while resistance of two antibiotics was the predominant mode of β-lactamase non-producing S. aureus (35.71%) with no resistance against more than four antibiotics. The study found that vancomycin, cefotaxime, and gentamicin were the most effective antibiotics against β-lactamase-producing S. aureus strains isolated from both clinical and inanimate samples of ICUs having percentages of resistance as follows: 42.22%, 44.44%, and 44.44 %, respectively, and the antibiotic tetracycline had the biggest percentage of resistance (82.22%) against S. aureus strains under study.Although vancomycin and cefotaxime were the most effective antibiotics for β-lactamase non-producing S. aureus strains, they had the lowest percentages of resistance in comparison to the first above group that recorded 13.33% and 20.0% of resistance, respectively, and tetracycline still being the weakest antibiotic having great resistance of 53.82% of isolates.The plasmid profiles in β-lactamase-producing and non-producing MDRSA were also determined in this study. When the band molecular weight ranged between 300 and 600 base pairs (bp), a clear main band appeared in the range 550-570 bp for β-lactamase-producing S. aureus. When the band molecular weight ranged between 200 and 700 bp, a clear main band appeared each in the band range 450-470 bp and 690-700 bp for β-lactamase-producing S. aureus.

Key words: Staphylococcus aureus, antibiotics, β-lactamase, intensive care units

INTRODUCTION

The hospital environment is uniquely suited to the spread of infections as it houses both susceptible patients and patients with difficult-to-treat infections. There is a great risk that some patients may contract hospital-asso-

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ciated infections other than those who were admitted for because of nosocomial pathogens around them (1). The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomial infections caused by drug-resistant microbes (2).

Staphylococcus aureus, a spherical aerobic Gram positive, catalase positive, oxidase positive, non-motile, spore-forming coccus, is an opportunistic pathogen in human beings and animals, and is one of the most frequent sources of hospital- and community-acquired infections. Generally, *S. aureus* is responsible for superficial infections and toxic epidermal necrolysis, systemic infections such as endocarditis inflammation of bone or bone marrow and pneumonia, and toxinoses such as food poisoning or toxic shock syndrome. However, among gram-positive cocci, only β-lactamase of major clinical significance is staphylococcal β-lactamase, which rapidly hydrolyzes benzylpenicillin, ampicillin, cephalosporins, and related antimicrobials (3,4). Methicillin-resistant *S. aureus* (MRSA) is a special strain of S. aureus that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class. Although, MRSA has traditionally been seen as a hospital-associated infection, community-acquired MRSA strains have appeared in recent years, notably in the USA and Australia (5). Several new strains of MRSA have been found showing antibiotic resistance even to vancomycin and teicoplanin; these new evolutions of the MRSA bacteria are called vancomycin intermediate-resistant *S. aureus* (VISA) (6,7). Community-acquired MDRSA (multidrug-resistant *S. aureus*) infections in the absence of identified risk factors have been reported. Many outbreaks of infections due to MDRSA have occurred, and it has now become endemic in several centers in the world (8,9). The emergence of community-acquired MDRSA that is capable of causing infections in otherwise healthy people has also been reported (10) Staphylococcal antibiotic resistance has been associated with resistant plasmids that have the ability to mediate the production of drug-inactivating enzymes such as β-lactamases (11) and other functions (12). MDRSA also differ in their resistance to antibacterial agents and in the genetic location of these resistance determinants. Studies have shown that the genetic determinants for antibiotic resistance reside on plasmids, chromosomal DNA, or transposable elements (13,14). In Bangladesh, as reported previously, the frequency of MDRSA was alarming due to indiscriminate and incomplete uses of antibiotics (15). In 2002, a total of 47.2% MDRSA was reported in an investigation on clinical *S. aureus* isolates (13). Both of these prevalence rates of MRSA were higher than the rate in some developed countries like Austria 21.6%, Belgium 25.1%, Spain 30.3%, and France 33·6%.16 Therefore, the current situation of the susceptibility patterns of local strains is essential for the judicious use of antibacterial agents as well as to become aware of the MDRSA in hospitals and community arenas. *S. aureus* is the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants (2,9). The *S. aureus* infection may also be superimposed on superficial dermatological diseases such as eczema, pediculosis, and mycosis (17). They live as commensals in anterior naves of over half the population of humans (18). The cocci are spread from these sites into the environment by the hands, handkerchief, clothing, and dust. *S. aureus* is an opportunistic pathogen in the sense that it causes infections most commonly in tissues and sites with lowered host resistance such as in individuals with diabetes, old malnourished persons, and other chronic cases (3,14). *S. aureus* causes folliculitis, boil, furnculosis, scalded skin syndrome, conjunctivitis, paronychia, mastitis, and toxic shock syndrome for menstruating women who use tampons. Staphylococcal pneumonia can occur if the staphylococcal infection spreads to the lungs (3). Hospital -acquired staphylococcal infections are common in newborn babies, surgical patients, and hospital staff. Patients develop sepsis in operation wounds, which take place in the theater during operation, and others postoperations in the ward (19). Attempts to control these diseases by chemotherapy through the use of antimicrobial agents, particularly antibiotics, have resulted in increased prevalence of resistance to these agents (20). Several investigations have been conducted to study the antimicrobial resistance pattern of *S. aureus*, and it has been shown that the organism is resistant to β-lactam antibiotics, amino glycoside, and macrolides (21,22). *S. aureus* strains carry a wide variety of multidrug-resistant genes on plasmids, which can be exchanged and spread among different species of staphylococci (23). The multiresistant determinants can be transferred to new bacterial hosts. The situation is made more difficult in developing countries such as Iraq where antimicrobial drugs are readily available to consumers across the counter with or without prescription from a medical practitioner. Such a practice has led to misuse of antimicrobial drugs with the associated high prevalence of drug resistance among the staphylococci

(3,24,25). Hospital strains of *S. aureus* are usually resistant to a variety of different antibiotics. A few strains are resistant to all clinically useful antibiotics except vancomycin. Some workers have reported, however, the presence of vancomycin-resistant strains (11,26).

This work was undertaken to determine the prevalence of β-lactamase-producing and non-producing. *S. aureus* associated with patients and inanimate sources in intensive care of hospital populations in Basrah hospitals of Iraq.

MATERIALS AND METHODS

Sample collection

Various clinical and inanimate swabs were collected from hospitalized patients in intensive care units (ICUs) of two main hospitals in Basrah city (Al-Sadder teaching and general Basrah hospitals) during the period between June and November 2011using sterile swab saturated with brain–heart infusion. All specimens were transported immediately to the laboratory and cultured within 3-4 h of collection.

Isolation and characterization of bacteria

The swab specimens were inoculated on various ordinary media; blood agar base, nutrient agar, MacConkey agar (Hi Media, India) to obtain discrete colonies. The plates were incubated at 37°C for 24 h under aerobic conditions,after which the culture plates were examined recording the appearance, size, color, and, morphology of the colonies. Gram stain reaction, catalase test, and coagulase test growth on differential and selective media – such as mannitol salt agar, triple sugar iron agar, eosin methylene blue agar (Hi Media, India) – and other biochemical tests were carried out according to standard techniques (27,28).

Isolates that were gram-positive, catalase positive, and coagulase positive cocci, and formed yellow colonies on mannitol salt agar were considered *S. aureus* in this study.

Susceptibility of isolates to various antibiotics

Antibiotic sensitivity test was carried out on all isolates using paper disk diffusion technique. A total of eight antibiotics were tested. About 0.1 ml of 18-h brain–heart infusion culture of the test organism was used to inoculate on a dry sterile Mueller–Hinton agar plate using an L-shaped sterile glass spreader and allowed to dry for about 15-30 min. The antibiotic disks were placed on the agar using sterile forceps. Each disk was placed far from each other to avoid their zones of inhibition from coalescing into the other. The plates with the antibiotic disks were then incubated at 37°C for 24 h to observe the zones of growth inhibition produced by the antibiotics.

The antimicrobial disks were sourced from the Hi Media Laboratories Ltd., Mumbai, India, as follows:

tetracycline (TET), gentamicin (GEN), amoxicillin (AMOX), ciprofloxacin (CIP), cefotaxime (CEF),amoxycillin/clavulanic acid (AMOX/CLA), vancomycin (VAN), and methicillin (METH).

The zone diameters measured around each disk were interpreted on the basis of (29) 29 according to guidelines by the NCCLS 2002 (30).

β-Lactamase test (31,32)

β-Lactamase production was assayed by the acid-formation method. A piece of Whatman No.1 filter paper (5x6) was briefly placed in a sterile Petri dish. The bluish penicillin solution was added dropwise to saturate the paper. Thick masses of bacterial colonies of the test organism were transferred with a bacteriological loop from the test culture to the filter paper and spread over an area of 5-mm diameter. The paper was then incubated at 37°C for 30 min with the Petri dish covered. The paper was examined and yellow zones formed by β-lactamase-producing strains were noted.

Plasmid profile and molecular studies on MDRSA strains (33,34,35)

Plasmid profiles of 14 MDR S. aureus strains were determined in the laboratory of biotechnology – College of Veterinary Medicine, and Oklahoma laboratory of biotechnology – College of Science, University of Basrah, by the miniprep method followed by band separation on horizontal gel electrophoresis in 1.5% agarose in 1X Tris–Borate–EDTA (TBE) buffer at room temperature (36). Single purified bacterial colonies were seeded each into 10 ml Mueller-Hinton broth (Hi Media) in screw cap tubes and incubated overnight at 37°C. After centrifugation of 1.5 ml of the overnight culture for 1 min, the pelleted cells were dissolved in 300 μl of TENS solution (Tris 25 mM, EDTA 10 mM, NaOH 0.1 N, and SDS 0.5%), the tube inverted a few times for thorough mixing, and iced for 5 min. An addition of 150 μl of 3.0 M sodium acetate (pH 5.2) was made and the tube vortexed till completely mixed. The solution was microcentrifuged for 5 min at 13,000 rpm to pellet cell debris and chromosomal DNA. Supernatant (400 μl) was decanted into fresh Eppendorf tube, mixed with 800 μl ice-cold absolute ethanol, and centrifuged for 10 min to pellet the plasmid DNA. The supernatant was discarded, pellet rinsed twice in 1 ml of 70% ice-cold ethanol, and dried at 45°C for 15 min. The dried pellet was resuspended in 40μl TE buffer and stored at 4°C till further analysis. For the separation of plasmid DNA, a horizontal tank loaded with 5 mm agarose gel stained with 20 μl of 1 mg/ml ethydium bromide was connected to a power supply at 80 V for 4 h. The loading dye used was bromocresol purple. For each well, 15 μl of plasmid DNA solution was mixed with 2 μl loading dye, carefully loaded onto the gel, and allowed to run for 2 h. DNA bands were visualized and photographed using digital camera (Sony. 7.2 mega-

Table 1: Numbers of samples collected from clinical and inanimate sites

Samples type		Number of samples*	%
1. Clinical samples	Skin	16**	12.80
	Blood	10	8.00
	Eye	11	8.80
	Nose	11	8.80
	Wounds	14	11.20
	Urine	12	9.60
2. Inanimate samples	Bed	13	10.4
	Wall	12	9.60
	Instruments	15	12.00
	Dresses	11	8.80
Total		125	100

* : One sample was taken from each type at a time.
** : There are no statistical differences between sites of postoperative wounds ($P \geq 0.05$) .

pixel). The molecular weight of unknown plasmid DNA was extrapolated using the band mobilities in the gel.

Statistical analysis

The results were statistically analyzed by using ANOVA and T-test in the SPSS (Statistical Package for the Social Sciences) (Version 17).The present study was carried out with approvement and agreement of Ethical and Medical Committee in College of Medicine – University of Basrah.

RESULTS

A total of 125 samples were collected from ICUs of two main hospitals in Basrah (74 clinical samples and 51 inanimate samples) as follows and are shown in Table 1 with no statistical differences $P \geq 0.05$:

Clinical samples: Skin 16, blood 10, eye 11, nose 11, wounds 14, and urine 12.

Inanimate samples: Bed 13, wall 12, instruments 15, and addresses 11.

Table 2 shows the total of 334 isolates of bacterial types isolated from various clinical and inanimate sources of ICUs, including the following numbersof isolates and percentages: *S. aureus* 45(13.47%), *S. epidermidis* 31 (9.28%), *S. saprophyticus* 18(5.38%), *S. xylosus* 11 (3.29%), *S. capitis* 7(2.09%), *S. pyogenes* 28 (8.38%), *Viridans streptococci* 35 (10.47%), *S. pneumonia* 12 (3.59%), *Pseudomonas aeruginosa* 41 (12.27%), *E. coli* 19 (5.68%), *Klebsiella spp* 20 (5.98%), *Proteus spp* 10 (2.99%), *Enterobacter* 9(2.69%), *Propionibacterium acnes* 24 (7.18%), *Acinetobacter spp* 9 (2.69%) and *Corynebacterium spp* 15(4.49%) .

There are high statistically differences between numbers of isolates and various clinical and inanimate sites $P<0.01$. *S. aureus* was the main predominant bacterial pathogens isolated from both types of samples, so we used this bacteria to demonstrate its ability to produce β-lactamase and determine the antibiotics profile.

The Categorization of β-lactamase-producing and non-producing S. aureus isolates isolated from various sources is illustrated in Table 3. A total of 31 isolates of S. aureus (68.89%) were isolated as β-lactamase producers, while 14 isolates (31.11%) were β-lactamase non-producers.

There are high statistically significant differences between numbers of β-lactamase-producing and non-producing *S. aureus* isolates in various clinical and inanimate sites $P<0.01$.

Table 4 illustrates the prevalence of multidrugs resistance of *S. aureus* against eight antibiotics; we found that resistance against three antibiotics had the biggest percentage (25.8%) for β-lactamase-producing *S. aureus* with resistance of eight antibiotics, while resistance of two antibiotics was the predominant mode of β-

Table 2: Bacterial types isolated from different clinical and inanimate sources of intensive care units.

Bacterial types	Total number of isolates (%)	Number of isolate (%)									
		Skin	Blood	Eye	Nose	Wounds	Urine	Bed	Wall	Instruments	Dresses
Staphylococcus aureus	45(13.47) **	5	3	2	4	7	5	5	3	9	2
Staphylococcus epidermidis	31(9.28)	7	0	5	0	2	0	4	4	6	3
Staphylococcus saprophyticus	18(5.38)	2	0	0	2	4	4	1	0	3	2
Staphylococcus xylosus	11(3.29)	2	0	1	1	2	2	0	0	2	1
Staphylococcus capitis	7(2.09)	2	0	0	0	1	0	1	0	2	1
Streptococcus pyogenes	28(8.38)	4	3	2	1	6	0	3	2	5	2
Viridans streptococci	35(10.47)	7	3	4	3	3	0	3	4	5	3
Streptococcus pneumoniae	12(3.59)	2	2	2	0	2	0	1	0	2	1
Pseudomonas aeruginosa	41(12.27)	4	0	2	2	6	4	4	6	10	3
Escherichia coli	19(5.68)	3	0	0	2	4	3	1	0	4	2
Klebsiella spp	20(5.98)	2	0	2	0	2	3	2	2	5	2
Proteus spp	10(2.99)	0	0	0	1	3	2	0	0	2	2
Enterobacter	9(2.69)	1	0	0	1	2	2	1	0	2	1
Propionibacterium acnes	24(7.18)	7	0	2	2	3	0	2	2	4	2
Acinetobacter spp	9(2.69)	2	0	0	1	2	1	1	0	2	0
Corynebacterium spp	15(4.49)	3	2	3	0	2	0	0	0	3	2
Total	334	53 (15.86)	13 (3.89)	25 (7.48)	20 (5.98)	51 (15.26)	26 (7.78)	28 (8.38)	23 (6.88)	66 (19.76)	29 (8.68)

** : There are high statistically significant differences between numbers of isolates and various clinical and inanimate sites P<0.01.

lactamase non-producing *S. aureus* (35.71%) with no resistance against more than four antibiotics.

There are high statistically significant differences between numbers of resistant isolates of β-lactamase-producing and non-producing *S. aureus* P<0.01.

Tables 5 and 6 illustrate the profile of antibiotic resistance of β-lactamase-producing and non-producing *S. aureus* strains isolated from clinical and inanimate samples of ICUs. The study found that vancomycin, cefotaxime, and gentamicin were the most effective antibiotics against β-lactamase-producing *S. aureus* strains isolated from both clinical and inanimate samples of ICUs having percentages of resistance as follows: 42.22%, 44.44%, and 44.44 %, respectively. The antibiotic tetracycline had the biggest percentage of resistance (82.22%) against *S. aureus*. Although vancomycin and cefotaxime were the most effective antibiotics for β-lactamase non-producing S. aureus strains, they had the

Table 3: Categorization of β-lactamase-producing and non-producing *S. aureus* isolates.

Types of isolates	Total number of strains (%)	Numbers of isolates (%)									
		Skin	Blood	Eye	Nose	Wounds	Urine	Bed	Wall	Instruments	Dresses
β-lactamase producing *S. aureus*	31** (68.89)	3 (9.67)	3 (9.67)	1 (3.22)	2 (6.45)	7 (22.58)	3 (9.67)	3 (9.67)	2 (6.45)	6 (19.35)	1 (3.22)
β-lactamase non-producing *S.aureus*	14 (31.11)	2 (14.28)	0	1	2 (14.28)	0	2 (14.28)	2 (14.28)	1 (7.14)	3 (21.43)	1 (7.14)
Total	45 (100.00)	5 (11.11)	3 (6.66)	2 (4.44)	4 (8.89)	7 (15.55)	5 (11.11)	5 (11.11)	3 (6.66)	9 (20.0)	2 (4.44)

** : There are high statistically significant differences between numbers of β-lactamase-producing and non-producing *S. aureus* isolates in various clinical and inanimate sites P<0.01.

lowest percentages of resistance in comparison to the first above group that recorded 13.33% and 20.0% of resistance, respectively, and tetracycline still being the weakest antibiotic having great resistance of 53.82% of isolates.

To look into the plasmid profiles in β-lactamase-producing and non-producing MDRSA, we selected 14 multidrug-resistant strains, isolated the plasmid DNA by alkaline lysis miniprep method, and analyzed them using agarose gel electrophoresis (Figures 1 and 2). When the band molecular weight ranged between 300 and 600 base pairs (bp), a clear main band appeared in the range 550-570 bp for β-lactamase-producing *S. aureus*. When the band molecular weight ranged between 200 and 700 bp, a clear main band appeared each in the range 450-470 bp and 690-700 bp for β-lactamase-producing *S. aureus*.

DISCUSSION

Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to escalating healthcare costs worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world (37). The present study was able to isolated a total of 334 isolates of bacterial types

Table 4: Prevalence of multiple drugs-resistant isolates of β-lactamase-producing and non-producing *S. aureus.*

Types of isolates	Total number of tested strains (%)	Number of resistant isolates (%)								
		0	1	2	3	4	5	6	7	8
β-lactamase producing *S.aureus*	31** (68.89)	3 (9.67)	3 (9.67)	5 (16.13)	8 (25.80)	5 (16.13)	3 (9.67)	2 (6.45)	1 (3.22)	1 (3.22)
β-lactamase non-producing *S. aureus*	14 (31.11)	3 (21.43)	2 (14.28)	5 (35.71)	3 (21.43)	1 (7.14)	0	0	0	0
Total	45 (100.00)	6 (13.33)	5 (11.11)	10 (22.22)	11 (24.44)	6 (13.33)	3 (6.66)	2 (4.44)	1 (2.22)	1 (2.22)

**: There are high statistically significant differences between numbers of resistant isolates of β-lactamase-producing and non-producing *S. aureus* P<0.01.

Table 5: Profile of antibiotic resistance of β-lactamase-producing *S. aureus* strains isolated from clinical and inanimate samples of intensive care units.

Samples type		Total number of tested strains	Number of resistant isolates							
			TET	GEN	AMOX	CIP	CEF	AMOX /CLA	VAN	METH
1.Clinical samples	Skin	5	5	3	4	3	2	3	2	5
	Blood	3	2	0	2	2	1	2	0	3
	Eye	2	2	1	2	2	0	2	1	2
	Nose	4	3	2	3	3	2	3	1	2
	Wounds	7	5	5	4	6	4	5	3	5
	Urine	5	3	3	4	5	3	3	2	3
2.Inanimate samples	Bed	5	4	2	3	4	3	0	3	2
	Wall	3	3	0	3	2	2	1	2	2
	Instruments	9	8	4	7	7	3	5	4	7
	Dresses	2	2	0	1	2	0	0	1	2
Total		45 (100.00)	37 (82.22)	20 (20.00)	33 (73.33)	36 (80.00)	20 (44.44)	24 (53.33)	19 (42.22)	33 (73.33)

TET: Tetracycline , GEN: Gentamicin, AMOX: Amoxicillin, CIP: Ciprofloxacin,
CEF: Cefotaxime, AMOX/CLA: Amoxycillin/Clavulanic Acid, VAN: Vancomycin, METH: Methicillin

from various clinical and inanimate sources of ICUs, including the following number of isolates and percentages: *S. aureus* 45 (13.47%), *S. epidermidis* 31 (9.28%), *S. saprophyticus* 18 (5.38%), *S. xylosus* 11 (3.29%), *S. capitis* 7 (2.09%), *St. pyogenes* 28 (8.38%), *Viridans streptococci* 35 (10.47%), *St. pneumonia* 12 (3.59%), *P. aeruginosa* 41(12.27%), *E. coli* 19(5.68%), *Klebsiella spp* 20(5.98%), *Proteus spp* 10 (2.99%), *Enterobacter* 9 (2.69%), *P. acnes* 24(7.18%), *Acinetobacter spp* 9 (2.69%), and *Corynebacterium spp* 15 (4.49%). All these isolates have been shown to cause different nosocomial infections, especially in ICUs infections. The results of our study were approved by results of recent studies (14,15, 38-41). The test for β-lactamase production

Table 6: Profile of antibiotic resistance of β-lactamase non-producing *S. aureus* strains isolated from clinical and inanimate samples of intensive care units.

Samples type		Total number of tested strains	Number of resistant isolates							
			TET	GEN	AMOX	CIP	CEF	AMOX /CLA	VAN	METH
1.Clinical samples	Skin	5	5	1	2	1	0	2	1	3
	Blood	3	1	0	0	0	0	0	0	0
	Eye	2	2	1	1	1	1	0	0	0
	Nose	4	2	1	2	1	2	1	0	1
	Wounds	7	4	3	4	3	2	3	2	3
	Urine	5	2	1	2	2	0	2	0	2
2.Inanimate samples	Bed	5	2	1	2	2	1	0	1	1
	Wall	3	2	0	2	2	2	1	0	0
	Instruments	9	3	2	5	3	2	3	2	4
	Dresses	2	1	0	1	1	0	0	0	2
Total		45 (100.00)	24 (53.82)	10 (22.22)	21 (46.66)	16 (35.55)	9 (20.00)	12 (26.66)	6 (13.33)	16 (35.55)

TET: Tetracycline , GEN: Gentamicin, AMOX: Amoxicillin, CIP: Ciprofloxacin,
CEF: Cefotaxime, AMOX/CLA: Amoxycillin/Clavulanic Acid, VAN: Vancomycin, METH: Methicillin

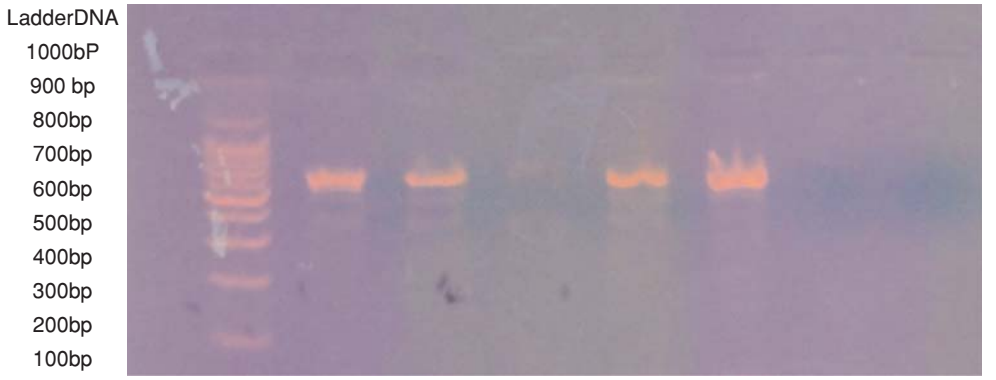

Figure 1: Gel electrophoresis result of multidrug resistant β-lactamase-producing *S. aureus* strains.

revealed that 68.89%isolates produced β-lactamase. The highest number of isolates was from wounds and instruments.

Our data found that resistance against three antibiotics had the biggest percentage (25.8%) for β-lactamase-producing *S. aureus* with resistance of eight antibiotics, while resistance of two antibiotics was the predominant mode of β-lactamase non-producing *S. aureus* having percentage 35.71% with no resistance against more than four antibiotics. And vancomycin, cefotaxime, and gentamicin were the most effective antibiotics against β-lactamase producing *S. aureus* strains isolated from both clinical and inanimate samples of ICUs having percentages of resistance as follows (42.22%, 44.44% and 44.44 %) respectively, and the antibiotic tetracycline had the antibiotics has a biggest percentage of resistance (82.22%) against *S. aureus*. Although vancomycin and cefotaxime were also the most effective antibiotics for β-lactamase non-producing S. aureus strains, they had the lowest percentages of resistance in comparison to the first above group that recorded 13.33% and 20.0% of resistance, respectively, and tetracycline still being the weakest antibiotic having great resistance of 53.82% of isolates.

The selection of an antimicrobial agent is determined by the most likely pathogen and its expected susceptibility pattern. Monitoring antibiotic susceptibility patterns of bacterial pathogens at a local level will yield important information regarding emerging problems of antibiotic resistance and provide assistance in managing empirical therapy (21,24). The widespread use of antibiotics has been responsible for the development of numerous problems, including the emergence of multidrug-resistant bacteria, increased number of nosocomial-acquired infections, and increased health care costs (42). Rising to the challenge posed by hospital-acquired

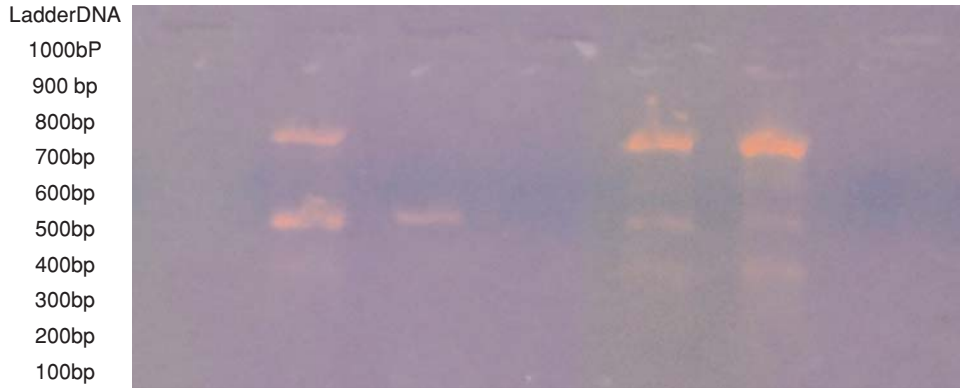

Figure 2: Gel electrophoresis result of multidrug-resistant β-lactamase non-producing *S. aureus* strains.

infections, which are emerging as a global health concern, over 1.4 million people worldwide are suffering from hospital-acquired infections. In this study, a few isolates have been found susceptible to tetracycline. However, all the isolates were susceptible to vancomycin. These findings are similar to the findings of References 15 and 43; however, they observed less percentage of MDRSA, which is much lower than the present study. The treatment of this infection is a major community indication of antibiotic usage (44). In this study, *S. aureus* isolates were resistant to tetracycline. The indiscriminate use of antibiotics may be a cause for this multidrug resistance (18). Among the eight drugs used in the present study, vancomycin and cefotaxime are the best choice for the treatment of ICU infections caused by *S. aureus*. *S. aureus* is capable of causing a variety of human infections, including fatal invasive and toxic conditions, and also possesses a differential ability to spread and cause hospital-associated outbreaks of infections (3,20).

Reports from the International Infection Control Consortium (INICC) surveillance study show that the nosocomial infection is markedly higher in the ICUs of the INICC hospitals (31). The emergence of multidrugs resistant *Staph.aureus* (MDRSA) strains, has posed a challenge in the treatment of this infection (32).

In India, the ICU infection rate is over 25% and is responsible for more mortality than any other form of accidental death. The prospective observational study describes that isolates of Acinetobacter, Pseudomonas, Klebsiella and *E. coli* are resistant to the third generation cephalosporins, and it also states that the increased duration of the time spent ICUs and days of intervention are associated with the incident (38). *S. aureus* is a pathogen of greater concern because of its virulence its ability to cause a diverse array of life threatening infections, and its capacity to adapt different environment conditions (45). A recent report about the rate of MDRSA in nosocomial infections in Isfahan, Iran, showed that 67.2% of isolates were MRSA. Geographical, health system capability in running an infection control program has a role in variability of prevalence MDRSA (46). Most isolates of MDRSA were observed in wound specimens (43%). Instead of many similar and independent studies that are not showing any relation between sex, age, site of infection and rate of MDRSA (14,20). In this study, the highest level of resistance is observed in tetracycline , which is in agreement with the other reports. Therefore, in the present investigation, the variation that occurs in the antibiotic sensitivity pattern of *S. aureus* confirms the emergence of antibiotic resistance. The resistance in bacterial pathogens to antibiotics increases the chance of severe infections in human beings (3,17).

However, the data indicate that among the eight antibiotics used in the present study, vancomycin and cefotaxime should be the drug of choice to treat the *S. aureus* infection. For proper treatment, the physician should perform the antibiotic sensitivity test before antibiotic treatment. Most of the antibiotics tested showed increased resistance with increasing age. These results suggest that clinicians should consider age and sites of infections while prescribing antibiotics.

In this study, the investigation was carried out to know the prevalence of multidrug-resistant (MDR) gene carrying plasmids in the MDRSAs, but no vivid result was found. However, multidrug-resistant isolates showed more plasmid bands, and all the isolates that did not show any plasmid were sensitive to almost all the antimicrobials. Our studies showed a 24.44% prevalence of MDRSA in the tested clinical samples, which was almost similar to that reported in Reference 34. Such high rates of MDRSA have also been reported in India – 20% and 32.8% MDRSA in some regions of India (38).

In this present study, most of the isolates that showed plasmids were found to be resistant to three antibiotics. On the other hand, no correlation was observed between tetracycline resistance and plasmid profiles. However, no inter-relation was found between the second and third generation cephalosporin resistance used in this investigation and plasmid profiles. Although the present studyshowed a tendency of multidrug-resistant isolates containing plasmids, no solid evidence could be provided. To clarify this issue, further studies are to be initiated. Abuse and irrational use of antibiotics will lead to development of drug resistance. In a developing country like Iraq, there is lack of guidelines in the practice of antibiotic prescriptions. However, our studies might provide a platform for physicians to choose and prescribe rational antibiotics in the treatment of MRSA in hospital and community infections (47-49).

In conclusion, this study indicates that some antibiotics commonly used in the treatment of ICU infections are still effective. These may be of immense value for use to determine drugs of choice in the treatment of ICU infections prior to the outcome of laboratory investigations.

However, vancomycin, cefotaxime, and gentamicin could be considered for the first-line therapy for ICU infections, which is in agreement with previous reports. Although there are some other "old antibiotics" – such as tetracycline – with a role that may be underestimated for ICU infections (50), prudent and rationale use of antibiotics must encourage prescribing vancomycin and other indicated antibiotics parsimoniously for uncomplicated UTIs.

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