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Original Research

Genetic basis of resistance waves among methicillin resistant *Staphylococcus aureus* isolates recovered from milk and meat products in Egypt

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Abstract: Antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious problem for clinicians worldwide. The present study attempted to evaluate the susceptibility patterns of MRSA to various antimicrobials and the prevalence of inducible clindamycin resistance as well as the relevant antibiotic and antiseptic resistance genes among these isolates. Totally, 40 MRSA isolates were recovered from examined milk and meat product samples (18.60%). Multi-drug resistance (MDR) was remarkably observed among 85% of these isolates. There was a good correlation between phenotypic determination of methicillin, amoxicillin/clavulinic acid and tetracycline resistances and PCR detections of *mec*A, *bla*Z and *tet*(K) genes, respectively, but *nor*A gene was not detected in the four ciprofloxacin resistant isolates. Although, 55% of MRSA expressed resistance to benzalkonium chloride (BC), neither *qac*A/B nor *smr* gene was detected. Of 20 isolates exhibiting erythromycin- clindamycin discordant resistance pattern, 8 displayed positive double disk diffusion (D-zone) test denoting inducible macrolide-lincosamide-streptogramin B (MLS_B) resistance phenotype with the inducibly expressed *erm*(A) and *erm*(C) genes in 87.5% of these isolates. Besides, the remaining 12 isolates showed MS phenotype (resistant to macrolides and type B streptogramins only) with a variety of *erm*(A), *mph*(C), *msr*(A) or a combination of these genes including *erm*(C). Finally, the constitutive MLS_B phenotype with the constitutive expression of *erm*(A), *erm*(B) and *erm*(C) genes was comprised in 2 isolates with higher minimum inhibitory concentration (MIC) values for erythromycin (512 and 1024 µg/ml) and clindamycin (16 and 32 µg/ml). These findings suggested the importance of monitoring the evolution of MRSA resistance.

Key words: MRSA, D-test, *qac*A/B, inducible MLS_B, MS phenotype, *erm*(A).

Introduction

Staphylococcus aureus has emerged as one of the most important pathogens over the past several decades. It has been a leading cause of food-poisoning outbreaks and contagious bovine mastitis (1).

The success of this pathogen is due to its potential virulence besides its remarkable ability to overcome most of antibiotics developed in the recent years with the emergence of multi-drug resistance (MDR) pattern (2). Infection caused by antibiotic resistant strains and particularly methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasingly serious medical problem threatening public health (3) owing to the difficulties of treatments and the ease with which MRSA spreads. This is mainly due to the dissemination of certain determinants that encode resistance to antimicrobials.

There are several classes of antibiotic resistance genes that confer resistance to different groups of antibiotics. Resistance to methicillin has been reported to be associated with the presence of an alternative low-affinity penicillin-binding protein 2a (PBP2a) which is encoded by the *mec*A gene. Another gene involved in penicillin resistance is *bla*Z, which encodes β-lactamase (4). Bacteria can use different mechanisms of resistance to antibiotics, however, many bacterial efflux pumps are able to extrude several, unrelated classes of antimicrobial compounds from the cell promoting the development of MDR phenotypes (5). For tetracycline resistance, *tet*K is the most often found in *S. aureus* among a variety of different *tet* genes coding for efflux mechanisms (6). In the vast majority of *S. aureus* isolates, resistance to quinolone is due to overexpression of *nor*A gene which encodes a multidrug efflux protein (NorA) capable of transporting fluoroquinolone outside the bacteria (7).

The increased frequency of staphylococcal infections along with augmented problem of resistance has led to the renewed interest to determine which of therapeutic alternatives are suitable to treat the infections. The usage of macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics with clindamycin being the preferred agent serves as one such alternative option that inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells. Clindamycin is a good substitute among the limited choices of antimicrobials effectively against MRSA due to its excellent pharmacokinetic properties (8).

The widespread use of MLS_B antibiotics has led again to the development of resistance which is the major barrier in their usage. It is well known that bacterial resistance to this group may be expressed through different mechanisms. Firstly, modification of ribosomal target which is mediated by *erm* (erythromycin ribosome methylase) genes leading to cross-resistance to macrolides, lincosamides and streptogramins B, the

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so-called MLS_B phenotype. Secondly, an active macrolide-specifc efflux mechanism which is encoded by *msr*(A) (methionine sulfoxide reductase) gene conferring resistance to macrolides and type B streptogramins only (MS phenotype). Lastly, macrolide inactivation by genes concerned in antibiotics inertness such as *mph*(C) (macrolide 2'-phosphotransferase) gene, which codes for a phosphotransferase that inactivates some macrolide antibiotics (9). The appearance of the MLS_B phenotype can be constitutive macrolide-lincosamide-streptogramin B ($cMLS_B$) resistance and inducible macrolide-lincosamide-streptogramin B ($iMLS_B$) resistance. *In vitro*, *S. aureus* isolates with constitutive resistance are resistant to all members of MLS_B and isolates with inducible resistance are resistant to 14- and 15-membered macrolides only, but appear to be susceptible to clindamycin. The risk of treatment failure was commonly reported during therapy with clindamycin in the inducible resistance phenotype. In this case, the *erm* genes require an inducing agent to express resistance to clindamycin. Erythromycin can act as a strong inducer of methylase synthesis (10). Constitutive resistance can be readily detected, but inducible resistance is not recognized by routine antimicrobial susceptibility methods, while it can be detected with a simple disk approximation test, commonly referred as the D-test (8) according to the recommendation of Clinical and Laboratory Standards Institute (CLSI) (11).

Because of the high resistance of *S. aureus* and the outbreaks of nosocomial infections due to MRSA worldwide, hygienic measures have concentrated on the prevention of infection rather than on medical treatment. A variety of antiseptic and disinfectant agents based on quaternary ammonium compounds (QACs) have been extensively used in hospitals and other health care settings (12). Overuse of antiseptic agents has led to decreased susceptibility to QAC and the emergence of alarming antiseptic resistance which is attributable to *qac* determinants that are mainly plasmid borne and confer resistance by a means of proton motive force-dependent multidrug efflux (13).

On the whole, this study sheds light on the *in vitro* resistance situation in *S. aureus* from milk and meat products against various kinds of antibiotics with special reference to inducible and constitutive clindamycin resistance and aims to critically record the current status of MRSA response to commonly used antiseptic in Egypt with assessing the genetic basis of the relevant resistance to guide therapy.

Materials and Methods

Samples and microbiological analysis

A total of 173 milk samples from apparently healthy and mastitic cows in addition to 42 samples of different meat products including sausage (15), burger (7) and minced meat (20) were randomly collected from Abu Kabeer, al-salhia and Zagazig cities in Sharkia province, Egypt during the period from December 2012 to December 2013. The samples were then transferred as early as possible to the laboratory in an icebox, where they were analyzed for *S. aureus* screening. Routine preliminary phenotypic characterization of *S. aureus* isolates were conducted initially on the basis of standard microbiological techniques (14).

Susceptibility testing

In vitro antimicrobial susceptibility testing of all *S. aureus* isolates to a panel of 14 antimicrobial agents including erythromycin (E), clindamycin (DA), tetracycline (TE), ciprofloxacin (CIP), chloramphenicol (C), methicillin (ME), amoxicillin/clavulinic acid (AMC), cefoxitin (FOX), vancomycin (VA), trimethoprim/sulfamethoxazole (SXT), rifampicin (RF), gentamycin (CN), imipenem (IPM) and ceftriaxone (CRO) (Oxoid Ltd., Basingstoke, Hampshire, UK) was carried out by disc diffusion method and interpreted according to the breakpoint values defined by CLSI (11). The resistance rate to each antibiotic was calculated as the number of resistant *S. aureus* isolates divided by the total number of isolates. Multi-drug resistance was defined as resistance to at least three various classes of selected antibiotics.

Furthermore, all *S. aureus* isolates were initially tested for their susceptibilities to QAC by studying their growth on Mueller Hinton agar (MHA) plates (Oxoid Ltd., Basingstoke, Hampshire, UK) containing 12 different concentrations of BC (Sigma-Aldrich, Co., St. Louis, MO, USA) ranging from 1 to 12 µg/ml as was previously described (15). A control MHA plate containing no QAC was used for each isolate. Isolates showing confluent or semi-confluent growth on MHA containing BC at ≥4 µg/ml were considered resistant to QAC.

Minimum inhibitory concentrations (MICs) of erythromycin and clindamycin (Sigma-Aldrich, Co., St. Louis, MO, USA) were then determined for all *S. aureus* isolates identified earlier on disc diffusion test as resistant to erythromycin and susceptible to clindamycin by a standardized broth microdilution method in accordance with the CLSI guidelines (11).

D-test

The presence of inducible clindamycin resistance due to expression of inducible *erm* genes in *S. aureus* was sought in those isolates with discordant resistance pattern for clindamycin and erythromycin (clindamycin susceptible and erythromycin resistant on the basis of their MICs) using the D-test. Briefly, this test was performed by placing erythromycin (15 µg) and clindamycin (2 µg) discs in the center of Mueller–Hinton agar plates previously inoculated with 0.5 McFarland equivalent bacterial suspensions at an edge-to-edge distance of approximately 15 to 20 mm. Following overnight incubation at 37°C, interpretation of the inhibition zone diameters was analyzed as per CLSI guidelines (11). Three different phenotypes were assessed and explained (16) as following:

1. Constitutive MLS_B phenotype: *S. aureus* isolates showing resistance to both erythromycin and clindamycin with a circular shaped zone of inhibition if any around clindamycin.
2. Inducible MLS_B phenotype: *S. aureus* isolates exhibiting resistance to erythromycin, while being sensitive to clindamycin and giving a blunted or truncated D shaped zone of growth inhibition around clindamycin disc with flattening on the side adjacent to erythromycin disc (D test positive).
3. MS phenotype: *S. aureus* isolates which were resis-

tant to erythromycin and susceptible to clindamycin giving a circular zone of inhibition around clindamycin (D test negative), which shows that the strain is truly susceptible to clindamycin, but has an active efflux pump.

Molecular detection of antibiotic and antiseptic resistance genes

Plasmid DNA extraction was carried out using QIAprep Spin Miniprep Kit (Qiagen GmbH, Hilden, Germany) according to manufacturers` instructions.

The presence of genes involved in resistance of *S. aureus* isolates to beta-lactams (*bla*Z and *mec*A), macrolides, lincosamides and streptogramins B [(*erm*(A), *erm*(B), *erm*(C), *mph*(C) and *msr*(A)], quinolones (*nor*A), tetracyclines (*tet*(K)] and benzalkonium chloride (*qac*A/B and *smr*) was determined by PCR amplifications using the specific primers and the PCR conditions outlined in Table 1. All PCR amplifications were performed in a PTC-100 TM programmable thermal cycler (MJ Research Inc., Waltham, USA) with a total reaction volume of 25 µl consisting of 12.5 µl of DreamTaq TM Green Master Mix (2X) (Fermentas, Inc. Hanover, MD, USA), 0.1 µl of 100 pmol of each primer (Sigma-Aldrich, Co., St. Louis, MO, USA), 2 µl of the extracted template and water nuclease-free up to 25 µl. Appropriate positive and negative controls were an integral part of any PCR assay. The amplified PCR products (5 µl) were analyzed on 1.5 % agarose gel stained with ethidium bromide, visualized and photographed using an ultraviolet transilluminator (Spectroline, Westbury, New York, USA). A 100 bp DNA ladder (Fermentas, Inc. Hanover, MD, USA) was used as a molecular size marker.

Statistical analysis

In order to compare the antibiotic resistance profiles of *S. aureus* isolates from milk and meat products, Fisher's exact test was applied through cross tab. Procedure of Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA). The value of $P < 0.05$ was used to indicate a statistical significance.

Results

Occurrence and distribution of S. aureus among analyzed samples

Following preliminary phenotypic identification, 40 *S. aureus* isolates were recovered from 215 examined samples (18.60%). For clarity, 30 of 173 milk samples (17.34%) and 10 of 42 meat product samples (23.81%) were contaminated with *S. aureus* with high frequencies from burger (71.43%), followed by sausage (26.67%), but minced meat samples displayed a rather low level of contamination with *S. aureus* relative to the total number of isolates from meat products (5%).

Antimicrobial susceptibility testing

Antibiotic susceptibility patterns of all *S. aureus* isolates are illustrated in Table 2. Analysis of methicillin resistance confirmed that all the recovered isolates were MRSA. Totally, all MRSA isolates tested were susceptible to imipenem and vancomycin and over 90% of the isolates were susceptible to trimethoprim/sulfamethoxazole (95%). However, the highest levels of resistance were obtained against amoxicillin/clavulinic acid, tetracycline and erythromycin (65, 60 and 57.5%, respectively) and the proportion of isolates resistant to clindamycin, ciprofloxacin, chloramphenicol trimetho-

Table 1. Oligonucleotide primers used for specific PCR amplifications of antibiotic and antiseptic resistance genes among *S. aureus* isolates.

Target gene/specificity	Primer pair	Primer sequence (5`→3`)	Amplicon size (bp)	PCR cycles and conditions*	Reference
*bla*Z/ β-lactamase	blaZ-1 blaZ-2	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173	95°C 30 s, 54°C 30 s, 72°C 30 s; 30x	17
tet(K)/ tetracycline efflux protein	tet(K)-1 tet(K)-2	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360		17
*mec*A/PBP2a	mecA-1 mecA-2	GTAGAAATGACTGAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	310	94 °C 30 s, 64 °C 30 s, 72 °C 45 s; 10x and 94 °C 30 s, 50 °C 45 s, 72 °C 2 min; 25x	18
erm(A)/ methylase	erm(A)-1 erm(A)-2	GCGGTAAACCCCTCTGAG GCCTGTCGGAATTGG	434		18
erm(B)/ methylase	erm(B)-1 erm(B)-2	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG	425	94 °C 60 s, 51 °C (*erm*A, *erm*B, *erm*C), or 55 °C (*msr*A, *mph*C) 60 s, 72 °C 60 s; 30x	18
erm(C)/ methylase	erm(C)-1 erm(C)-2	ATCTTTGAAATCGGCTCAGG CAAACCCGTATTCCACGATT	295		18
mph(C)/ phosphotransferase	mph(C)-1 mph(C)-2	GAGACTACCAAGAAGACCTGACG CATACGCCGATTCTCCTGAT	722		18
msr(A)/ hydrophilic protein (Msr(A))	msr(A)-1 msr(A)-2	GCAAATGGTGTAGGTAAGACAACT ATCATGTGATGTAAACAAAAT	400		18
*nor*A/ multidrug efflux protein	norA-1 norA-2	TTCACCAAGCCATCAAAAAG CTTGCCTTTCTCCAGCAATA	620	94°C 30 s, 45°C 30 s, 72°C 1 min; 35x	19
*qac*A/B/ multidrug efflux pumps: membrane proteins	qacA/B-1 qacA/B-2	GCAGAAAGTGCAGAGTTCG CCAGTCCAATCATGCCTG	361		20
smr/ efflux proteins belonging to the small multidrug resistance family	smr-1 smr-2	GCCATAAGTACTGAAGTTATTGGA GACTACGGTTGTTAAGACTAAACCT	195	95°C 30 s, 52°C 30 s, 72°C 1.5 min; 25x	20

PBP2a, penicillin-binding protein 2a;* Initial heating was always performed at 94°C for 5 min, final elongation at 72°C for 5 min.
x: Number of cycles

Table 2. Antibiotic susceptibility profiles of *S. aureus* isolates.

Antibiotic	No of *S. aureus* isolates (%)		
	S	I	R
Erythromycin	6 (15)	11 (27.5)	23 (57.5)
Clindamycin	35 (87.5)	1 (2.5)	4 (10)
Tetracycline	8 (20)	8 (20)	24 (60)
Ciprofloxacin	34 (85)	2 (5)	4 (10)
Chloramphenicol	30 (75)	5 (12.5)	5 (12.5)
Methicillin	0 (0)	0 (0)	40 (100)
Amoxicillin/clavulinic acid	12 (30)	2 (5)	26 (65)
Cefoxitin	18 (45)	0 (0)	22 (55)
Vancomycin	40 (100)	0 (0)	0 (0)
Trimethoprim/sulfamethoxazole	38 (95)	1 (2.5)	1 (2.5)
Rifampicin	33 (82.5)	2 (5)	5 (12.5)
Gentamycin	36 (90)	0 (0)	4 (10)
Imipenem	40 (100)	0 (0)	0 (0)
Ceftriaxone	11 (27.5)	15 (37.5)	14 (35)

S, susceptible; I, intermediate; R, resistant.

prim/sulfamethoxazole, rifampicin and gentamycin was less than 15%. More precise interpretation of antibiotic resistance revealed that all the isolates were resistant to at least one of the screened antibiotics, but none of them were resistant to all antibiotics. In addition, majority of MRSA (85%) had phenotypic resistance to at least three antimicrobial drugs of three different groups being MDR with a reduced gradient of multiple antibiotic resistance.

The antibiotic susceptibility profiles of MRSA isolates from milk and meat product samples were found to be variable. Almost all MRSA strains (96.67%) from milk samples were sensitive to trimethoprim/sulfamethoxazole, 93.33% to gentamycin, 90% to clindamycin and 80% to each of ciprofloxacin and rifampicin. However, 73.33% of the strains recorded resistance to amoxicillin/clavulinic acid, followed by 66.67% to tetracycline. In general, 86.67% of these MRSA isolates proved MDR pattern, in which only 3 strains were resistant to 8 drugs. Among MRSA strains from meat products, 100% sensitivity was observed to ciprofloxacin, followed by 90% to each of trimethoprim/sulfamethoxazole and rifampicin and 80% to each of clindamycin and gentamycin. Overall, 80% of these isolates expressed MDR, while no isolates exhibited resistance to more than 6 antibiotics. Distribution of antimicrobial resistance among *S. aureus* from milk and meat products is clarified in Figure 1. It is worth noting that there were no significant differences observed in the resistance rates of MRSA isolates from both sources to all tested antibiotics ($P > 0.05$).

According to the MHA method used for initial screening of MRSA susceptibilities to BC, 22 of the 40 isolates (55%) expressed resistance to this QAC.

Interestingly, MIC testing confirmed the erythromycin resistance pattern among 23 MRSA isolates yielding various erythromycin MIC values ranging from 8 to 1024 µg/ml with only one isolate exhibiting the high-level resistance (1024 µg/ml). Moreover, all 35 disc diffusion clindamycin sensitive isolates were further affirmed using a standardized broth microdilution method displaying clindamycin MIC values ≤0.5 µg/ml.

Interpretative criteria of D-test

Out of the total MRSA isolates, 4 (10%) were susceptible to both clindamycin and erythromycin. Meanwhile,

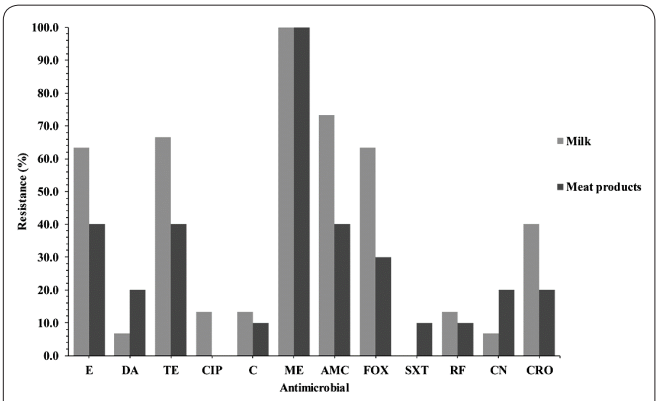

Figure 1. Antimicrobial resistance patterns of *S. aureus* isolates from milk and meat products. E, erythromycin; DA, clindamycin; TE, tetracycline; CIP, ciprofloxacin; C, chloramphenicol; ME, methicillin; AMC, amoxicillin/clavulinic acid; FOX, cefoxitin; SXT, trimethoprim/sulfamethoxazole; RF, rifampicin; CN, gentamycin; CRO, ceftriaxone.

of all 23 erythromycin resistant MRSA, only 2 isolates from milk samples exhibited resistances to both erythromycin and clindamycin indicating $cMLS_B$ resistance phenotype. This phenotype was not detected in MRSA from meat products. Phenotypic evaluation of inducible resistance to clindamycin revealed that the clindamycin-erythromycin discordant resistance pattern compatible for realization of the D test was obtained by a total of 20 MRSA isolates (16 from milk samples and 4 from meat products) with MIC values of 8-256 and 0.03-0.50 µg/ml for erythromycin and clindamycin, respectively. Among these 20 isolates, 8 (40%) displayed positive D-test denoting $iMLS_B$ resistance phenotype [7/16 (43.75%) from milk and 1/4 (25%) from meat products] with an overall percentage of 20% from all MRSA isolates, while the remaining 12 isolates (60%) demonstrated true sensitivity to clindamycin (negative D-test) suggesting MS phenotype [9/16 (56.25%) from milk and 3/4 (75%) from meat products]. D test positive isolates showed higher resistance rates to ceftriaxone (75%) and cefoxitin (62.5%) as compared to D test negative isolates (16.67 and 25%, respectively), but both isolates showed full resistances to erythromycin, tetracycline, methicillin and amoxicillin/clavulinic acid (100%). It was noticed that the MS phenotype predominated over the $cMLS_B$ and $iMLS_B$ resistance phenotypes among MRSA isolates from both milk and meat pro-

Table 3. Susceptibility patterns of MRSA isolates from milk and meat products against erythromycin and clindamycin .

Susceptibility pattern (phenotype)	Source of MRSA		Total (40)
	Milk (30)	Meat products (10)	
E^S, DA^S	3 (10)	1 (10)	4 (10)
E^R, DA^R ($cMLS_B$)	2 (6.67)	0 (0)	2 (5)
E^R, DA^S (D test positive, $iMLS_B$)	7 (23.33)	1 (10)	8 (20)
E^R, DA^S (D test negative)	9 (30)	3 (30)	12 (30)
E^S, DA^R	0 (0)	2 (20)	2 (5)
Total	21 (70)	7 (70)	28 (70)

MRSA, methicillin resistant *S. aureus*; E, erythromycin; DA, clindamycin; S, sensitive, R, resistant; $cMLS_B$, constitutive macrolide lincosamide and streptogramin B; $iMLS_B$, inducible macrolide lincosamide and streptogramin B.
Data are presented as No. (%).

duct samples. Moreover, the percentages of both $cMLS_B$ and $iMLS_B$ resistance phenotypes were higher amongst MRSA isolates from milk as compared to MRSA isolates from meat products, but percentages of susceptible and MS phenotype were equal among both sample types. Frequency of different susceptible phenotypes for erythromycin and clindamycin among all screened isolates are provided in Table 3.

Analysis of the genetic basis of resistance pattern

Twenty two multi-drug and antiseptic resistant MRSA isolates exhibiting all D-test resistance phenotypes were selected to interpret their genotypic characteristics using PCR screening of some antibiotic and antiseptic resistance genes. With regards to PCR determination of antiseptic resistance genes, both *qac*A/B and *smr* genes were not detected in any antiseptic resistant strains. However, all isolates held more than one antibiotic resistance gene and a high proportion of these isolates (59.09%) possessed five genes confirming the large spread of MDR isolates. The PCR analysis gave data consistent with phenotypic resistance patterns of the isolates via amplifications of the respective antibiotic resistance genes with the yield of the expected amplicons sizes (Table 4). In particular, *mec*A gene was detected in all methicillin resistant isolates confirming them as MRSA and *blaZ* and *tet*(K) genes were amplified in all phenotypically amoxicillin/clavulinic acid and tetracycline resistant isolates (100%), respectively. Conversely, no amplification was observed with primer sets for *nor*A gene in the four ciprofloxacin resistant isolates.

Besides, the PCR based detection of five erythromycin resistance genes showed that all the isolates harbored *erm*(C) gene and the majority of them (81.82%) were positive for *erm*(A) gene. The distribution of *erm*(A) in milk samples was higher than in meat product samples (88.89 and 50%, respectively). On the other hand, the prevalence of *mph*(C), *msr*(A) and *erm*(B) genes were considerably lower than the *erm*(A) gene. Six (27.27%)

Table 4. Correlation between phenotypic antibiotic resistance and PCR results among 22 MRSA isolates categorized by D-test.

Sample origin	Isolate number	Resistence phenotype (disk diffusion test)	MIC (µg/ml)		D-test result	PCR results [a] (resistance genes)
			E	DA		
Milk (18)	1	E, TE, ME, AMC, CRO	16	0.03	$iMLS_B$	*erm*(A)
	2	E, TE, ME, AMC	8	0.50	-	*msr*(A)
	3	E, TE, ME, AMC, FOX, CRO	16	0.25	$iMLS_B$	*erm*(A)
	4	E, TE, CIP, ME, AMC, CRO	32	0.03	$iMLS_B$	*erm*(A)
	5	E, TE, CIP, ME, AMC	32	0.25	-	*mph*(C)
	6	E, TE, ME, AMC, CRO	64	0.03	-	*erm*(A), *mph*(C)
	7	E, TE, ME, AMC, FOX	128	0.06	-	*erm*(A), *mph*(C)
	8	E, TE, CIP, ME, AMC, FOX, CRO	32	0.06	$iMLS_B$	*erm*(A)
	9	E, TE, CIP, ME, AMC	64	0.25	-	*erm*(A), *msr*(A)
	10	E, TE, ME, AMC, FOX	32	0.50	-	*erm*(A)
	11	E, DA, TE, ME, AMC, FOX, CRO	512	16	$cMLS_B$	*erm*(A), *erm*(B)
	12	E, TE, ME, AMC, CRO	128	0.12	-	*erm*(A), *mph*(C)
	13	E, TE, C, ME, AMC	32	0.25	-	*erm*(A)
	14	E, TE, ME, AMC, FOX	64	0.03	-	*erm*(A), *msr*(A)
	15	E, TE, C, ME, AMC, FOX, RF, CRO	256	0.06	$iMLS_B$	*erm*(A), *erm*(B)
	16	E, TE, C, ME, AMC, FOX, RF, CRO	32	0.25	$iMLS_B$	*erm*(A)
	17	E, TE, ME, AMC, FOX, CN	16	0.03	$iMLS_B$	*erm*(A)
	18	E, DA, TE, C, ME, AMC, FOX, CRO	1024	32	$cMLS_B$	*erm*(A), *erm*(B)
	19	E, TE, ME, AMC	128	0.03	-	*mph*(C), *msr*(A)
Meat products (4)	20	E, TE, ME, AMC	8	0.03	-	*mph*(C)
	21	E, TE, ME, AMC	32	0.12	-	*erm*(A)
	22	E, TE, ME, AMC	16	0.50	$iMLS_B$	*erm*(A)

E, erythromycin; DA, clindamycin; TE, tetracycline; CIP, ciprofloxacin; C, chloramphenicol; ME, methicillin; AMC, amoxicillin/clavulinic acid; FOX, cefoxitin; RF, rifampicin; CN, gentamycin; CRO, ceftriaxone; $iMLS_B$, inducible macrolide lincosamide and streptogramin B; $cMLS_B$, constitutive macrolide lincosamide and streptogramin B; -, negative; [a] *mec*A, *blaZ*, *tet*(K) and *erm*(C) genes were detected in all isolates, but *nor*A gene was not identified in ciprofloxacin resistant isolates number 4, 5, 8 and 9.

among the 22 tested isolates contained *mph*(C), 4 isolates (18.18%) harbored *msr*(A) and only 3 isolates from milk samples (13.64%) were positive for *erm*(B) gene. Furthermore, nine isolates (40.91%) carried 3 erythromycin resistance genes.

Based on analysis of the association between phenotypic erythromycin resistance patterns identified on the basis of MIC values and the distribution of erythromycin resistance determinants among MRSA isolates displaying all categories of D-test (Table 4), it was noted that all 22 erythromycin resistant isolates (MIC range of 8 to 1024 μg/ml) had at least two of the respective resistance genes. The $iMLS_B$ phenotype was detected in 8 isolates with susceptibility to clindamycin (MIC range of 0.03–0.50 μg/ml). It is noteworthy that the inducibly expressed *erm*(A) and *erm*(C) genes were present in combination in majority of these isolates [7/8 (87.5%], while *erm*(A), *erm*(B) and *erm*(C) genes were detected in only one isolate from milk sample with a higher MIC value for erythromycin (256 μg/ml). Moreover, the $cMLS_B$ phenotype corresponded in two isolates with the constitutive expression of *erm*(A), *erm*(B) and *erm*(C) genes displayed higher MIC values for both erythromycin (512 and 1024 μg/ml) and clindamycin (16 and 32 μg/ml). Finally, the remaining 12 isolates showing MS phenotype contained a variety of *erm*(A), *mph*(C), *msr*(A) or a combination of these genes including *erm*(C). Surprisingly, *erm*(A) gene occurred in these isolates more often than other genes either alone (3 isolates) or in combination with the *mph*(C) gene (3 isolates) or *msr*(A) gene (2 isolates). In addition to the high resistance to erythromycin (MIC ≥16 μg/ml) in previous cases, which accurately differentiated *erm* positive isolates, resistances to at least three other antimicrobial agents were also observed. Multiple resistance to erythromycin, tetracycline, methicillin and amoxicillin/clavulinic acid was noted in all isolates and concurrent resistance to other antimicrobial agents was recorded in most of the isolates. The *mph*(C) or *msr*(A) genes, independently or in combination were detected in 4 isolates. The presence of either *mph*(C) or *msr*(A) gene led to lower MIC values (8 and 32 μg/ml) than reported for the isolates showing the simultaneous presence of both genes (128 μg/ml).

Discussion

S. aureus is considered as one of the major clinically significant pathogens worldwide. The emergence of MDR is a stumbling block for antimicrobial chemotherapy especially after the introduction of new classes of antimicrobial agents (21). Moreover, antiseptics resistance is an increasingly serious threat since the early 1990. Therefore, concerns have particularly arisen to update our knowledge about the prevalence and distribution of antibiotic and antiseptic resistance genes among MRSA isolates is of utmost importance for controlling their dissemination and management of severe infections.

In this study, 18.60% of the 215 analyzed samples were contaminated with *S. aureus*; 17.34% from milk samples and 23.81% from meat product samples. The contamination rates of *S. aureus* herein were lower than those reported in another study conducted in Turkey, where a higher prevalence rate of *S. aureus* (33.4%); 23.2% from milk samples and 48.7% from meat and meat product samples was observed (1). The variation in different geographical regions may be due the efficacy of infection control practices, healthcare facilities and antibiotic usage.

Analyzing the antibiogram results of the current study proved methicillin resistance in all recovered *S. aureus* isolates unveiling the huge existence of MRSA in milk and meat products. Isolation of MRSA in this study was significant and considered an extension of previous studies conducted recently by another research groups in Bangladesh (22) and Egypt (23) signifying that the occurrence of MRSA in food samples has been a major concern for the consumer health. The full susceptibility of the isolates observed in this study to imipenem and vancomycin agreed with data reported for *S. aureus* isolates from food samples in Turkey (24) and *S. aureus* strains isolated from raw milk in Tehran and Mashhad (25) indicating these antibiotics as excellent and effective agents for treatment of *S. aureus* infections. On the other hand, all the strains were resistant to at least one of the antibiotics with the highest levels of resistance obtained against amoxicillin/clavulinic acid, tetracycline and erythromycin (65, 60 and 57.5%, respectively). The prevalence of resistance of *S. aureus* from food samples to the aforementioned antibiotics was also comparable with data from previous reports in major countries; 52% against amoxicillin/clavulinic acid in Egypt (23), 60% against tetracycline in Romania (26) and 58.6% against erythromycin in Turkey (1). A remarkable level of MDR was not surprising as 85% of MRSA have been accounted as MDR strains. This finding is consistent with a previous report demonstrating this emerging feature in the city of Nairobi, where most *S. aureus* isolates from milk and meat samples (80.2%) were multiply resistant to between two and six antibiotics (27). This trend suggests that the contamination of milk and meat with resistant *S. aureus* posing a potential risk to consumers. Therefore, comprehensive control measures during processing of these products and judicious application of effective antibiotics should be adopted to overcome this critical situation and avoid the risk of human infection. It is worth mentioning that the resistance patterns of MRSA isolates from milk and meat product samples were variable with a high proportion of resistance to most antibiotics among milk isolates compared to meat isolates. On the contrary, another study performed in Kenya recorded elevated resistances to antimicrobial agents among *S. aureus* isolates from meat than those from milk samples attributing to excessive handling of the meat with contaminated hands during mincing process (27).

Regarding the susceptibilities of MRSA to antiseptics, 55% of MRSA isolates expressed resistance to BC. However, a previous study carried out in Iran detected only 9% of MRSA isolates with MIC values higher than 2 μg/ml (28).

Confirming the results of disc diffusion test for erythromycin resistance and clindamycin susceptibility was performed by broth microdilution method with erythromycin MIC values ranging from 8 to 1024 μg/ml and clindamycin MIC values ≤0.5 μg/ml. Our data are consistent with the results of a previous study carried

out in the Czech Republic with MIC range of 8 to >128 mg/l in erythromycin resistant isolates and MIC range of ≤0.5 mg/l in clindamycin susceptible isolates (29).

In the light of increasing frequency of a variety of staphylococcal infections, especially the expanding role of MRSA and changing patterns in their antimicrobial resistance, resurgence in interest in clindamycin therapy should be considered to treat such infections. Reporting *S. aureus* as susceptible to clindamycin without accurate checking for inducible resistance using D-test may result in therapeutic failures, but negative result for inducible resistance confirms true clindamycin susceptibility and provides a very good therapeutic option to improve the empirical approaches to the therapy of serious infections caused by staphylococci (30).

In this study, a total of 20% of MRSA demonstrated $iMLS_B$ phenotype. This is in concordance with studies published by other authors in Turkey (24.4%) (31) and in India (27.6%) (30). However, contrary to our data, 50% of MRSA isolates in a study conducted in Alabama were determined to have the inducible phenotype (32) and 0.65% of MRSA isolates in a later report in Bangalore demonstrated inducible resistance (33). The striking differences in the incidence of inducible clindamycin resistance among MRSA could be attributed to rapid changes in antimicrobial resistance patterns and vary widely by the geographical specificity.

We agree in principle that only erythromycin resistant but clindamycin susceptible isolates should be tested. Thus, we tested 20 isolates with clindamycin-erythromycin discordant resistance pattern and detected inducible clindamycin resistance (D test positive) in 8 isolates, while the rest ones were D test negative. This observation suggests that if the D test had not been performed, nearly half of the erythromycin resistant isolates (40%) would have been misidentified as clindamycin sensitive and could be converted to a constitutively resistant phenotype during treatment resulting in therapeutic failure as was previously observed in another study conducted in India (30). This further reaffirms the critical role of D-test as an auxiliary and alternative method for clinical microbiology laboratories to provide routine detection of inducible clindamycin resistance enabling us to guide the clinicians towards the judicious use of clindamycin to retain confidence in clindamycin when erythromycin resistance is present.

In this study, all MRSA isolates have a low prevalence of constitutive resistance (5%) which is within the limits of two studies conducted in different parts of India; 7.3% (30) and 8.6% (34). The low constitutive clindamycin resistance in our study may be attributed to the fact that this drug is not commonly used and hence there is a less prevalence of resistant strains.

It was noticeable that the MS phenotype predominated over the $cMLS_B$ and $iMLS_B$ resistance phenotypes among MRSA isolates as was previously reported (35). An important fact observed in our study regarding the increasing frequency of resistance rates with *in vitro* inducible clindamycin resistance among D test positive isolates has left very few therapeutic options for clinicians.

Accurate detection of the most frequently antibiotic resistance genes associated with resistance of *S. aureus* to clinically relevant antibiotics is essential for the treatment of overt infections and the implementation of infection control practices. A major problem in the treatment of *S. aureus* infections is the ability of this pathogen to be resistant to a number of antibiotics. Apparently, all isolates carried more than one antibiotic resistance gene and 59.09% of them harbored five genes. These results are in agreement with the findings of an earlier report in USA (36), whereby all MRSA isolates carried two to six antibiotic resistance genes with the majority (72%) carrying four to six different genes emphasizing the considerable spread of MDR isolates.

In this study, the observed phenotypic methicillin, amoxicillin/clavulinic acid and tetracycline resistances were justified by PCR detections of *mec*A, *bla*Z and *tet*(K) genes. This affirms the close correspondence between the results of PCR and those of classical resistance testing as reported before (37). Conversely, a discrepancy between genotypic and phenotypic detection of antimicrobial sensitivity patterns was evidenced by the absence of *nor*A gene in the four ciprofloxacin resistant isolates. The reason for this difference is the possibility of carrying other drug resistance genes or harboring some other antimicrobial resistance phenomenon. This result is in contrast to a recent report in Baghdad, where *nor*A gene was detected in 47% of *S. aureus* isolates (38).

Other significant observations in this study were that all 22 erythromycin resistant isolates had at least two of the respective resistance genes and *erm*(C), followed by *erm*(A) genes were the most prevalent ones. Similarly, in a study performed in South of Turkey, all erythromycin resistant strains contained at least one of the erythromycin resistance genes and *erm*A was the predominant one (17).

The percentages of *mph*(C), *msr*(A) and *erm*(B) genes in this study (27.27, 18.18 and 13.64%, respectively) were lower than those observed in a recent survey conducted in Turkey (30.4, 78 and 52.2%, respectively) (39).

The multiplicity of mechanisms which is conferred resistance to MLS_B antibiotics reflects the complexity of the resistant phenotypes as well as the clinical situation. The most widespread and clinically important resistance mechanisms encountered with inducible clindamycin resistance are evidenced by the production of *erm* genes. The results of the present investigation revealed that the inducibly expressed *erm*(A) and *erm*(C) genes were present in combination in majority of the isolates and the constitutive expression of *erm*(A), *erm*(B) and *erm*(C) genes were corresponded in two isolates. Likewise, the simultaneous presence of more than one *erm* gene in the genome of *S. aureus* is possible and has previously been detected in another similar study performed in the Czech Republic (29).

For isolates showing MS phenotype, a variety of *erm*(A), *mph*(C), *msr*(A) or a combination of these genes including *erm*(C) detected in the present study was just as another research done in Atlanta, where *msr*A-mediated resistance is representative for *S. aureus* isolates with MS phenotype (16).

In addition, the presence of either *mph*(C) or *msr*(A) gene herein led to lower MIC values than reported for the isolates showing the simultaneous presence of both genes. On the contrary, the simultaneous presence of the

msr(A) and *mph*(C) genes led to lower MIC values than noted for the isolates carrying only *msr*(A) gene (29).

In accordance with another study in the Czech Republic (29), the high resistance to erythromycin in *erm* positive isolates herein was accompanied by resistances to at least three other antimicrobial agents.

Finally, although antiseptic-resistant MRSA (55%) are already identified in our study, *qac*A/B and *smr* genes were not detected in these strains. Contemporary literature in Asia detected 137 antiseptic-resistant MRSA (26.5%) without *qac*A/B and *smr* genes (40). These results suggest that the antiseptic resistant MRSA lacking both genes may be intrinsically tolerant to the antiseptics and their resistance may be due to other genes presumably located on the chromosome of *S. aureus* as was previously published (41). Alternatively, the presence of *qac*A/B and *smr* genes in MRSA has been documented in a number of studies performed in Japan (41) and Iran (28).

Based upon the results of the present investigation, we can conclude that the fairly high rate of inducible clindamycin resistance with evidences of the wide occurrence of antibiotic resistance genes among multi-drug resistant MRSA strains is a warning for public health in Egypt. Therefore, periodical surveillance of antimicrobial susceptibility patterns of MRSA strains and the implementation of the D-test for delineation of phenotypic pattern of inducible clindamycin resistance are of utmost importance in reducing the present scourge of MRSA infections and alleviating the situation of failures in clindamycin therapy. However, a further investigation is warranted to provide an exploration of anti staphylococcal agents with alternative and multiple modes of antibacterial activity in the future. Ultimately, as a consequence of the absence of antiseptic resistance genes, the present study advocated the use of antiseptic agents as appropriate control strategies with a higher potential to prevent the infections of MRSA in Egypt.

References

1. Guven K, Mutlu MB, Gulbandilar A, Cakir P. Occurrence and characterization of *Staphylococcus aureus* isolated from meat and dairy products consumed in Turkey. Journal of Food Safety 2010; 30:196–212.
2. Diep BA, Chambers HF, Graber CJ, Szumowski JD, Miller LG, Han LL, *et al.* Emergence of multidrug-resistant, community-associated, methicillin-resistant *Staphylococcus aureus* clone USA300 in men who have sex with men. Ann Intern Med 2008; 148:249-57.
3. Witte W. Antibiotic resistance in gram-positive bacteria: epidemiological aspects. J Antimicrob Chemother 1999; 44:1-9.
4. Zapun A, Contreras-Martel C, Vernet T. Penicillin-binding proteins and beta-lactam resistance. FEMS Microbiol Rev 2008; 32:361-85.
5. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell 2007; 128:1037-50.
6. Schmitz FJ, Krey A, Sadurski R, Verhoef J, Milatovic D, Fluit AC. Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates. J Antimicrob Chemother 2001; 47:239-40.
7. Kaatz GW, Seo SM. Mechanisms of fluoroquinolone resistance in genetically related strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 1997; 41:2733-7.
8. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. J Clin Microbiol 2003; 41:4740-4.
9. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis 2002; 34:482-92.
10. Drinkovica D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J Antimicrob Chemother 2001; 48:315-6.
11. Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement, CLSI document M100-S24, Wayne, Pennsylvania, USA, 2014; 34:134-6.
12. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999; 12:147–79.
13. Chapman JS. Biocide resistance mechanisms. Intern Biodet Biodeg 2003; 51:133-8.
14. Baird D. Staphylococcus: Cluster forming gram positive cocci, Chapter 11. In: Mackie and McCartney Practical Medical Microbiology, 14th ed. Vol. 2. Collee JG, Fraser AG, Marmion BP and Simmons A. (eds.), New York, Churchill Livingstone, 1996, pp. 245–61.
15. Bjorland J, Sunde M, Waage S. Plasmid-borne *smr* gene causes resistance to quaternary ammonium compounds in bovine *Staphylococcus aureus*. J Clin Microbiol 2001; 39:3999-4004.
16. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, *et al.* Testing for induction of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus.* J Clin Microbiol 2005; 43:1716-21.
17. Duran N, Ozer B, Duran GG, Onlen Y, Demir C. Antibiotic resistance genes & susceptibility patterns in staphylococci. Indian J Med Res 2012; 135:389–96.
18. Adegoke AA, Okoh AI. Species diversity and antibiotic resistance properties of staphylococcus of farm animal origin in Nkonkobe Municipality, South Africa. Folia Microbiol 2014; 59:133-40.
19. Couto I, Costa SS, Viveiros M, Martins M, Amaral L. Efflux-mediated response of *Staphylococcus aureus* exposed to ethidium bromide. J Antimicrob Chemother 2008; 62:504-13.
20. Noguchi N, Nakaminami H, Nishijima S, Kurokawa I, So H, Sasatsu M., Antimicrobial agent of susceptibilities and antiseptic resistance gene distribution among methicillin-resistant *Staphylococcus aureus* isolates from patients with impetigo and staphylococcal scalded skin syndrome. J Clin Microbiol 2006; 44:2119-25.
21. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol 2001; 9:486-93.
22. Nusrat J, Ifra TN, Mrityunjoy A. Detection of methicillin-resistant *Staphylococcus aureus* within raw milk and cheese samples. International Food Research Journal 2015; 22:2629-33.
23. Ammar AM, Attia AM, Abd El-Aziz NK, Abd El Hamid MI, El-Demerdash AS Class 1 integron and associated gene cassettes mediating multiple-drug resistance in some food borne pathogens. International Food Research Journal 2016; 23:332-9.
24. Aydin A, Muratoglu K, Sudagidan M, Bostan K, Okuklu B, Harsa S. Prevalence and antibiotic resistance of foodborne *Staphylococcus aureus* isolates in Turkey. Foodborne Pathog Dis 2011; 8:63-9.
25. Sahebekhtiari N, Nochi Z, Eslampour MA, Dabiri H, Bolfion M, Taherikalani M, *et al.* Characterization of *Staphylococcus aureus* strains isolated from raw milk of bovine subclinical mastitis in Tehran and Mashhad. Acta Microbiol Immunol Hung 2011; 58:113-21.
26. Brînda M, Herman V, Faur B. Antimicrobial sensitivity of some *Staphylococcus aureus* strains from bovine mastitis. Lucrări Stiinłifice Medicină Veterinară 2010; XLIII:102-5.
27. Ombui JN, Kimotho AM, Nduhiu JG. Antimicrobial resistance

patterns and plasmid profiles of *Staphylococcus aureus* isolated from milk and meat. East Afr Med J 2000; 77:463-7.
28. Hasanvand A, Ghafourian S, Taherikalani M, Jalilian FA, Sadeghifard N, Pakzad I. Antiseptic resistance in methicillin sensitive and methicillin resistant *Staphylococcus aureus* isolates from some major hospitals, Iran. Recent Pat Antiinfect Drug Discov 2015; 10:105-12.
29. Schlegelova J, Vlkova H, Babak V, Holasova M, Jaglic Z, Stosova T, *et al.* Resistance to erythromycin of *Staphylococcus* spp. isolates from the food chain. Veterinarni Medicina 2008; 53:307-14.
30. Deotale V, Mendiratta DK, Raut U, Narang P. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Indian J Med Microbiol 2010; 28:124-6.
31. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol 2007; 56:342-5.
32. Patel M, Waites KB, Moser SA, Cloud GA, Hoesley CJ. Prevalence of inducible clindamycin resistance among community- and hospital-associated *Staphylococcus aureus* isolates. J Clin Microbiol 2006; 44:2481–4.
33. Sasirekha B, Usha MS, Amruta JA, Ankit S, Brinda N, Divya R. Incidence of constitutive and inducible clindamycin resistance among hospital-associated *Staphylococcus aureus*. 3 Biotech 2014; 4:85-9.
34. Mittal V, Kishore S, Siddique ME. Prevalence of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* detected by phenotypic method: A preliminary report. Journal of Infectious Diseases and Immunity 2013; 5:10-2.
35. Lyall KDS, Gupta V, Chhina D. Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus*. Journal of Mahatma Gandhi Institute of Medical Sciences 2013; 18:112-5.
36. Roberts MC, Soge OO, No D. Comparison of multi-drug resistant environmental methicillin-resistant *Staphylococcus aureus* isolated from recreational beaches and high touch surfaces in built environments. Front Microbiol 2013; 4:74.
37. Gao J, Ferreri M, Liu XQ, Chen LB, Su JL, Han B. Development of multiplex polymerase chain reaction assay for rapid detection of *Staphylococcus aureus* and selected antibiotic resistance genes in bovine mastitic milk samples. J Vet Diagn Invest 2011; 23:894-901.
38. Al-Marjani MF, Abdal AK, Edan DS. Identification of an efflux pump gene, *nor*A, in methicillin resistant *Staphylococcus aureus* in Baghdad. World journal of pharmaceutical research 2015; 4:346-56.
39. Aslantaş Ö, Öztürk F, Ceylan A. Prevalence and molecular mechanism of macrolide and lincosamide resistance in staphylococci isolated from subclinical bovine mastitis in Turkey. J Vet Med Sci 2011; 73:1645-8.
40. Noguchi N, Suwa J, Narui K, Sasatsu M, Ito T, Hiramatsu K, *et al.* Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes *qac*A/B and *smr* of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. J Med Microbiol 2005; 54:557-65.
41. Noguchi N, Hase M, Kitta M, Sasatsu M, Deguchi K, Kono M. Antiseptic susceptibility and distribution of antiseptic-resistance genes in methicillin-resistant *Staphylococcus aureus*. FEMS Microbiol Lett 1999; 172:247-53.