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۱ **Molecular analysis of accessory gene regulator functionality and virulence**
۲ **genes in *Staphylococcus aureus* derived from pediatric wound infections**

۳
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۳۵ **Abstract**

۳۶ *Staphylococcus aureus* is a major human pathogen causing infections with high morbidity and
۳۷ mortality in both healthcare and community settings. The accessory gene regulator (Agr) is a key
۳۸ genetic element controlling the expression of numerous virulence factors in *S. aureus*. The
۳۹ significance of a functional Agr system in clinical *S. aureus* isolates derived from pediatric wound
۴۰ infections is still unclear. Therefore, the present study was conducted to identify virulence genes
۴۱ and determine Agr functionality from this cohort of patients. A total of 48 *S. aureus* wound isolates
۴۲ were collected from patients referred to Tehran Children's Medical Center Hospital from April
۴۳ 2017 to April 2018. In addition, *in vitro* antimicrobial susceptibility of the isolates was assessed
۴۴ using the disk diffusion and E-test methods. Conventional PCR was performed for the detection
۴۵ of toxins (*tsst-1*, *hla*, *hlb*, *hld*, *eta*, *etb*, *etd*, *edin-A*, *edin-B*, *edin-C*) and Agr typing (*agrI*, *agrII*,
۴۶ *agrIII*, *agrIV*). Agr functionality was assessed by quantitative reverse transcriptase real-time PCR
۴۷ (qRT-PCR). All *S. aureus* isolates were found to be susceptible to linezolid and vancomycin. The
۴۸ most frequently detected toxin gene was *eta* (100%), and the most prevalent Agr type was *agrIII*
۴۹ (56.3%). Importantly, qRT-PCR revealed that Agr was functional in 28 (58%) of wound isolates.
۵۰ Consequently, our data suggests that a functional Agr system may not be required for the
۵۱ development of *S. aureus* wound infections.

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۵۳ **Keywords:** *Staphylococcus aureus*, Agr functionality, wound infections, pediatrics.

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76 **1. Introduction**

77 *Staphylococcus aureus* is an ever-present opportunistic pathogen that can cause a variety of
78 diseases. The severity of *S. aureus*-associated infections ranges from benign localized skin
79 abscesses to life-threatening diseases, such as arthritis, osteomyelitis, and endocarditis (Francois
80 *et al.*, 2006; von Eiff *et al.*, 2004). In recent decades, methicillin-resistant *S. aureus* (MRSA) strains
81 have emerged as a predominant cause of invasive diseases, namely skin and soft tissues, as well
82 as musculoskeletal infections in children (Kaushik and Kest, 2018). This bacterium is one of the
83 most dominant commensals on human skin and nasal mucosa and can express a multitude of
84 virulence factors, such as surface adhesins, enterotoxins and hemolysins which are central in the
85 development of disease. (Kassam *et al.*, 2017; Stevens *et al.*, 2017). The synchronized expression
86 of these virulence determinants is tightly controlled by the cumulative action of several regulatory
87 elements, such as the accessory gene regulator (*agr*), staphylococcal accessory regulator A (*sarA*),
88 and the alternative sigma factor B (σ B) (Manna and Cheung, 2001).

89
90 The Agr system plays a central role in the growth-phase dependent modulation of virulence
91 gene expression (Bronner *et al.*, 2004; Sakoulas *et al.*, 2003a). The *agr* operon is an autocatalytic
92 system controlled in a cell density-dependent fashion through the production and sensing of auto-
93 inducing peptides (AIP). At high cell density, the Agr system increases the production of many
94 secreted virulence factors, including Toxic shock syndrome toxin -1 (TSST-1), delta-hemolysin
95 and exfoliative toxins A and B (ETA and ETB). In contrast, Agr decreases the expression of several
96 colonization factors such as fibronectin binding proteins, important in adhesion and biofilm
97 formation (Li *et al.*, 2018). The *agr* locus consists of two distinct transcripts, RNAII and RNAIII,
98 which are transcribed by two promoters, P2 and P3 respectively. The activation of P2 induces the
99 expression of the components involved in cell-to-cell quorum-sensing communication
100 (AgrBDCA) (Bibalan *et al.*, 2014a). Both AgrB and AgrD function to process and secrete the auto-
101 inducing peptide (AIP), which acts as the chemical messenger critical for Agr activity (Wang *et al.*
102 *et al.*, 2014). Upon reaching a critical density, AIPs interact with the sensor kinase, AgrC which
103 promotes phosphorylation of the DNA binding response regulator AgrA. Phosphorylated AgrA
104 undergoes a conformational change permitting interaction and binding to the intergenic region
105 between P2 and P3 facilitating their expression. P3 activation leads to the expression of RNAIII,
106 the effector of target gene regulation (Novick and Geisinger, 2008).

97 Several studies have demonstrated a correlation between *agr* types and particular diseases. For
98 example phylogenetic group AF1 (*agr* group IV) strains are closely related to generalized
99 exfoliative syndromes and bullous impetigo whereas endocarditis is mainly caused by
100 phylogenetic group AF2 (*agr* groups II and I) strains (Jarraud *et al.*, 2002). In addition, it has been
101 suggested that *agr* group III and IV strains are associated with toxic shock syndrome (Gomes *et*
102 *al.*, 2005). To the best of our knowledge, there is no published study evaluating Agr functionality
103 among Iranian *S. aureus* isolates. The present study was conducted to determine dominant Agr
104 types, Agr activity and presence of specific virulence genes in *S. aureus* isolates derived from
105 pediatric wound infections.

106

107 **2.1 Materials and Methods**

108 *2.1 Bacterial isolation and identification*

109 In the present study, 48 *S. aureus* isolates were collected from wound infections of pediatric
110 patients referred to the Children's Medical Center Hospital Tehran, Iran over one year from April
111 2017-2018. The School of Medicine, Shahid Beheshti University of Medical Sciences ethics
112 committee approved this study (IR.SBMU.MSP.REC.1395.369). The isolates were identified as
113 *S. aureus* according to phenotypic (colonial morphology and Gram-stain), biochemical (catalase,
114 haemolysis, oxidase, coagulase, DNase, and mannitol fermentation tests) and genetic (polymerase
115 chain reaction (PCR) detection of *S. aureus* specific nuclease A (*nucA*) gene) analysis. The isolates
116 were stored in Tryptic Soy Broth (TSB) (Merck, Germany) containing 20% glycerol at -80C for
117 further investigation.

118

119 *2.2 Antimicrobial susceptibility testing*

120 Antibiotic susceptibility testing was performed using the Kirby-Bauer disc-diffusion method as
121 recommended by the Clinical and Laboratory Standards Institute (CLSI) (Huse *et al.*, 2017).
122 Commercially available antibiotic disks (Mast Co., UK) used in this study included penicillin (10
123 units), ciprofloxacin (5 µg), clindamycin (2 mg), gentamicin (10 µg), erythromycin (15 µg),
124 linezolid (30 µg), oxacillin (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and
125 ceftaroline (30 µg). In addition, susceptibility to vancomycin was determined using minimum
126 inhibitory concentration (MIC) E-test strips (Liofilchem Co., Roseto, Italy).

127 *2.3 MRSA identification*

128 For detection of MRSA isolates, an MIC of cefoxitin was determined using E-test (Liofilchem
129 Co., Italy). *S. aureus* ATCC 25923 was used as a quality control reference strain. Additionally, for
130 the molecular detection of MRSA, PCR analysis of the *mecA* gene was performed (Table 1).

131
132 *2.4 DNA extraction*
133 Genomic DNA was isolated from bacterial strains grown in Mueller-Hinton broth (Merck Co.,
134 Germany) overnight at 37°C. Pelleted bacterial cells were resuspended in 200 µl of phosphate-
135 buffered saline (PBS) and then DNA extraction was carried out using High Pure PCR Template
136 Preparation Kit (Roche Co., Germany) according to the manufacturer's instructions. Evaluation of
137 the concentration and purity of extracted DNA was measured by Nanodrop (DeNovix Inc., USA).
138 Extracted DNA was stored at -20°C for later analysis.

139
140 *2.5 Molecular detection of toxin genes and Agr typing*
141 The presence of the virulence genes encoding toxins, (*tsst-1*, *hla*, *hly*, *hld*, *eta*, *etb*, *etd*, *edin-A*,
142 *edin-B*, and *edin-C*) were investigated using PCR. The product size and annealing temperature of
143 each primer sets are provided in Table 1. Agr typing was conducted using a pan forward primer
144 and four specific reverse primers (Table 1). The PCR reaction was performed in a total volume of
145 25 µl containing 12.5 µl of 2X master mix (BIOFACT, Korea), 1µl (10 pM/ µl) of each primer,
146 8.5 µl of distilled water, and 2µl of DNA (10 ng) template. The cycling programs, was preceded
147 by 4 min at 94° C and consisted of 30 cycles of 94° C for 2 min, 1 min annealing at specific
148 temperature for each primer set (Table 1) and 72° C for 1 min, followed by a final extension step
149 at 72° C for 5 min. PCR amplicons were separate using 1.2% agarose gels and visualized by
150 staining with gel red stain (CinnaGen Co., Iran).

151
152 *2.6 Reverse transcription and quantitative RT-PCR*
153 *S. aureus* isolates grown overnight in TSB were diluted 1:1000 in fresh TSB and grown at 37°C
154 for 6 h. The cultures were treated with two volumes of RNAlater (Sigma-Aldrich, Germany),
155 immediately mixed by gentle vortexing for 5 s and incubated for 10 min at room temperature.
156 Next, cultures were centrifuged at 7000 × g for 10 min, supernatant discarded and the resulting
157 pellet was stored at -70°C until required. Following thawing on ice, the pellet was resuspended in
158 Tris-EDTA (TE) buffer (pH 8) containing 200 µg/mL lysozyme (Sigma-Aldrich Co., Germany)

109 and 250 µg/mL lysostaphin (Sigma-Aldrich Co., Germany) followed by incubation at 37°C for 2
110 h. During the incubation period, the suspension was mixed for 10s in 10 min intervals. Following
111 incubation, the suspension was treated with proteinase K (Roche Co., Germany) for 20 min at
112 37°C with mixing for 10 s at 3 min intervals. RNA extraction was performed using the RNeasy
113 Mini Kit (Roche Co., Germany) according to the manufacturer's instructions with the addition of
114 an extra DNase treatment (CinnaGen Co., Iran) following RNA purification. The absence of DNA
115 contamination was verified by PCR amplification of the housekeeping *gyrA* gene. Reverse
116 transcription was carried out using the cDNA synthesis kit (Wizbio Co., South Korea) according
117 to the manufacturer's instructions. Real-time PCR was performed with SYBR green PCR master
118 mix (Amplicon Co., Denmark) using specific primers for both *gyrA* [*gyrAF*: 5'-
119 CCAGGTAAATTAGCCGATTGC-3'; *gyrAR*: 5'-AAATCGCCTGCGTTCTAGAG-3'] and
120 RNAlII [*rnaIIIF*:5'-GAAGGAGTGATTTCAATGGCACAAG-3', *rnaIIIR*: 5'-
121 GAAAGTAATTAATTATTCATCTTATTTTTTAGTGAATTTG-3']. Cycling conditions were
122 95°C for 10 min followed by 40 cycles of 95°C for 20 s and 54°C for 1 min and a dissociation step
123 72°C for 20 s. The relative expression was normalized to the value of the positive control (*S. aureus*
124 strain NCTC8325) as described previously (Gomes-Fernandes *et al.*, 2017a). Accordingly, Agr
125 functionality was determined as RNAlII expression of within 10-fold of the positive control as
126 described previously (Gomes-Fernandes *et al.*, 2017a). Experiments were performed using three
127 biological replicates.

128

129 2.7 Statistical analysis

130 The data was analyzed with SPSS version 22.0 (IBM Corp., USA). Gene expression analysis was
131 performed using REST® 2009 (Qiagen, Germany) software. Independent-samples t-test was used
132 to evaluate differences between test groups. A *p-value* of less than 0.05 was considered statistically
133 significant.

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190 **3. Results**

191 *3.1 Bacterial strains and antimicrobial resistance profiles*

192 In this study, 48 *S. aureus* clinical isolates were collected from pediatric wound infections from
193 children aged between 1 day and 14 years. 14 (29.2%) samples were collected from patients
194 admitted to the infectious disease ward, 9 (18.8%) from post-surgery ward, 15 (31.2%) from
195 infants, 6 (12.5%) from OPD, 3 (6.3%) from in-patient ward, 2 (4.2%) from emergency cases, and
196 3 (6.3%) from gastrointestinal, 2 (4.2%) from neurosurgery, 2 (4.2%) from intensive care unit, 1
197 (2.1%) from coronary intensive care unit and 1 (2.1%) from nephrology wards. The antibiotic
198 susceptibility profile showed that all isolates were susceptible to linezolid and vancomycin.
199 Clinical isolates were highly susceptible to ceftaroline (89.6%, n=43), trimethoprim-
200 sulfamethoxazole (83.3%, n=40), and gentamicin (77.1%, n=37). Lastly, the susceptibility rate for
201 ciprofloxacin, ceftazidime, erythromycin, and penicillin was determined 68.8% (n=33), 43.8%
202 (n=21), 39.6% (n=19), and 2.1% (n=1), respectively (Table 2). The frequency of MRSA and
203 MSSA were 57.6% and 43.7%, respectively based on both ceftazidime susceptibility and presence of
204 *mecA* gene.

205

206 *3.2 Distribution of toxin-encoding genes*

207 We evaluated the prevalence of *S. aureus* toxin-encoding genes from strains isolated from pediatric
208 wound infections using PCR and primers outlined in Table 1. Additionally, the resultant amplicons
209 generated were sequenced and submitted to GENBANK, and the accession numbers for *tsst-1*, *eta*,
210 *etb*, and *edin-C* genes are shown in Table 4. Our results showed that the *eta* was the most prevalent
211 gene (100%), followed by *hld* (97.9%), *hla* (72.9%), *hly* (60.4%), *edin-B* (47.9%), *tsst* (41.7%),
212 *edin-C* (33.3%), *etd* (22.9%), *etb* (14.6%), *edin-A* (2.1%). Based on statistical analysis, there was
213 no significant correlation between antibiotics resistance and toxins genes in MRSA and MSSA
214 isolates (Table 3).

215

216 *3.3 Agr typing*

217 Agr type were determined by PCR using specific primers (Table 1). Generated PCR amplicons
218 were sequenced and submitted to GENBANK with the accession numbers of *agrII*, *agrIII* shown
219 in Table 4. The results indicate that *agrIII* gene (56.3%) was the predominant Agr type followed

220 by *agrI* (41.7%), *agrII* (8.3%), and *agrIV* (8.3%). Statistical analysis of virulence genes and Agr
221 type indicated no significant association.

222

223 3.4 Agr functionality evaluation

224 In order to measure Agr activity, RNAIII expression was evaluated and compared with *S. aureus*
225 strain NCTC8325 as a control. In total 48 wound isolates of *S. aureus* were assessed. Figure 1
226 illustrates the expression level of RNAIII among MRSA (Fig 1a) and MSSA (Fig 1b) isolates
227 using qRT-PCR. Agr functionality was observed in 28 (58%) wound isolates with 56% of MRSA
228 (15/27) and 61% of MSSA (13/21) being classed as Agr functional. We observed a significant
229 correlation between the presence of *tsst-I* gene with Agr functionality ($p=0.05$). However no
230 statistically significant association was observed between Agr functionality and the presence of
231 toxin genes, methicillin resistance or Agr class type.

232

233 4. Discussion

234 The present study was conducted to evaluate the activity of the Agr system among clinical isolates
235 of *S. aureus* derived from pediatric wound infections. In addition, the association between Agr
236 activity and the presence of several virulence determinants and antibiotic susceptibility was
237 examined. Several techniques can be used to determine Agr function including the CAMP
238 synergistic haemolysis assay, the Vesicle Lysis Test (VLT) and qRT-PCR detection of RNAIII.

239 Agr activity is traditionally evaluated using the CAMP test, which reports on the expression of

240 delta haemolysin, a 26-amino acid toxin translated from the *maIII* transcript (Novick and
241 Geisinger, 2008). A previous study reported that the interpretation of the CAMP test for evaluation

242 of Agr activity may be unreliable with results varying between different laboratories, particularly

243 observed for *S. aureus* strains exhibiting weak haemolytic activity (Traber *et al.*, 2008).

244 Alternatively it is shown that the VLT method, a highly sensitive assay specific to toxins strictly

245 regulated by Agr system, is more reliable than CAMP assay for Agr functionality assessment

246 (Laabei *et al.*, 2014). However, the evaluation of RNAIII expression using qRT-PCR is still

247 considered the gold-standard for Agr activity assessment (Gomes-Fernandes *et al.*, 2017b; Laabei

248 *et al.*, 2014). Accordingly, we investigated the expression of RNAIII as a marker for Agr activity

249 using qRT-PCR in our cohort of *S aureus* strains.

250

201 Our results demonstrated that 58% of tested *S. aureus* isolates were Agr functional. The
202 expression of virulence genes by *S. aureus* is influenced by the Agr system, which controls the
203 balance of virulence factors known to be important during the colonization and invasive phases
204 of infection (Papakyriacou *et al.*, 2000). To the best of our knowledge, there are no published
205 studies examining Agr functionality among clinical isolates of *S. aureus* derived from pediatric
206 wound infections. A recent study reported that Agr activity was high (82.2%) in *S. aureus* strains
207 isolated from lower respiratory tract infections (Gomes-Fernandes *et al.*, 2017b). These findings
208 suggest the importance of a functional Agr system in lower respiratory tract colonization and
209 infection. In contrast, previous work has highlighted that genes encoding the Agr system are down-
210 regulated in cases of persistent bacteremia (Malachowa *et al.*, 2011). However, a recent review of
211 numerous studies highlighted that the percentage of Agr dysfunctional strains isolated from
212 bacteraemia varies widely (3-82%) (Painter *et al.*, 2014). This most likely reflects the different
213 methods in testing Agr and different genetic backgrounds of *S. aureus* strains. Different infections
214 may promote the emergence of Agr dysfunction. It has also been demonstrated that apolipoproteins
215 in human blood can interfere and inhibit Agr activity (Reuter *et al.*, 2016) whereas this selection
216 for downregulating Agr function may not be as strong in other infections.

217
218 The Agr typing results revealed that the majority of isolates belonged to Agr Group III followed
219 by Agr Group I, Agr Group II, and Agr Group IV. In accordance with our data, two recent studies
220 highlighted that Agr Group III was the predominate Agr group derived from hospital clinical
221 isolates (Bibalan *et al.*, 2014b) (Ben Ayed *et al.*, 2006). The exact relationship between specific
222 Agr groups and particular infections is not clear, however past studies have highlighted significant
223 associations between the two factors. For instance, past work reported that the majority of
224 menstrual toxic shock strains belonged to Agr specificity Group III and exfoliative toxin producers
225 responsible for staphylococcal scalded skin syndrome (SSSS) and bullous impetigo were
226 designated Agr Group IV (Jarraud *et al.*, 2000). Furthermore, it was observed that TSST-1
227 producing strains belonged to *agr* Groups I and III (Chini *et al.*, 2006). Additionally, isolates taken
228 from patients suffering from endocarditis were mainly associated with with *agr* Group I (Gomes
229 *et al.*, 2005). Finally in a study investigating Agr activity in bloodstream isolates it was reported
230 that more than half of strains belonged to *agr* group II (Sakoulas *et al.*, 2003b). Our analysis

۲۸۱ showed that there was no significant correlation between Agr types and *S. aureus* isolates from
۲۸۲ wound infection however the majority of isolates belonged to Agr group III.

۲۸۳
۲۸۴ Previous studies have focused on examining the relationship between Agr functionality and
۲۸۵ susceptibility to some antimicrobial agents. The most prominent observations highlighted a
۲۸۶ reduction in vancomycin susceptibility in Agr dysfunctional isolates (Soon *et al.*, 2017; Tsuji *et*
۲۸۷ *al.*, 2012; Tsuji *et al.*, 2007). In this study, we observed no association between antibiotic resistance
۲۸۸ and Agr dysfunction. One limitation of this study was the relatively low sample size of isolates
۲۸۹ tested. Additionally, we did not screen for nasal carriage nor genotype the *S. aureus* isolates. These
۲۹۰ additionally tests would indicate whether the infecting *S. aureus* isolate was part of the patient's
۲۹۱ microflora or had been introduced externally from the hospital environment. Future studies will
۲۹۲ incorporate these analyses and improve our understating of *S. aureus* wound infections.

۲۹۳

۲۹۴ **5. Conclusion**

۲۹۵ In the present study, the data revealed that there was no significant correlation between Agr
۲۹۶ activity and the ability to cause wound infections by *S. aureus* strains.

۲۹۷

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441 **Table 1.** Oligonucleotide primers used in this study.

Target	Sequences(5'-3')	Annealing temperature (C°)	Product size (bp)	Reference
<i>nuc</i>	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACTAAAGC	54	270	(Stuhlmeier and Stuhlmeier, 2003)
<i>mecA</i>	F: GTAGAAATGACTGAACGTCCGATAA R CCAATTCACATTGTTTCGCTCTAA	60	310	(Seni <i>et al.</i> , 2013)
<i>tsst-1</i>	F: TTATCGTAAGCCCTTTTGTTG R: TAAAGGTAGTTCTATTGGAGTAGG	46	398	(Benvidi <i>et al.</i> , 2017)
<i>hla</i>	F: CTGATTACTATCCAAGAAATTCGATTG R: CTTTCCAGCCTACTTTTTTATCAGT	53	210	(Suryadevara <i>et al.</i> , 2012)
<i>hlb</i>	F: GTGCACTTACTGACAATAGTGC R: GTTGATGAGTAGCTACCTTCAGT	53	310	(Suryadevara <i>et al.</i> , 2012)
<i>Hld</i>	F: GAATTTGTTCACTGTGTCG R: TTTACACCACTCTCCTCAC	49	357	(Kiran <i>et al.</i> , 2009)
<i>eta</i>	F: TTTGCTTTCTTGATTTGGATTG R: GATGTGTTCCGGTTTGATTGAC	51	464	(Koosha <i>et al.</i> , 2014)
<i>etb</i>	F: ACGGCTATATACATTCAATT R: TCCATCGATAATATACCTAA	51	226	(Suryadevara <i>et al.</i> , 2012)
<i>etd</i>	F:GGGGAGACTATAGCTTCTGGTGTATTA R: TCCAACATGAATACCAACTAACTCT	55.5	477	(Franke <i>et al.</i> , 2010)
<i>edinA</i>	F: TAAATGGGGGAATAAACTTA R: CGATACTTGTCAAATAATCT	43	248	(Yamashita <i>et al.</i> , 2013)
<i>edinB</i>	F: CATAAATACTCCTCTAAG R: GCATATTCTGTCCCTCTA	40	444	(Ohkura <i>et al.</i> , 2009)
<i>edinC</i>	F: TATTAAGCATTCAATCAA R: AGTGTAGTCTGTTCCCTCT	45	629	(Ohkura <i>et al.</i> , 2009)
<i>agr</i>	Pan F: ATGCACATGGTGCACATGC R1: GTCACAAGTACTATAAGCTGCGAT R2: TATTACTAATTGAAAAGTGCCATAGC R3: GTAATGTAATAGCTTGTATAATAATACCCAG R4: CGTAATGCCGTAATACCCG	54.5 54 54.5 56	- 439 573 406 657	(Suryadevara <i>et al.</i> , 2012)

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٤٤٥ **Table 2.** The antimicrobial susceptibility patterns of *S. aureus* isolated from wound infection of
 ٤٤٦ pediatric patients
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Antibiotics	(%) Resistance	(%)Intermediate	(%) Susceptible
Penicillin	(97.9%)	0	(2.1%)
Erythromycin	(45.8%)	(14.6%)	(39.6%)
Clindamycin	(41.7%)	(12.5%)	(45.8%)
Cefoxitin	(56.3%)	0	(43.8%)
trimethoprim- sulfamethoxazole	(16.7%)	0	(83.3%)
Oxacillin	(54.2%)	0	(45.8%)
Linezolid	0	0	(100%)
gentamicin	(22.9%)	0	(77.1%)
ceftaroline	(8.3%)	(2.1%)	(89.6%)
ciprofloxacin	(27.1%)	(3.4%)	(69.5%)
vancomycin	0	0	(100%)

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462 **Table 3.** The frequency of the virulence and Agr genes determinant among the MRSA and MSSA
 463 isolated in *S. aureus*

gene			P value *
	MRSA	MSSA	
<i>tsst-1</i>	37.28%	38.09%	0.771
<i>hla</i>	66.6%	80.9%	0.338
<i>hlb</i>	66.6%	52.38%	0.380
<i>hld</i>	100%	95.23%	0.438
<i>eta</i>	100%	100%	1
<i>etb</i>	7%	23.8%	0.118
<i>etd</i>	25.92%	19.04%	0.733
<i>edinA</i>	3.7%	0%	1
<i>edinB</i>	40.7%	57.14%	0.383
<i>edinC</i>	25.92%	42.85%	0.237
<i>agrI</i>	40.74%	42.85%	1
<i>agrII</i>	7.4%	9.5%	1
<i>agrIII</i>	66.6%	42.85%	0.144
<i>agrIV</i>	7.4%	9.5%	1

464 * p values were measured using a Chi-squared analysis

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474 **Table 4.** Accession numbers of genes

Target gene	Accession Numbers
<i>tsst-1</i>	MH805860
<i>eta,</i>	MH727607
<i>etb</i>	MH818223
<i>edin-C</i>	MH750913
<i>agrII</i>	MH805858
<i>agrIII</i>	MH805859

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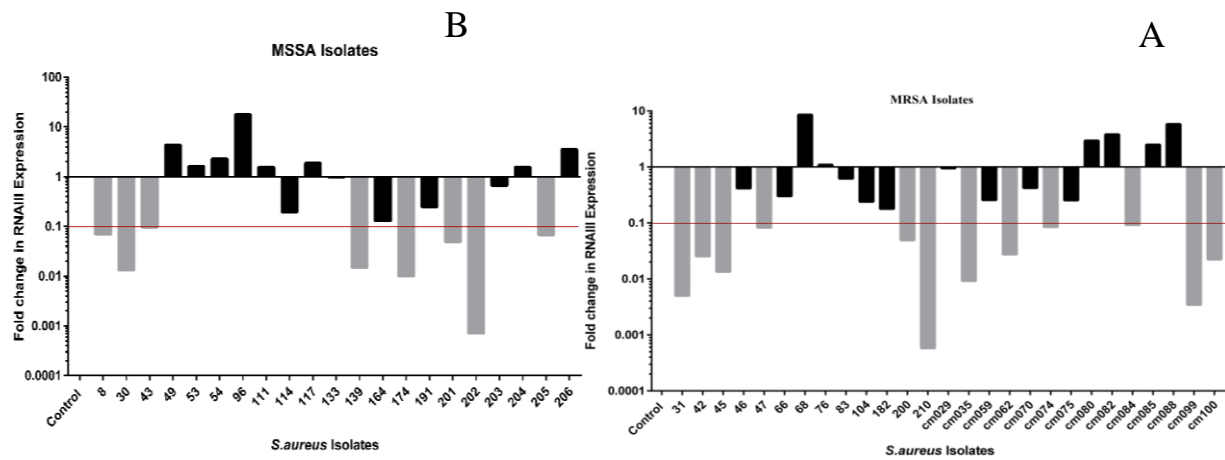
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٤٩٢ **Figure 1. The expression level of RNAIII among MRSA (A) and MSSA (B) strains.**
 ٤٩٣ 27 MRSA (A) and 21 MSSA (B) isolates were analyzed for Agr activity using qRT-PCR. Fold change of
 ٤٩٤ RNAIII expression was normalized to the housekeeping gene *gyrB*. The red line depicts the cut off for
 ٤٩٥ functional Agr activity based on a 10-fold difference to a positive Agr control.
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