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Full Length Research Paper

Prevalence of β-lactamase producing and non-producing *Staphylococcus aureus* associated with patients in intensive care unit

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A total of one hundred and twenty five samples were collected from the intensive care unit of two main hospitals in Basrah: 74 clinical samples; Skin, blood, eye, nose, wounds and urine and 51 inanimate samples; bed, wall, instruments and addresses. A total of 334 isolates of bacterial types isolated from various sources and their percentages are shown as follows: *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%), Viridance streptococci 35 (10.47%), *Streptococcus pneumonia* 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%). 31 isolates of *S. aureus* in percentage 68.89 were isolated as β-lactamase producers, while 14 isolates (31.11%) were β-lactamase non-producers. The prevalence of multiple drugs resistance *S. aureus* against eight antibiotics were carried in the present study, the resistance against three antibiotics has a 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* in percentage 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 intensive care unit in percentages of resistance (42.22, 44.44 and 44.44%), respectively and tetracycline was the antibiotics that has the highest percentage of resistance (82.22%) against the above strain. While for β-lactamase non- producing *S. aureus* strains, vancomycin and cefotaxime were also the most effective antibiotics but have the lowest percentages of resistance in comparison to the first above group which recorded (13.33 and 20.0%) of resistance respectively. And tetracycline still the weakest antibiotic has great resistance of 53.82% of isolates. The plasmid profiles in β-lactamase producing and non-producing MDRSA was also determined in this study. The ranges of band molecular weight were between 300 to 600 base pairs with clear main band in 550-570 bp for β-lactamase producing *S. aureus.* While the ranges of band molecular weight were between 200 to 700 base pairs with clear main band in 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-associated infections other than those they were admitted for because of nosocomial pathogens around them (Chambers, 2001). The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomial infections caused by drug resistant microbes (Anupurba et al., 2003).

Staphylococcus aureus, a spherical aerobic Gram positive, catalase positive, oxidase positive, non-motile, spore-forming coccus, is an opportunistic pathogen in human and animal, 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, 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 hydrolyses benzylpenicillin, ampicillin, cephalosporins, and related antimicrobials (Foster, 1996; Boyce, 1994). 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 (Shakibaie et al., 2002). 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 *Staphylococcus aureus* (VISA) (Tuo et al., 1995; Sampathukumar, 2007). Community-acquired MDRSA (multi drugs 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 (Schito, 2006; Mansouri and Khaleghi, 1997). The emergence of community acquired MDRSA that is capable of causing infections in otherwise healthy people has also been reported (Sieradzki and Tomasz, 1997). 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 (Daini and Akano, 2009) and other functions (Diep et al., 2008). 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 on transposable elements (Diep et al., 2006; Maltezou and Giamarellou, 2006). In Bangladesh, as reported previously, the frequency of MDRSA was alarming due to indiscriminate and incomplete uses of antibiotics (Rahman et al., 2002). In 2002, 47.2% MDRSA was reported in an investigation on clinical *S. aureus* isolates (Diep et al., 2006). 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% (Gradie et al., 2001). 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.

Staphylococcus aureus is the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants (Anupurba et al., 2003; Mansouri and Khaleghi, 1997). *S. aureus* infection may also be superimposed on superficial dermatological diseases such as eczema, pediculosis and mycosis (Herwaldt and Wenzel, 1996). They live as commensals in anterior naves of over half the population of humans (Hotu et al., 2007). 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 infection most commonly in tissues and sites with lowered host resistance such as in individuals with diabetes, old malnourished persons and other chronic cases (Foster, 1996; Maltezou and Giamarellou, 2006). *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 staphylococcal infection spreads to the lungs (Foster, 1996). 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 theatre during operation, and others post-operations in the ward (Hsueh et al., 2005). 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 (King et al., 2006). 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 (Francis et al., 1997; Marples and Reith, 1996). *S. aureus* strains carries a wide variety of multi-drug resistant genes on plasmids, which can be exchanged and spread among different species of Staphylococci (Mehta et al., 1998). The multi-resistance 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 (Foster, 1996; O'Brien et al., 1999; Okuma et al., 2002).

Hospital strains of *S. aureus* are usually resistant to a variety of different antibiotics. Few strains are resistant to all clinically useful antibiotics except vancomycin. Some workers have reported however the presence of vancomycin resistant strains (Daini and Akano, 2009; Olukoya et al., 1995).

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 unit of two main hospitals in Basrah city (Al-Sadder teaching and general Basrah hospitals) from June to November, 2011, using sterile swab saturated with Brain – Heart Infusion. All specimens were transported immediately to the laboratory and cultured within 3 to 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℃ for 24 h under aerobic conditions. After 24 h of incubation, 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 (Forbes et al., 2002; Cowan and Steel, 2004).

Isolates that were gram-positive, that is, cocci, catalase positive, coagulase positive, and form 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 disc diffusion technique. A total of eight antibiotics were tested. A 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 by using a sterile glass L-shaped spreader and allowed to dry for about 15 to 30 min. The antibiotic discs were placed on the agar using sterile forceps. Each disc was placed far from each other to avoid their zones of inhibition from coalescing into the other. The plates with the antibiotic discs were then incubated at 37℃ for 24 h to observe the zones of growth inhibition produced by the antibiotics. The antimicrobial disks were sourced from the HiMedia Laboratories Ltd., Mumbai, India as follow:

Tetracycline (TET), Gentamicin (GEN), Amoxicillin (AMOX), Ciprofloxacin (CIP), cefotaxime (CEF), Amoxycillin/Clavulanic Acid (AMOX/CLA), Vancomycin (VAN), Methicillin (METH).

The zone diameters measured around each disk were interpreted on the basis of Bauer et al. (1966), according to guidelines by the NCCLS (2002).

β-Lactamase test (Adegoke and Komolafe, 2009; Ekramul et al., 2011)

β-Lactamase production was assayed by the acid-formation method. A piece of Whatman No.1 filter paper (5×6) was briefly placed in a sterile Petri dish. The bluish penicillin solution was added drop wise 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℃ 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 (Maniatis et al., 1989; Adeleke and Odelola, 1997; Hamid et al., 2011)

Plasmid profiles of 14 MDR *S. aureus* strains were determined in Laboratory of Biotechnology, College of Veterinary Medicine and Oklahoma Laboratory of Biotechnology, College of Science, University of Basrah by the Mini Prep method followed by band separation on horizontal gel electrophoresis in 1.5% agarose in 1X Tris-Borate-EDTA (TBE) buffer at room temperature (Lech and Brent, 1987; Kraft et al., 1988). Briefly, single purified bacterial colonies were seeded each into 10 ml Mueller-Hinton broth (HiMedia) in screw cap tubes and incubated overnight at 37℃. After centrifugation of 1.5 ml of the overnight culture for 1 min, the pelleted cells was 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 until it was completely mixed. The solution was micro-centrifuged 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℃for 15 min. The dried pellet was re-suspended in 4 0 µl TE buffer and stored at 4℃ 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 megapixel). 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) package (Version 17).

RESULTS

A total of one hundred and twenty five samples were collected from the intensive care unit of two main hospitals in Basrah (74 clinical samples and 51 inanimate samples) (Table 1), with no statistical differences at $P \geq 0.05$:

1. Clinical samples: Skin 16, blood 10, eye 11, nose 11, wounds 14, and urine 12 samples.
2. Inanimate samples: bed 13, wall 12, instruments 15 and addresses 11 samples.

Table 2 show the total of 334 isolates of bacterial types isolated from various clinical and inanimate sources of

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

Samples type		Numbers of samples*	%
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
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 types at time once. #: there are no statistical differences between sites of postoperative wounds ($P \geq 0.05$).

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

Bacterial types	Total number of isolates (%)	Numbers 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
Viridance streptococci	35(10.47)	7	3	4	3	3	0	3	4	5	3
Streptococcus pneumonia	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 a highly statistically differences between numbers of isolates and various clinical and inanimate sites $P<0.01$.

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

Type of isolates	Total no 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 a highly statistically differences between numbers of β-lactamase producing and non-producing *S. aureus* isolates in various clinical and inanimate sites *P<0.01*.

Table 4. Prevalence of Multiple drugs resistance isolates of β-lactamase producing and non- producing *Staph. aureus*.

Type of isolates	Total no of tested strains (%)	Numbers of resistant isolates (%)								
		0	1	2	3	4	5	6	7	8
β-lactamase producing *Staph. 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 *Staph. 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 a highly statistically differences between numbers of resistant isolates of β-lactamase producing and non- producing *S. aureus* P<0.01.

the intensive care unit. The number of their isolates and percentages are shown as follows: *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%), Viridance streptococci 35 (10.47%), *Streptococcus pneumonia* 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%).

There is a highly statistically differences between numbers of isolates and various clinical and inanimate sites (P<0.01).

Staphylococcus aureus was the main predominant bacterial pathogens isolated from both types of samples, so we used these 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 from various sources are illustrated in Table 3. 31 (68.89%) isolates of *S. aureus* were isolate of β-lactamase producers, while 14 isolates (31.11%) were β-lactamase non-producers. There was a highly statistically differences between numbers of β-lactamase producing and non-producing *S. aureus* isolates in various clinical and inanimate sites *P<0.01*.

Table 4 illustrated the prevalence of multiple drugs resistance of *S. aureus* against eight antibiotics. We found that resistance against three antibiotics has the highest 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.

There is a highly statistically differences between numbers of resistant isolates of β-lactamase producing and non- producing *S. aureus* (*P<0.01*).

Tables 5 and 6 illustrates profile of antibiotic resistance of β-lactamase producing and non-producing *S. aureus* strains isolated from clinical and inanimate samples of intensive care unit. The study found that vancomycin, cefotaxime and gentamicin were the most effective antibiotics

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

Samples type		Total no of tested strains	Number of resistant isolates							
			TET	GEN	AMOX	CIP	CEF	AMOX/CLA	VAN	METH
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
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)

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

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

Samples type		Total no of tested strains	Number of resistant isolates							
			TET	GEN	AMOX	CIP	CEF	AMOX/CLA	VAN	METH
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	0	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
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)

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

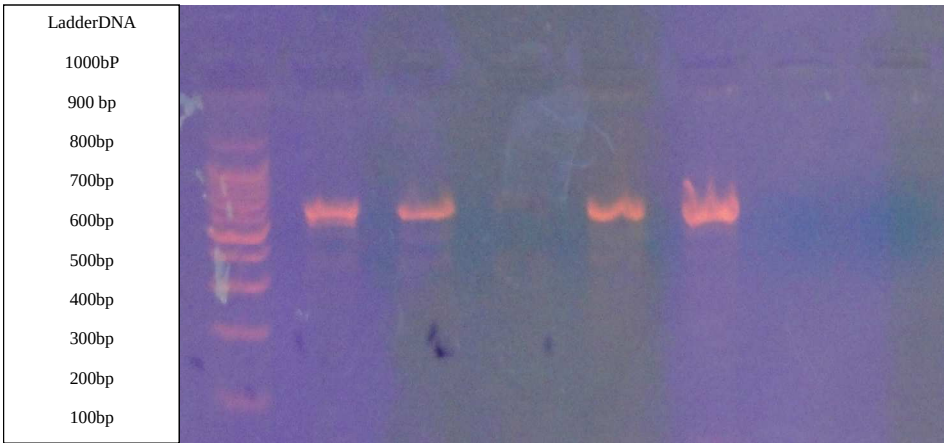


Figure 1. Gel electrophoresis result of multidrug resistant β-lactamase producing *Staphylococcus aureus* strains.

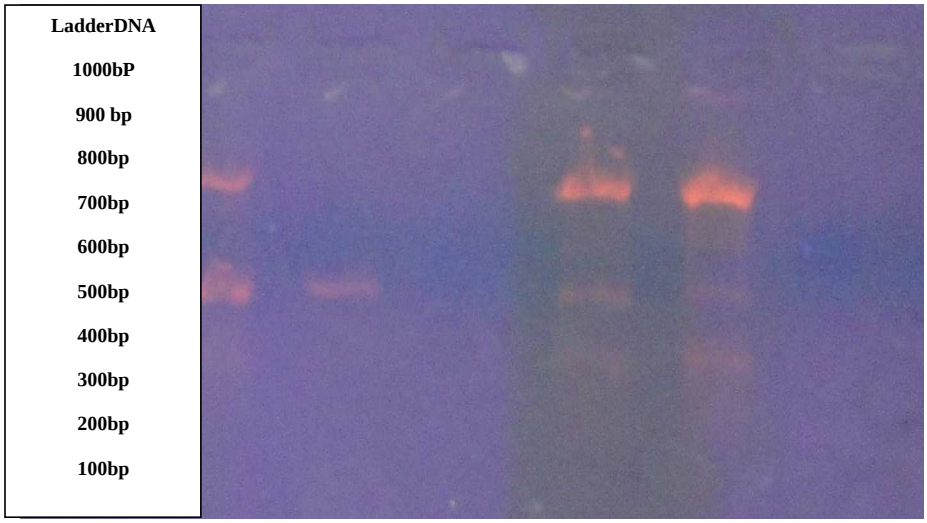


Figure 2. Gel electrophoresis result of multidrug resistant β-lactamase non-producing *Staphylococcus aureus* strains.

against β-lactamase producing *S. aureus* strains isolated from both clinical and inanimate samples of intensive care unit in percentages of resistance (42.22, 44.44 and 44.44%) respectively. And tetracycline as antibiotics has the highest percentage resistance (82.22%) against the aforementioned strain. While for β-lactamase non-producing *S. aureus* strains, also vancomycin and cefotaxime were the most effective antibiotics, but have the lowest percentage resistance compared to the first aforementioned group, which recorded 13.33 and 20.0% of resistance, respectively, and tetracycline as the weakest antibiotic has great resistance of 53.82% of isolates.

To look into the plasmid profiles in β-lactamase producing and non-producing MDRSA, we selected 14 multi-drug resistant strains, isolated the plasmid DNA by alkaline lysis miniprep method and analyzed using agarose gel electrophoresis (Figures 1 and 2). The ranges of band molecular weight were between 300 to 600 base pairs with clear main band in 550-570 bp forβ-lactamase producing *S. aureus,* while the ranges of band molecular weight were between 200 to 700 base pairs with clear main band in 450-470 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 (Udo et al., 1993). The present study isolated a total of 334 isolates of bacterial types from various clinical and inanimate sources of intensive care unit (ICU); with the numbers of isolate and thier percentages given as follows: *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%), Viridance streptococci 35 (10.47%), *Streptococcus pneumonia* 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%). All these isolates have been shown to cause different nosocomial infections, especially intensive care unit infections. The result of our study were approved by results of recent studies (Maltezou and Giamarellou, 2006; Rahman et al., 2002; Vidhani et al., 2001; Karlowsky et al., 2003; Laupland et al., 2007; Levy, 1998). Test for β-lactamase production revealed that 68.89% isolates produced β-lactamase. The highest numbers of isolates were from wounds and instruments.

The data obtained in this study showed that resistance against three antibiotics has the highest 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. Vancomycin, cefotaxime and gentamicin were the most effective antibiotics against β-lactamase producing *S. aureus* strains isolated from both clinical and inanimate samples of intensive care unit with percentage resistance of 42.22, 44.44 and 44.44%, respectively and tetracycline as an antibiotic has the highest percentage resistance of 82.22% against the aforementioned strain. While for β-lactamase non- producing *S. aureus* strains, vancomycin and cefotaxime, were the most effective antibiotics, but have the lowest percentage resistance in comparison to the first aforementioned group, which recorded 13.33 and 20.0% resistance, respectively. And tetracycline as the weakest antibiotic has great resistance of 53.82%.

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 the emerging problems of antibiotic resistance and provide assistance in managing empirical therapy (Francis et al., 1997; O'Brien et al., 1999). The widespread use of antibiotics has been responsible for the development of numerous problems, including the emergence of multi drug resistance bacteria, increased number of nosocomial acquired infections and increased health care costs (Maple et al., 1989). Rising to the challenge posed by hospital acquired infections, which are emerging as a global health concern, over 1.4 million people worldwide are suffering from hospital acquired infections. In this study, few isolates have been found susceptible to tetracycline. On the other hand, all the isolates were susceptible to vancomycin. These findings are similar to the findings of Rahman et al. (2002) and Cunha (2006). But they observed less percentage of MDRSA, which is much lower than the present study. Treatment of this infection is a major community indication of antibiotic usage (Felmingham, 2002).

In this study, *S. aureus* isolates were resistant to tetracycline. The indiscriminate use of antibiotics may be a cause for this multidrug resistance (Hotu et al., 2007). Among the eight drugs used in the present study, vancomycin and cefotaxime are the best choice for the treatment of *S. aureus* caused ICU infections. *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 (Foster, 1996; King et al., 2006).

Reports from the International Infection Control Consortium (INICC) surveillance study showed that nosocomial infection is markedly higher in the ICUs of the INICC hospitals (Adegoke and Komolafe, 2009). The emergence of multidrugs resistant *S. aureus* (MDRSA) strains, has posed a challenge in the treatment of infection (Ekramul et al., 2011).

In India, 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 Escherichia coli* are resistant to the third generation cephalosporins, and it also states that the increased duration of the time spent in intensive care units and days of intervention are associated with the incident (Vidhani et al., 2001). *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 (Aubry-Damon et al., 1998). Recent report about the rate of MDRSA in nosocomial infections in Isfahan Iran showed that 67.2% of isolates were MRSA geographical, and health system capability in running infection control program has role in variability of prevalence MDRSA (Fatholahzadeh et al., 2008). Most isolates of MDRSA were observed in wound specimens (43%). Instead of many similar and independent studies that is not showing any relationship between sex, age, site of infection and rate of MDRSA (Maltezou and Giamarellou, 2006; King et al., 2006).

In this study, the highest level of resistance is observed in tetracycline, which is in agreement with the other

reports. So, 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 (Foster, 1996; Herwaldt and Wenzel, 1996).

However, the data indicated that among eight antibiotics used in the present study, vancomycin and cefotaxime should be the drug of choice to treat *S. aureus* infection. For proper treatment, the physician should perform the antibiotic sensitivity test before antibiotic treatment. It is as an important factor while prescribing antibiotics also observed that resistance of most of the antibiotics tested shows increased resistance with increasing age. These results suggest that clinicians should consider age and sites of infections.

In this study, investigation was carried out to know the prevalence of multiple-drug resistant (MDR) gene carrying plasmids in the MDRSAs but no vivid result was found. However, multi-drug resistant isolates showed more plasmid bands and all the isolates which 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 by Adeleke and Odelola (1997). Such high rates of MDRSA have also been reported in India: 20 and 32.8% MDRSA in some regions of India (Vidhani et al., 2001).

In this present study, most of the isolates which 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 2nd and 3rd generation cephalosporin-resistance used in this investigation and plasmid profiles. Although, in the present study, it was observed that there is a tendency that multi-drug resistant isolates contain plasmids but no solid evidence could be provided. In order to clarify this issue, further studies are to be initiated. Abuse and irrational use of antibiotics will lead to development of drug resistance. In 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 (Khorvash et al., 2008; Kohner et al., 1999; Zhang et al., 2005).

In conclusion, this study indicates that some antibiotics commonly used in treatment of ICU infections are still effective. They 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 while vancomycin, cefotaxime and gentamicin could be considered for first-line therapy for ICU infections, in agreement with previous reports. Although there are some others "old antibiotics" such as tetracycline with a role that may be underestimated for ICU infections (Honderlick et al., 2006), prudent and rationale use of antibiotics must encourage prescribing vancomycin and other indicated antibiotics parsimoniously for uncomplicated UTIs.

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