Comparative phenotypic and genotypic study of MSSA and MRSA wound infections in Babylon

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Abstract

The study aim to evaluate and compare phenotypic and genotypic features of methicillin sensitive (MSSA), and methicillin resistance (MRSA) *S. aureus* isolates. Out of 113 infected wound swabs from outpatient clinic in Babylon/Iraq, only (31) (27.43%) *S. aureus* isolates were isolated by selective medium, and cefoxitine disk diffusion was used to differentiate MSSA from MRSA in order to study the comparative phenotypic and genotypic features. Vancomycin minimum inhibitory concentration (MIC) was used also. The PCR assay was used for direct detection of methicillin (mec A) and vancomycin (van A) antibiotics resistance gene in S. aureus isolates. Results showed that (17) out of (31) isolates were MRSA, fifteen of them harbor Mec A gene. While only (14) out of (31) isolates were MSSA, two of them harbor the Mec A gene, and there was no single vancomycin resistance in all isolates. Two (2) MRSA isolates have intermediate vancomycin susceptibility (MIC 8-16µg/ml) and just two (2) isolates having Van A gene. MRSA nearly resist all β lactam, cloxacillin, gentamycin and ciprofloxacin, while the MSSA isolates were sensitive for the commonly used antibiotic with high resistant rate to penicillin, amoxicillin and amoxicillin clavulante. In conclusion, MRSA has become a major public health problem with decreased susceptibility to antibiotics that necessate the availability of highly sensitive diagnostic test like PCR with routine laboratory techniques (based on the detection of the mec A gene) (or cefoxitine disc diffusion method) to differentiate MSSA from MRSA and the availability of the highly active antibiotic in order to control their spreads as early as possible.

Key words: *Staphylococcus aureus*, wound infection, MRSA, antibiotic sensitivity of *S. aureus*, antibiotic resistance gene of *S. aureus*

دراسة مقارنة الصفات المظهرية والوراثية بين بكتريا المكورات العنقودية الحساسة والمقاومة لعقار الميثسلين في عدوى الجروح/محافظة بابل

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الخلاصة

تهدف الدراسة الى تقييم ومقارنة الصفات المظهرية والوراثية لبكتريا المكورات العنقودية بنوعيها الحساس والمقاوم للميثسلين. تضمنت هذه الدراسة أخذ 113مسحة ، حيث تم عزل 31 عزلة من المكورات العنقودية الذهبية المعزولة من مرضى يعانون من التهاب الجروح من العيادات الخارجية في بابل/العراق من خلال أوساط انتقائية واستخدام قرص cefoxitine لتمييز MSSA عن MRSA من أجل دراسة ومقارنة الصفات المظهرية والوراثية. وقد استخدم الفانكومايسين لتحديد الحد الأدنى للجرعة المثبطة (MIC) أيضا. تم استخدام فحص PCR للكشف المباشر عن جين مقاومة الميثسلين والفانكومايسين. أظهرت النتائج أن (31) عزلة من أصل (113) عزلة كانت بكتريا المكورات العنقودية (27.43%) وكانت 17 عزلة مقاومة للميثسلين منها 15 عزلة تحمل جين مقاومة الميثسلين بينما مجرد 14 من اصل 31 عزلة كانت حساسة للميثسلين وأثنان فقط تحمل جين المقاومة ولم تكن هنالك عزلة مقاومة للفانكومايسين. وجد كذلك ان عزلتين من هذه العزلات كانت تحمل حساسية متوسطة للفانكومايسين واثنان فقط تحمل جين المقاومة للفانكومايسين. كانت جرثومة MRSA مقاومة تقريبا لكل البيتالاكتام، الكلوكساسيللين، الجنتاميسين، والسيبروفلوكساسين. بينما كانت جرثومة MSSA حساسة للمضادات الحيوية التي تستخدم عادة مع ارتفاع معدل مقاومة للبنسيلين ، الاموكسلين والاموكسيلين مع

الكلوفانيت. يستنتج من هذه الدراسة بأن هذه الجرثومة أصبحت تشكل مشكلة صحية رئيسية مع انخفاض الحساسية للمضادات الحيوية ومن الواجب تقديم اختبار تشخيصي حساس للغاية مثل PCR ضمن الطرق الروتينية المختبرية (على أساس الكشف عن الجين) (أو القرص cefoxitine بطريقة الانتشار) لتمييز جرثومة MSSA عن MRSA و توافر المضادات الحيوية النشطة للغاية من أجل السيطرة على أنتشار الجرثومة بصورة مبكرة قدر المستطاع.

الكلمات المفتاحية: بكتريا المكورات العنقودية الذهبية ، عدوى الجروح ، بكتريا المكورات العنقودية الذهبية المقاومة للمثسلين ، الحساسية الدوائية لبكتريا المكورات العنقودية الذهبية ، الجينات المقاومة للمضادات الحيوية لبكتريا المكورات العنقودية الذهبية.

Introduction

S. aureus is the most common cause of pyogenic infection, causing a range of infections that includes boils, abscesses, septic fingers, impetigo and sticky eye in neonate (1) and it is one of important cause of nosocomial infections, including bacteremia, surgical wound infections, as well as pneumonia (2,3,4). The first isolate of methicillin-resistant *S. aureus* (MRSA) was reported in 1961 in England (5), then it has become a major cause of hospital acquired infection, and is being recognized with increasing frequency in community acquired infections throughout the world (6), moreover, half of *S. aureus* in many centers are methicillin resistant (multidrug resistant) posing major therapeutic challenge (7). MRSA is usually acquired during exposure to hospitals and other healthcare facilities and causes a variety of serious healthcare-associated infections (8). Its determine by the availability of weak patients, selective pressure exert by antimicrobial use, increased potential for transmission from larger numbers of colonized or infected patients (colonization pressure), and the impact of implementation and adherence to prevention efforts (9). MRSA determine by (mec A) gene which composed of 50 kb of DNA chromosome which there was cross-resistant to all currently license β-lactam antibiotics (10). The MRSA infections are usually treated by vancomycin, linezolid, daptomycin, teicoplanin, quinupristine-dalfopristine and tigecycline. The glycopeptides vancomycin has been regarded as the drug of choice for the treatment of infections due to methicillin-resistant strains (11). Currently MRSA had been acquire resistance even to vancomycin, so in the near future the treatment options for MRSA infections are going to shrink further. It is epidemiologically important, so prompt diagnosis of MRSA infection is necessary. So the aim of the study was to use polymerase chain reaction (PCR) technique to detect methicillin and vancomycin antibiotics resistance gene in *S. aureus* and to study the antibiotic susceptibility as the development of multiple antibiotics resistance and control of disease transmission had been recognized as a major challenge. Also knowledge of prevalence of methicillin (MRSA) and vancomycin (VRSA) resistant *S. aureus* and their antimicrobial profile become necessary in the selection of appropriate empirical therapy to early limit the emerging multidrug-resistant *S. aureus.*

Materials and methods

Sample collection: (113) infected wound swab samples were collected from outpatient clinic in Babylon/Iraq. Samples were directly transferred into microbiology laboratory for bacterial isolation.

Bacterial isolation: *S. aureus* was isolated by inoculation of samples on Brain Heart Infusion Broth medium at 37°C overnight for primary enrichment culture and then the bacterial growth were inoculated on mannitol salt agar (MSA) at 37°C overnight for selective isolation of pure culture *S. aureus* strains. All *S. aureus* strains were identified according to (12, 13).

Detection of methicillin resistant *S. aureus* (MRSA): An Oxacillin and Cefoxitine disc was used as an alternative to methicillin. 0.5 McFarland standard suspension of the isolate was made on MHA plate. Plates were incubated at 37C˚ for 18 hours. Inhibition zone diameters < 19 mm was reported as methicillin resistant and >20 mm was considered as methicillin sensitive (14).

Detection of penicillin-resistant *S. aureus* (PRSA): Cefoxitine-resistant *staphylococci*

were resistant to all currently available β-lactam antibiotics. Thus, susceptibility or resistance to a wide-ranging array of β-lactam antibiotics might be deduced from testing only penicillin and cefoxitine (14)

Vancomycin susceptibility: It was done in broth dilution test; the results were compared with standard break points values. Vancomycin minimum inhibitory concentration determination (MIC) test was achieved to determine the susceptibility of staphylococcal isolates to vancomycin (18); it was achieved according to (25).

Antibiotic susceptibility test: All *S. aureus* isolates were tested for detection of susceptibility of them for the commonly used antimicrobial agents by kirby-Bauer method on Muller-Hinton agar (MHA) (Hi-media) (26). Plates were incubated at 37°C for 18 hrs. Following the incubation, the diameter of inhibition zone was used as parameter for determination of sensitivity as compared with standard zones of growth inhibition table:
penicillin (30µg), amoxicillin (25µg), amoxicillin-clavulanate (20/10µg), imipenem (10µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), meropenem (10µg), gentamycin (l0µg), rifampin (30µg), erythromycin (15µg), doxycycline (30µg), tetracycline (30µg), ciprofloxacin (CIP 30µg), amikacin (30µg) and norfloxacin. The results were interpreted according to the standard inhibition zone diameter as recommended by (16).

Bacterial genomic DNA extraction: Bacterial genomic DNA (gDNA) was extracted from *S. aureus* isolates by using (PrestoTM Mini gDNA Bacteria Kit. Geneaid USA). One ml of overnight bacterial growth on BHI broth was placed in 1.5ml micro centrifuge tubes and then transferred in centrifuge at 10000 rpm for 1 minute. After that the supernatant was discarded and the bacterial cells pellets were used in genomic DNA extraction according to company instruction. The extracted gDNA was checked by Nano drop spectrophotometer and store at -20°C in refrigerator until perform PCR assay.

Polymerase chain reaction (PCR): PCR assay was performed by using specific primer for detection methicillin (Mec) and vancomycin (Van) antibiotics resistance gene. Primes were designed by using NCBI-GenBank recorded sequence for mecA gene (Genbank: KC243783.1) and Van A gene (Genbank:GQ273978.1) and by using primer 3 plus design online. Primers were provided by (Bioneer company / Korea), and the sequences of primers as below.

Primer	Sequence		Product size
van A	F	AGCTGTACTCTCGC CGGATA	284bp
	R	CCACCGGCCTATCA TCTTTA	
mec A	F	GGCCAATACAGGA ACAGCAT	421bp
	R	AACGATTGTGACA CGATAGCC	

PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified gDNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Mygene Bioneer. Korea) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 58 °C for 30 s, and extension 72 °C for 1min and then final extension at 72 °C for 10 min. The PCR products were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and visualized under UV trans illuminator.

Statistical analysis: Data were presented as means ± standard deviation (SD) and the results were analyzed using SPSS version 14 statistical system. Student t test was used to compare between results. $P<0.05$ was considered as statistically significant value.

Results

Methicillin resistance gene (mecA) had been detected by PCR technique. positive isolates had been seen (Fig. 1). Also PCR technique had showed the presence of van A gene in two isolates (Fig. 2). No isolate was found to be VRSA by phenotypic methods.

The study displayed presence of risk factors associated with MSSA and MRSA wound infections attainment. The patients with MRSA have gained a history of prolonged hospitalization, prior antimicrobial therapies and prior surgery in a ratio of (70.85, 76.47 and 64.7%) respectively, while they were (21.42, 21.42& 14.28%) respectively for MSSA wound infections which were statistically significant $P \leq 0.05$ (Table 1). The frequency of MSSA and MRSA among *S. aureus* wound infections with regard to risk factor shown four (4) out of (14) cases had been recognized as MSSA according to risk factor, while in case of MRSA wound infections, (15) out of (17) (88.23%) gave history of one or more of the above mentioned risk factors and hence they were regarded as hospital acquired infections, while only two (2) out of (17) (11.77%) did not give history of the above mentioned risk factors, hence they were regarded as community acquired wound infections (Table 2). The frequency of mec A and van A genes among MSSA and MRSA wound infections was seen significantly high (88.23%) in MRSA, in comparison to MSSA isolates (14.23%) (Table 3).

Antibiotic resistance and sensitivity:

A) Penicillin G. The study showed (85.71%) of the MSSA isolates were resistant to penicillin G. In addition to that, these isolate showed also resistant rate against amoxicillin and amoxicillin clavulanic acid (81.42% and 64.82 %) respectively**.** All MRSA were seen resistant (100%) to penicillin, amoxillin and amoxicillin clavulanic acid.

B) Cloxacillin: The resistance rate to cloxacillin was (21.42 %) in MSSA, while it is (82.35%) in MRSA.

C) Carbapenems: The study showed that (2.14%) of MSSA and (35.29%) of MRSA were resistant to imipenem, while it was (14.28%) and (76.47%) in case of meropnem respectively.

D) Cefoxitine: All MSSA isolates were sensitive to cefoxitine except those two isolates which had the mecA gene typical of methicillin resistant (14.28%). On the other hand, (88.23%) of MRSA isolates gave (100%) cefoxitine resistance and all of them carried the mecA gene (Table 4).

E) Trimethoprim/sulfamethoxazole: High resistance rate of MRSA was recorded against trimethoprim/sulfamethoxazole (94.11 %) in comparison to (53.3%) resistance rate of MSSA was found.

F) Resistance to aminoglycosides and macrolides: High resistance for gentamicin (82.35%) than amikacin (11.76%) in MRSA while they are (35.71%) and (2.41%) in MSSA respectively. MRSA show erythromycin resistant rate of (64.7%) while only (7.14%) of MSSA isolates was resist erythromycin. Also there was resistance to gentamicin and macrolide.

G) Rifampin: Resistance to Rifampin has been detected, and the percentage of *S. aureus* resistance to this antibiotic was found to be (7.14%) in MSSA while it was 11.76% in MRSA**.**

H) Tetracycline and Doxycycline: High resistance rate (58.82%) was detected to tetracycline, while resistance rate to doxycycline was (47.05%) in MRSA while they are (14.28%) in contrast to (7.14 %) in MSSA respectively.

I) Norfloxacin: There was a high frequency of norfloxacin resistance in MRSA in comparison to MSSA(70.58% versus 21.42%).

J) Ciprofloxacin: There was a high resistance rate for ciprofloxacin (76.47%) in MRSA in comparison to (7.14%) in MSSA isolates.

K) Vancomycin: Vancomycin was seems to be the only antimicrobial agent who showed (100%) sensitivity (Fig.3).

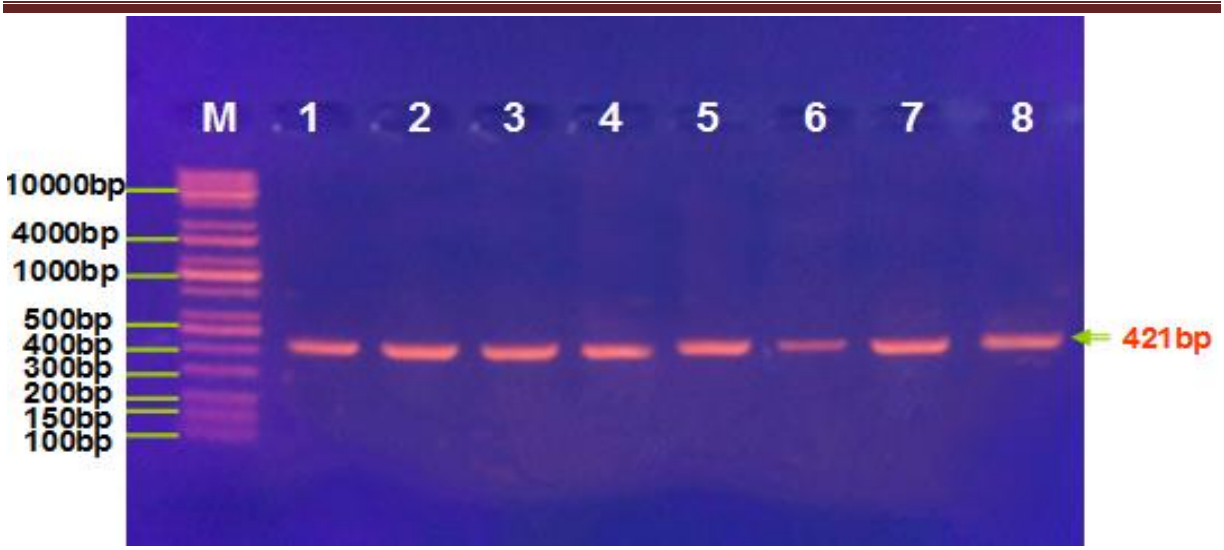

Fig. (1): Detection of methicillin resistance gene (mecA) PCR products by agarose gel electrophoresis, where, lane (M) DNA marker (100bp) and lane (1-8) mecA gene positive isolate.

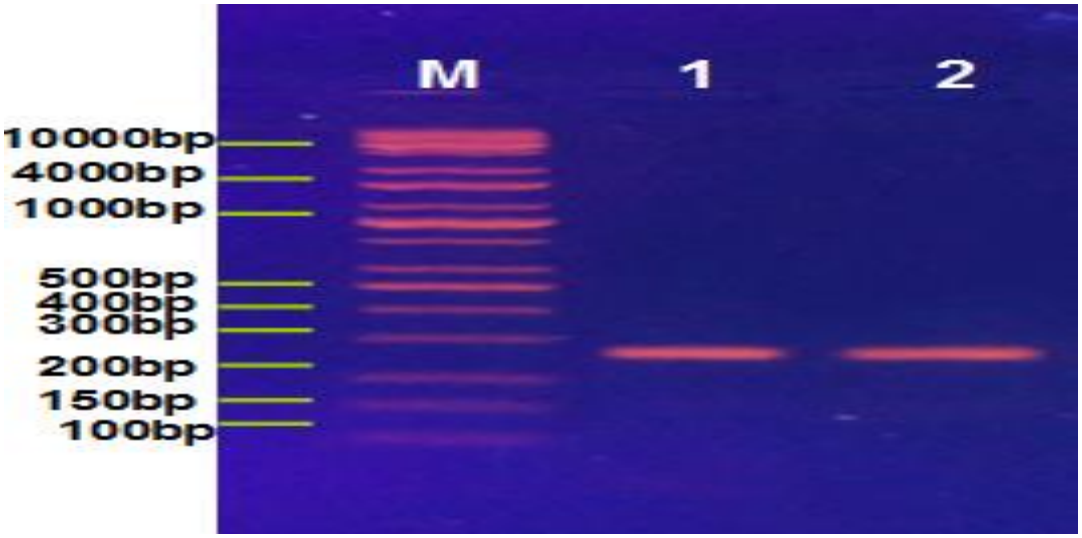

Fig. (2): Detection of Vancomycin resistance gene (van A) PCR products by agarose gel electrophoresis, where, lane (M) DNA marker (100bp) and lane (1&2) Vancomycin resistance isolates.

Table (1): Risk factors associated with MSSA and MRSA wound infections

Risk factors	MSSA	Total	%	MRSA	Total	%
Prolong hospitalization	3	14	21.42	12	17	70.85
Prior antimicrobial therapies	3		21.42	13		76.47
Prior surgery	2		14.28	11		64.70

Table (2): Frequency of MSSA and MRSA among S. aureus wound infections with regard to risk factors

Source of infections	MSSA	%	MRSA	%	total
Hospital acquired	4	28.57	15	88.23	19
Community acquired	10	71.43	2	11.77	12
Total	14	100	17	100	31

Table (3): Frequency of mec A and van A genes among MSSA and MRSA wound infections

Type of *S.aureus*	Total	mec A gene	%	van A gene	%
MSSA	14	2	14.28	0	0
MRSA	17	15	88.23	2	11.77

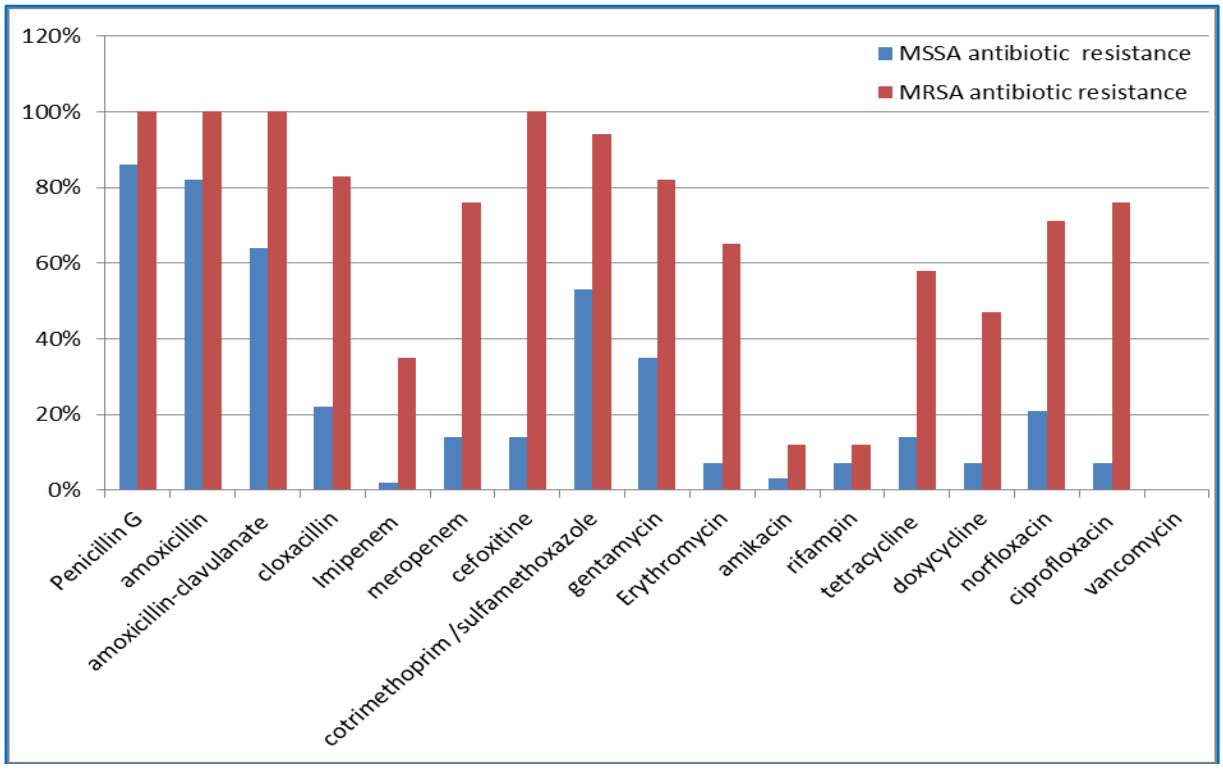

Fig. (3) :Antibiotic resistance among MSSA and MRSA wound infection

Table (4): Cefoxitine susceptibility in relation to mec A gene among MSSA and MRSA

Cefoxitine	MSSA (N=14)				MRSA (N=17)			
	mec A gene positive	%	mec A gene negative	%	mec A gene positive	%	mec A gene Negative	%
Susceptible	0	0	12	85.72	0	0	0	0
Resistant	2	14.28	0	0	15	88.2	2	11.8

Discussion

The study showed that the patients with MRSA and MSSA wound infections have a history of prolonged hospitalization, prior antimicrobial therapies, and prior surgery. These risk factors might be the causes behind the acquisition of *S. aureus* mec A gene (27). Any case had one or more of the previously mentioned risk factors were considered to be hospital acquired and if there was no risk factor at all, the infection was considered as community acquired (27). Although MRSA was until recently considered a healthcare-associated pathogen, several recent reports had documented the emergence of infections in non-healthcare settings, in patients with no established risk factors for MRSA acquisition. In contrast to nosocomial MRSA infection, infections caused by community-acquired MRSA were often mild and analogous to those caused by MSSA, but severe infections. This was consistent with (28,29). The frequency of mec A gene in MRSA was significantly high (88.23%) in comparison to MSSA isolates (14.23%)($P \leq 0.05$). This might be due to increase in the transmission rate of this gene among these

isolates (30) which was responsible for intrinsic resistance against penicillin binding protein (31). MRSA was determined by the availability of weak patients, selective pressure exerted by antimicrobial use, increased potential for transmission from larger numbers of colonized or infected patients (colonization pressure), and the impact of implementation and adherence to prevention efforts (9). Resistance resulted from the chromosomal acquisition of novel DNA, leading to production of a new penicillin-binding protein, termed PBP2a, with a low binding affinity for methicillin. PBP2a was encoded by mec A, part of the mobile genetic element, the staphylococcal chromosomal cassette mec (32).The source of mec A was unknown; however, it had been suggested that it was acquired from a coagulase-negative staphylococcal species (33,34). Waves of clonal dissemination of methicillin resistant *S. aureus* (MRSA) strains spread rapidly across the world, accounting for varying proportions of nosocomial *S. aureus* infections in different countries (33). However, if effective control measures are taken to prevent further MRSA transmission, MRSA prevalence might be reduced to sporadic levels (35,36).

Antibiotic resistance and sensitivity:

A)Penicillin G. This study showed (85.71%) of the MSSA isolates were resistant to this drug. In response to β-lactam chemotherapy, *S. aureus* had sequentially acquired resistance genes, *blaZ* that codes for a β-lactamase and confers resistance to penicillin only (37, 38, and 39). In addition to that, these isolate showed also resistant rate against amoxicillin and amoxicillin clavulanic acid. This possibly might be due to addition of clavulanic acid that could inhibit the β-lactamases enzyme action (40). This was consistent with (37,38).

B) Cloxacillin: The resistance rate to cloxacillin was (21.42 %) in MSSA while it is (82.35%) in MRSA.

C) Carbapenems: The study showed that the MSSA and MRSA were resistant to imipenem and meropnem. Although the Carbapenemswere highly resistant to the β-lactamase enzymes (41), but the resistance might arise due to penicillin-binding proteins mutation, resistance to diffusion across the bacterial outer membrane and production of metallo-β-lactamases (42), thus imipenem is considered better than meropnem in *S. aureus*e mpirical treatment.

D) Cefoxitine: All MSSA isolates were sensitive to cefoxitine except those two isolates which had the mec A gene typical of methicillin resistant. On the other hand, (88.23%) of MRSA isolates gave (100%) cefoxitine resistance and all of them carried the mec A gene. As the Mec A gene based PCR methods had accepted as gold standard for MRSA detection (43, 44, 45, 46, 47, 48), which considered as excellent inducer of mec A gene (49,50). All isolates which had mec A gene therefore gave similar results in case of cefoxitine disk diffusion, so cefoxitine disk diffusion might be used as alternative method for PCR which could be adapted for use in the reference health care institutions only. Since molecular methods are not always available for most medical institutions, thus phenotypic methods through the use of the cefoxitine disk diffusion might be helpful instead in these laboratories (51).

E) Trimethoprim/sulfamethoxazole: High resistance rate of MRSA was recorded against trimethoprim/sulfamethoxazole. This study was agreed with results obtained by (52).This may be due to mutation of the dihydrofolatereductase due to excessive use of trimethoprim/ sulfamethoxazole without doing culture and sensitivity, so it is among the antibacterial agents that had been rendered ineffective due to misuse and overuse of these antibiotics, for which there were serious concerns regarding bacterial resistance (53). Therefore, to prevent treatment failures in the absence of antibiotic susceptibility testing data, public insight on the uselessness of these antibiotics against *S. aureus* infections, and the performing of effective drug policies are urgently wanted.

F–Resistance to aminoglycosides and macrolides: In both MRSA and MSSA high resistance for gentamicin than amikacin was found. This possibly due to misuse of gentamycin especially without culture and sensitivity. Moreover, the usage of low dosages of antibiotics might inhibit the

susceptible bacterial growth but at the same time, they would leave smaller number of already resistant bacteria to grow and thrive. These bacteria would spread their resistant strain to other bacteria that were previously nonresistant cells (45). Resistance of MRSA to erythromycin confers cross resistant to other macrolides antibiotics this might be due to the ribosomal receptor site mutation or receptor modification (54, 55, 56, 57, 58, and 59). MRSA isolates show high resistant rate for erythromycin. The resistance to gentamicin and macrolide occurred because *blaZ* A encodes β-lactamase and is part of a plasmid transposable element, which often also contains genes resistant to other antibiotics (33).

G) Rifampin: MRSA show more resistance to rifampin than MSSA. This might be due to longtime bacterial requirement to develop antibiotic resistance at cell division with high rate (60, 61). Rifampin not use singly as antimicrobial treatment, so combined treatment with it leads to reduction of *S. aureus* antibiotic resistance (62).

H) Tetracycline and Doxycycline: In MRSA high resistance rate was detected to tetracycline, and less to doxycycline, while very low in MSSA isolates. This might be due to overuse of these antibiotics in skin diseases. (37) found that the tetracycline resistance of MRSA was 40% while (63) showed that it was 37.8%. The resistance rate for doxycycline in the present study was more than the result obtained by (37). The resistance to these antibiotics are plasmid mediated (64) and usually mobile genetic elements implanted and this differences might be due to geographical variations.

I) Norfloxacin: High frequency of norfloxacin resistance in MRSA in comparison to MSSA, might be due to poor patient compliance in norfloxacin use as sub inhibitory levels of this drug might result in the induction of fibronectin-binding proteins that resulted in induction offibronectin-coated surfaces (65) and finally lead to bacterial virulence increase (66), therefore, isolates that were norfloxacin susceptible might become resistant after initiation of therapy within (72-96) hours (62).

J) Ciprofloxacin: The high resistance rate for ciprofloxacin in MRSA in comparison to MSSA isolates might be due to empirical or indiscriminate use of these drugs and might reflect rapid emergence stepwise acquisition of chromosomal mutations in the quinolone-resistance-determining region that reduce the affinity of quinolone for its targets (33).

K) Vancomycin: Its seem to be the only antimicrobial agent which showed (100%) sensitivity and may be used as the drug of choice for treating multidrug resistant MRSA infections except (2) MRSA isolates which had represented intermediate susceptibility to vancomycin (VISA) (MIC values 8-16μg/ml). This might be reversed through the combine doxacillin treatment (67).The specific resistance genes for VISA strains are, as yet, unknown (68). No isolate was found to be VRSA by phenotypic methods. PCR technique had showed the presence of Van A gene in two isolates. The resistance of MRSA to vancomycin could be attributed to the presence of resistance genes like (Van A ,B,C1,C2,C3) genes (69) that needed to be confirmed by further study. MRSA possess genes encoding protein that lead to thicker cell wall which would lead to more vancomycin trapping molecules in the peptidoglycan layer before reaching the cytoplasmic membrane where peptidoglycan synthesis happens (70). Extensive use of vancomycin creates a selective pressure that favors the outgrowth of rare vancomycin-resistant clones (71, 72). Two (2) isolates had van A genes which suggests that the resistance determinate could acquire from a vancomycin resistant *Enterococcus* (73). Vancomycin resistance had been perceived as a fearsome threat to the already challenging therapy of MRSA (74, 75), so monitoring of vancomycin sensitivity and regular routine testing of other newer glycopeptides (like teicoplanin) should be carried out. Further, the regular and routine mandatory surveillance of hospital related infections using PCR technique to detect the possibly resistant genes of MRSA for formulation of definite antibiotic policy may be helpful for reduction of MRSA,VISA and VRSA infections incidence.

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