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Prevalence of *Agr* Specificity Groups among *in vitro* Biofilm Forming Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Nasal Carriers

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) has the ability of biofilm-formation associated with serious infections and decreased the susceptibility to antibiotics. In *Staphylococcus aureus (S. aureus)*; the accessory gene regulator (*agr*) globally controls the coordinated production of virulence factors. The aim of this study was to determine the prevalence of biofilm formation among MRSA isolated from nasal carriers by crystal violet microtitre plate assay and Congo red agar assay. PCR-based method was used to identify *agr* specificity groups among studied biofilm forming MRSA isolates. The study was conducted in 325 patients by using sterilized nasal swabs for collection of nasal specimens. Nasal bacteria were recognized as *S. aureus* strains by standard biochemical tests. MRSA isolates were detected phenotypically by disk diffusion method and genotypically by detecting *mecA* gene by PCR. *Agr* specificity groups among studied MRSA isolates were identified by PCR-based method. The susceptibility of MRSA isolates to different antibiotics was determined by using disk diffusion method. One hundred and six (32.6%) *S. aureus* were isolated from our participants. Forty seven (44.3%) of all *S. aureus* isolates were recognized as MRSA. Eighty three percentages were biofilm forming MRSA. Regarding *agr* locus distribution, *agr* group I is the most prevalent group in our nasal carriage patients (74.4%) and associated with MRSA biofilm forming strains and high resistance to antibiotics. While 15.4% is belonging to *agr* group II and 10.2% to *agr* group III. There was a significant difference amongst types of *agr* gene distribution in biofilm producer isolates ($P<0.05$). *Agr* group II (50%) and III (25%) are prevalent in non-biofilm forming strains and more sensitive to antibiotics in MRSA biofilm-forming strains. *Agr* group IV was not detected among all studied strains. Slime layer assay showed that 76.6% of MRSA isolates were slime layer producer. The highest distribution of antibiotic resistance was observed amongst *agr* group I followed by *agr* group II and was least with *agr* group III with significant difference ($P<0.001$). MRSA nasal carriage rate in our study was high and had the ability to form biofilm that is alarming for public health. *Agr* quorum sensing (Qs) warrants further investigation as novel drug targeting.

Key words: *Agr* Gene · Methicillin Resistant *Staphylococcus aureus* (MRSA) · Quorum Sensing (Qs) · Biofilm · Antimicrobials Susceptibility · Nasal Carriers

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INTRODUCTION

Among the regulatory systems of bacteria, cell–cell communication or quorum-sensing (QS) systems have gained broad attention in the scientific community. The signals of QS systems are small molecules called autoinducers (AIs). At low cell population density, AIs are low in concentration. When the cells reach a certain population density and AIs accumulate to a threshold concentration, a transcriptional regulator is activated. This transcriptional factor in turn regulates the expression of various genes, which often includes a series of virulence factors [1].

In *S. aureus*, the accessory gene regulator (*agr*) globally controls the coordinated production of virulence factors. It controls a large set of genes, including most of those encoding cell-wall-associated and extracellular proteins. The *agr* locus is composed of 2 divergent transcriptional units, RNAII and RNAIII, driven by the P2 and P3 promoters, respectively. The P2 operon encodes 4 proteins (agrA, agrB, agrC and agrD) that generate the *agr* sensing mechanisms [2]. The classical two-component signaling system (agrC as the signal receptor and agrA as the response regulator) serves as a quorum-sensing regulon to autoinduce RNA III, the principal effector of the *agr* response [3]. The agrB and agrD gene products are engaged in the production of AIs [4]. The AI is an autoinducing peptide of 8 amino acids in length, which is encoded within the agrD gene. It is synthesized as a larger polypeptide and then presumably trimmed by the agrB gene product [5, 6] to form a thiolactone-containing ring structure [7, 8]. AIs from different staphylococcal strains and species have a divergent primary amino acid sequence, but conserve the typical ring structure. The four allelic groups of *agr*, which generally inhibit the regulatory activity of each other, have been identified within the species. As *agr* controls a series of virulence factors, it has been proposed to exploit *agr* antagonism by cross-inhibiting AIs as a means to control staphylococcal infection [8].

According to some reports, over 65% of hospital-acquired infections occur by the infecting organisms that have the ability of producing biofilms [9]. A biofilm is a sessile microbial community of cells that are attached to a substratum. QS appears to influence biofilm formation at many of these stages (initial attachment, cell-to-cell adhesion and proliferation and finally detachment) [10]. Non-functionality of the *agr* system facilitates the initial attachment of staphylococci to a polystyrene surface [11]. While, *agr* up regulation expected to play a major role in the detachment process and revert cells to planktonic form [12, 13].

Numerous studies have demonstrated that biofilm-grown bacteria lack susceptibility to antimicrobials, whereas planktonic cultures of the same microorganism do not [14- 16]. Factors such as the biomaterial composition, biofilm permeability and microenvironmental (chemical composition and dynamic factors) characteristics of biofilm may affect the bactericidal activity of antimicrobials against bacteria forming biofilms [17]. This suggests that the selection of antimicrobial agents on the basis of traditional susceptibility methods, such as broth MIC or breakpoint, fails to predict efficacy in microbial biofilm eradication, possibly resulting in treatment failure.

Early identification and adopting efficient control protocol against biofilm forming MRSA can be one of the essential steps towards the prevention of the most serious nosocomial infections [18].

In this study, we tried to determine the prevalence of biofilm formation among MRSA isolated from nasal carriers referred to outpatient clinic of Otorhinolaryngology department in university hospitals by using a modified microtitre plate assay and Congo red agar assay. Also, we used PCR-based methods to identify *mec*A gene among *S. aureus* isolates and *agr* specificity groups among studied biofilm forming MRSA isolates.

MATERIALS AND METHODS

Specimens and MRSA Identification: Three hundred twenty five patients had undergone the study after informed consent obtained from them according to Ethics Committee of University Hospitals. The study was performed between December 2012 and May 2013. Sterile swabs were used for collecting samples from both anterior nares of each patient. The identification of isolates was done according to standard methods. Methicillin resistance was confirmed by oxacillin and cefoxitin disk test in accordance with CLSI [19]. All isolates were stored in brain-heart infusion broth containing 16% (w/v) glycerol at -80°C until use.

Biofilm and Slime Formation: Biofilm and slime formation were determined by crystal violet microtitre plate assay and Congo red agar assay, respectively. Briefly, biofilm formation was detected by using a modified microtitre plate test Stepanović *et al*. [20]; bacteria were grown

overnight on Müller Hinton agar (Oxoid) plates and then re-suspended in trypticase soy broth (TSB) plus 5% glucose. The optical density at 650nm (OD_{650}) of the bacterial suspensions was determined and aliquots of 100 µl were inoculated in nine parallel wells of a 96-well polystyrene plate. After incubation for 48 h at 37°C, the plates were softly shaked to remove planktonic bacteria. The wells were rinsed with phosphate buffer saline (PBS) and then fixed with 150 µl absolute methanol for 10 min. Attached bacterial material was then stained by adding 150 µl crystal violet (1% w/v) for 20 min. The plates were rinsed with tap water and the amount of attached material was measured by solubilisation of the crystal violet dye in 150 µl of 33% glacial acetic acid. The A_{570} was measured using an ELISA reader [20]. Congo red agar (CRA) assay was used for detecting slime producing isolates; MRSA isolates were cultured on the agar comprising 0.8 g of Congo red (Sigma) and 36 g of saccharose (Sigma) to one liter of brain heart infusion agar (Bacton, Dickinson, France) and incubated at 37°C for 48 h. Strains which produced black colonies considered as slime producers and strains with red colonies labeled as non-slime producers [21].

MecA Detection: The presence of *mecA* gene in MRSA isolates was confirmed by a PCR assay. DNA was extracted using illustra bacteria genomicPrep Mini Spin Kit (GE Healthcare, UK). The used primers were designed by Eurofins MWG Operon (Germany); Forward 5'TCCAGATTACAACTTCACCAGG3', Reverse 5'CCACTTCATATCTTGTAACG3'.Theseprimersamplify 162 bp of DNA fragment [22]. PCR was carried out in a total volume of 45µl with 30ng chromosomal DNA as a template. PCR conditions included: heating at 94°C for 15min; followed by 30 cycles of 94°C for 30s, 57°C for 90s and 72°C for 2min; and a terminal cycle of 72°C for 10 min using GeneAmp PCR System 9600 (Perkin-Elmer Cetus Corp., Norwalk, CT, USA). In each PCR run, *S. aureus* strain ATCC 33591 was used as a positive control and negative control was added to each PCR run. A 50 bp DNA ladder was used as a size marker. Synthesized DNA fragments were detected on 1.5% agarose gels by ethidium bromide staining and visualized under ultraviolet light and photographed.

Agr Grouping: Agr specificity groups were identified by PCR amplification of the hyper variable domain of the *agr* locus [23] using oligonucleotide primers specific for each of the four major specificity groups. A forward primer, pan-*agr* (5 - ATGCACATGGTGCACATGC-3), corresponding to conserved sequences from the *agrB* gene, was used in all reactions. Four reverse primers, each specific for amplification of a single *agr* group based on *agrD* or *agrC* gene nucleotide polymorphism, were as follows: *agr* I, 5'-GTC ACAAGTACTATAAGCTGCGAT-3' (in the *agrD* gene); *agr* II, 5'-GTATTACTAATTGAAAAGTGCCATAGC-3' (in the *agrC* gene); *agr* III, 5'-CTGTTGAAAAAGTCAACTAAAAGCTC-3 (in the *agrD* gene); and *agr* IV, 5'-CGATAATGCCG TAATAC CCG-3' (in the *agrC* gene).

DNA was extracted using illustra bacteria genomicPrep Mini Spin Kit (GE Healthcare, UK). PCR product size of *agr* groups I and III and groups II and IV were similar, so, we performed 2 duplex PCR reactions. The reverse primers I (product size: 440 bp) and IV (product size: 588 bp) were used in the first reaction and primers II (product size: 572 bp) and III (product size: 406 bp) were used in the second reaction. Amplification was performed with a Mycycler TM Thermal cycler (BioRad, USA). PCR conditions were: one cycle at 95°C for 2 min followed by 30 cycles of PCR, one cycle of denaturation (1 min at 94°C), annealing (1 min at 55°C) and extension (1 min at 72°C). A final elongation step was at 72°C for 5 min PCR products were separated by using QIAxcel system, (QIAGEN®, Germany) each sample was automatically loaded into an individual capillary. Separation was performed using the AM320 method (100 ng/µl sample was injected at voltage 5 kV for 10s and separation voltage was 6kV for 320s). The negatively charged nucleic acid molecules migrate through the capillary to the positively charged end. As the molecules migrate through the capillary, they pass a detector which detects and measures the fluorescent signal. A photomultiplier detector converts the emission signal into electronic data, which are then transferred to the computer for further processing using QIAxcel ScreenGel Software. After processing, the data are displayed as an electropherogram and gel images.

S. aureus strains RN6390 (*agr* group I), RN6607 (*agr* group II), RN8462 (*agr* group III), RN4550 (*agr* group IV) and RN6911 (*agr* negative) were used as reference strains for *agr* group identification provided by Novick RP (Shirball Institute, New work).

Antimicrobial Susceptibility Testing: By using the disk diffusion method, the antibiotic susceptibility of the isolates was performed according to the CLSI [19]. The tested antimicrobial disks were: penicillin (10 U), methicillin (5 µg), oxacillin (1 µg), amoxicillin/clavulanic acid (20/10 µg), cefoxitin (30 µg), ceftriaxone (30 µg), gentamicin (30 µg), amikacin (30µg), kanamycin (30 µg), teicoplanin (30 µg), tetracycline (30 µg), linezolid (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (2 µg), rifampin (5 µg), moxifloxacin (5 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gatifloxacin (5 µg), cotrimoxazole (30 µg), pristinomycin (15 µg), mupirocin (5 µg) and fusidic acid (30 µg). Vancomycin susceptibility was detected by MIC test [19].

Statistical Analyses: Computer SPSS program version 15 was used. The results were expressed by applying Chi-square test and P values. P value <0.05 was considered to be significant.

RESULTS

The study group consisted of 325 patients who were between ages 19 and 74 years (median 36.7). One hundred eighty six (57.2%) were males and 139 (42.8%) were females. Distribution of MRSA nasal carrier according to age group and sex was not significant (P>0.05).

One hundred and six (32.6%) *S. aureus* were isolated from our participants. Forty nine (46.2%) MRSA were detected by disk diffusion method among isolated *S. aureus* but *mecA* gene was seen in forty seven (44.3%) of them only. Figure 1, shows the amplification for presence of *mecA* gene.

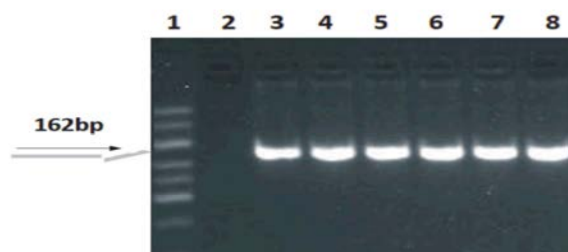

Fig. 1: Gel electrophoresis of the PCR amplification using *mecA* gene specific primers. Lane 1; DNA ladder, lane 2; negative control, lane 3; *S. aureus* strain ATCC 33591 (a positive control) and lanes 4 to 8: *mecA* gene positive strains.

Identification of biofilm production was observed according to the criteria of Stepanović *et al*. [25] (Table 1). The biofilm producer isolates were represented by 83% of the MRSA strains (39 isolates); while non-biofilm producers were represented by 17% of the studied MRSA isolates (8 isolates). Regarding *agr* locus distribution, 29 strains out of 39 biofilm producer isolates (74.4%) were belonged to *agr* group I, while 6 strains (15.4%) were belonged to *agr* group II and 4 strains (10.2%) were belonged to *agr* group III. *Agr* group IV was not detected among all studied biofilm producer strains. There was a significant difference amongst types of *agr* gene distribution in biofilm producer isolates (P<0.05). Twenty five percentage of non-biofilm producer isolates was *agr* group I, while 50% was *agr* group II, 25% was *agr* group III and no detectable *agr* group IV without significant differences (P>0.05). Slime layer assay showed that 36 out of 47 (76.6%) of MRSA isolates were slime layer producer and 11 out of 47 (23.4%) were classified as non-slime layer producer. Figure 2 shows colonies of slime layer and non-slime layer producer MRSA isolates on CRA.

Different antimicrobial agents were chosen to test the susceptibility pattern of 39 MRSA biofilm producer isolates in *agr* specific groups. For each *agr* specific group, the percentages of resistance of studied antimicrobial agents were obtained (Table 2). Our study reported that, all strains were highly resistant (100%) to penicillin, methicillin, oxacillin and cefoxitin.

Table 1: Biofilm forming pattern of MRSA and *agr* locus distribution

Biofilm Profile	Biofilm production (N)%	*agr* gene	Strains (N)%	P value
Non- biofilm producer	(8/47) 17	I	(2/8) 25	P>0.05
		II	(4/8) 50	
		III	(2/8) 25	
		IV	(0/8) 0.00	
Biofilm producer	(39/47) 83	I	(29/39) 74.4	P<0.05
		II	(6/39) 15.4	
		III	(4/39) 10.2	
		IV	(0/39) 0.00	

Table 2: Antibiotic resistance of MRSA biofilm producer isolates (N= 24) in *agr* specific groups

		Group				
Antibiotics		*Agr* I (N=29)	*Agr* II (N=6)	*Agr* III (N=4)	% resistance (N=39)	P value
Penicillin	Penicillin (10U)	29 (100%)	6 (100%)	4 (100%)	39 (100%)	P<0.001
	Methicillin (5µg)	29 (100%)	6 (100%)	4 (100%)	39 (100%)	
	Oxacillin (1µg)	29 (100%)	6 (100%)	4 (100%)	39 (100%)	
	Amoxicillin/clavulanic acid (20/10µg)	18 (62.1%)	0 (0.00%)	0 (0.00%)	18 (46.2%)	
Cephalosporins	Cefoxitin (30µg)	29 (100%)	6 (100%)	4 (100%)	39 (100%)	
	Ceftriaxone (30µg)	21 (72.4%)	4 (66.7%)	1 (25%)	26 (66.7%)	
Aminoglycosides	Gentamicin (30µg)	16 (55.2%)	0 (0.00%)	0 (0.00%)	16 (41.1%)	
	Amikacin (30µg)	3 (10.3%)	0 (0.00%)	0 (0.00%)	3 (7.7%)	
	Kanamycin (30µg)	13 (44.8%)	0 (0.00%)	0 (0.00%)	13 (33.3%)	
Glycopeptides	Vancomycin (breakpoint; = 2 - = 16)	4 (13.8%)	0 (0.00%)	0 (0.00%)	4 (10.3%)	
	Teicoplanin (30µg)	7 (24.1%)	0 (0.00%)	0 (0.00%)	7 (17.9%)	
Tetracyclines	Tetracycline (30µg)	3 (10.3%)	0 (0.00%)	0 (0.00%)	3 (7.7%)	
Oxazolidinones	Linezolid (30µg)	4 (13.8%)	0 (0.00%)	0 (0.00%)	4 (10.3%)	
Phenocols	Chloramphenicol (30µg)	6 (20.9%)	2 (33.3%)	0 (0.00%)	8 (20.5%)	
Macrolides	Erythromycin (15µg)	13 (44.8%)	0 (0.00%)	0 (0.00%)	13 (33.3%)	
Lincosamides	Clindamycin (2µg)	6 (20.9%)	2 (33.3%)	0 (0.00%)	8 (20.5%)	
Ansamycins	Rifampin (5µg)	21 (72.4%)	0 (0.00%)	0 (0.00%)	21 (53.8%)	
Quinolones	Moxifloxacin (5µg)	19 (65.5%)	2 (33.3%)	1 (25%)	22 (56.4%)	
	Ciprofloxacin (5µg)	24 (82.8%)	0 (0.00%)	0 (0.00%)	24 (61.5%)	
	Levofloxacin (5µg)	21 (72.4%)	5 (83.3%)	3 (75%)	29 (74.4%)	
	Gatifloxacin (5µg)	8 (27.6%)	0 (0.00%)	0 (0.00%)	8 (20.5%)	
Anti-Metabolites	Cotrimoxazole (30µg)	22 (75.9%)	0 (0.00%)	0 (0.00%)	22 (56.4%)	
Others	Pristinomycin (15µg)	3 (10.3%)	0 (0.00%)	0 (0.00%)	3 (7.7%)	
	Mupirocin (5µg)	3 (10.3%)	0 (0.00%)	0 (0.00%)	3 (7.7%)	
	Fusidic acid (30µg)	24 (82.8%)	2 (33.3%)	0 (0.00%)	26 (66.7%)	

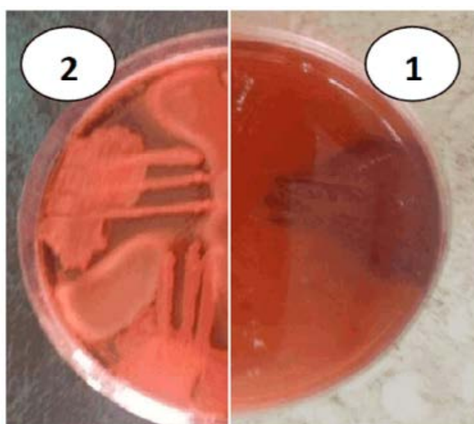

Fig. 2: Slime layer production of MRSA strains on CRA. Black colonies (1) on CRA show slime layer producer MRSA and red colonies (2) illustrate non-slime layer producer MRSA isolates.

Fig. 3: PCR assay for the identification of *agr* specificity groups. Lane 1; *S. aureus* RN6390 (*agr* group I), lane 2; *S. aureus* RN6607 (*agr* group II), lane 3; *S. aureus* RN8462 (*agr* group III), lane 4; *S. aureus* RN4550 (*agr* group IV), lane 5; *S. aureus* RN6911 (*agr* negative), lanes 6 to 9, PCR products of studied strains using reverse primers *agr* I, *agr* II, agr III and agr IV (440 bp for *agr* I, 572 bp for *agr* II, 406 bp for *agr* III and no band for *agr* IV, respectively).

Other antimicrobial agents showed variable degrees of sensitivity against the studied isolates. About 74.4% of strains were resistant to levofloxacin, 66.7% to ceftriaxone and fusidic acid, 61.5% to ciprofloxacin, 56.4% to moxifloxacin and cotrimoxazole, 53.8% to rifampin, 46.2% to amoxicillin/clavulanic acid, 41.1% to gentamicin, 33.3% to kanamycin and erythromycin, 20.5% to chloramphenicol, clindamycin and gatifloxacin, 17.9% to teicoplanin, 10.3% to vancomycin and linezolid and finally 7.7% to tetracycline, amikacin, pristinomycin and mupirocin. The highest distribution of antibiotic

resistance was observed amongst *agr* group I followed by *agr* group II and was least with *agr* group III with significant difference (P<0.001).

Figure 3 shows the capillary electrophoresis results of one isolate from each *agr* type as an example by using QIAxcel System and QIAxcel ScreenGel Software, as simulated bands on gel images. Gel images of amplified PCR products gave one DNA band at 440 bp for *agr* I, at 572 bp for *agr* II, at 406 bp for *agr* III and no band for *agr* IV.

DISCUSSION

Staphylococcus aureus is human opportunistic pathogen which can cause a plethora of infections ranging from cutaneous infections to more serious infections such as bacteremia and toxemic syndromes as toxic shock syndrome [24, 25]. MRSA have become a large economic problem worldwide. However, its rate varies widely between countries [26].

The pathogenic capacity of *S. aureus* is clearly dependent on its production of exoproteins. The synthesis of these virulence factors is globally regulated by an *S. aureus* quorum-sensing system called the *agr*. The expression of genes in *agr* varies in response to changes in cell density [24, 25]. The signal peptide interacts with the trans-membrane receptor protein (agrC) of a classical two-component regulatory system (agrC, agrA) that in turn activates the transcription of the *agr* locus. This induces the down-regulation of genes encoding surface proteins and the up-regulation of genes encoding secreted virulence factors [27, 28]. On the basis of autoinducer-receptor specificity, *S. aureus* can be divided into four different *agr* groups [29–32]. In 2002, *agr* alleles were identified by the amplification of a 1.234 pb *agr* fragment encompassing the 3' end of *agr*B, all of *agr*D and the 5' end of *agrC* followed by the sequencing [30, 31]. More recently, PCR amplification of the hypervariable domain of the *agr* locus was described in Dufour *et al.* [27] study. It has allowed classifying our strains in one of the four different *agr* groups defined by Ji *et al*. [29].

In this trial, we identified *agr* specificity groups among a collection of biofilm forming MRSA isolates obtained from nasal carriers referred to outpatient clinic of Otorhinolaryngology department in university hospitals. Three out of four allelic groups of *agr*, which generally inhibit the regulatory activity of each other, had been identified within our isolates. Most of our isolates belonged to *agr* group I (74.4%), followed by *agr* group II (15.4%) then *agr* group III (10.2%) and no *agr* group IV was detected in our strains. Shopsin and his colleagues [33] showed that *agr* specific group I (42%) was prevalent between children and their guardians while in the van Leeuwen and colleagues [34] study, 71% of strains belonged to *agr* group I. A higher prevalence of *agr* group II in the nosocomial infection group was detected in the study of Manago *et al*. [35]. No detection of *agr* specific group IV in our research as in many previously reported studies [33-36] was observed. This absence of *agr* group IV isolates suggests that competition does not favor these strains. However, *agr* specific group IV was detected in 9.6% of blood cultures, 3.2% of urine samples, 10.8% of respiratory tract specimens, 12.1% of cutaneous specimens and 6.6% of nasal swabs from healthy patients of Peerayeh *et al*. [2] study. Other studies revealed that, 44 from 87 (51%) of MRSA belonged to *agr* group I and 38 of 87 (44%) belonged to *agr* group II [37], in contrast to prior data showing that greater than 60% of MRSA bacteremia isolates collected from multiple centers around the United States belonged to *agr* group II [38]. Goerke *et al*, [39] revealed that, most of MRSA isolates belonged to *agr* group III (40.3%) followed by *agr* group I (15.7%). They explained that by the closely relationship between these two groups (80% sequence homology) [39] that would suggest a unique genetic characteristics of their isolates. The distribution of *agr* groups in our study differed from some data previously reported, perhaps reflecting ecological and geographical structuring or sampling bias.

Moreover, we investigated a possible relationship between *agr* specificity group and antibiotic resistance patterns. All *agr* specific group I isolates showed variable degrees of resistance to all studied antibiotics. While, *agr* specific group II and III isolates were susceptible to most studied antibiotics (with the exception of penicillin, methicillin, oxacilin and cefoxitin). Thus, in accordance with these results, there is provided a method of increasing sensitivity of biofilm-forming MRSA with an activator of an *agr* quorum-sensing system especially *agr* II and *agr* III. The activator may be an autoinducing peptide (AIP) by inhibiting MRSA biofilm formation, inhibiting MRSA biofilm growth, reducing MRSA biofilm size or promoting detachment of MRSA from a formed biofilm. Our data is similar to great extent with the patency of inventor Horswill [40] in 2012 who used activators of bacterial *Agr* QS systems to prevent or reverse biofilm formation in MRSA, or to restore sensitivity of MRSA biofilms to antibiotics [40]. That can be explained by decreasing the expression of surface adhesins through the *Agr* system activation in established biofilms that triggers a dispersal pathway, detaching cells from a

surface-bound biofilm and reverting them to a planktonic, antibiotic-susceptible state (induction of the agr system results in biofilm disassembly) [41]. So, studying of QS control in staphylococci represents a promising target for the development of novel antibacterial agents.

Our results showed that, tetracycline, amikacin, pristinomycin and mupirocin were the most effective antibiotic against nasal carriers biofilm-forming MRSA strains followed by vancomycin and linezolid. This is in agreement with the study of Rezaei *et al*. [18], who revealed that, tetracyclines seem to be good candidates for investigations as preventative agent of MRSA biofilms because tetracyclines are protein synthesis inhibitors, broad-spectrum, bacteriostatic antibiotics that target the 30S ribosome and prevent binding of tRNA and effective against both Gram-positive and Gram-negative bacteria [42].

The rate of MRSA nasal carriage in our study was high, 83% of MRSA isolates had the ability to form biofilm. Since biofilm–forming capacity increase the resistance to commonly used antibiotics, isolating biofilm-forming MRSA from nasal carrier that can be easily transmitted to other people in the community and hospital, is alarming for public health.

CONCLUSIONS

We concluded that; (1) *agr* grouping may represent a first subdivision of MRSA based on the fundamental biology, (2) *agr* group I is the most prevalent group in our nasal carriage patients and associated with MRSA biofilm forming strains and high resistance to antibiotics, (3) *agr* group II and III are prevalent in non-biofilm forming strains and more sensitive to antibiotics in MRSA biofilm-forming strains due to intrinsic sensitivity or reverting them to a planktonic-susceptible state, (4) *agr* QS system warrants further investigation as novel antibiotics agents, (5) tetracycline, amikacin, pristinomycin and mupirocin are the most effective antibiotic against nasal carriers biofilm-forming MRSA strains and (6) MRSA nasal carriage rate in our study is high and has the ability to form biofilm that is an alarming for public health.

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