JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Genes encoding adhesion factors and biofilm formation in methicillin-resistant *Staphylococcus aureus* in Morocco

Bahija Serray[1,2], Salwa Oufrid[2], Imane Hannaoui[3,2], Fatna Bourjilate[2], Nabila Soraa[4], Mostafa Mliji[5], Mohammed Sobh[1], Abderrahmane Hammoumi[1], Mohammed Timinouni[2], Mohamed El Azhari[2]

[1] Laboratory of Microbiology, Pharmacology, Biotechnology and Environment, Faculty of Sciences Ain-Chock, Casablanca, Morocco
[2] Molecular Bacteriology Laboratory, Institut Pasteur du Maroc, Casablanca, Morocco
[3] Laboratory of biological engineering, Faculty of Sciences and Technics, Sultane Moulay Slimane University, Beni Mellal, Morocco
[4] Departments of Microbiology, University Hospital Mohamed VI, Marrakech, Morocco
[5] Division of Microbiology and Hygiene Products and the Environment, Institut Pasteur du Maroc, Casablanca, Morocco

Abstract

Introduction: Infections involving methicillin-resistant *Staphylococcus aureus* (MRSA) remain a serious threat to hospitalized patients worldwide. MRSA is characterized by recalcitrance to antimicrobial therapy, which is a function not only of widespread antimicrobial resistance, but also the capacity to form biofilms. The present study evaluated the presence of genes encoding adhesion factors and the biofilm-forming capacity in MRSA.

Methodology: In this study, 53 isolates of MRSA, recovered from December 2010 to May 2014 in a mother and child hospital, CHU Mohamed VI in Marrakech, Morocco, were screened for the presence of *bap* and *ica* genes associated with biofilm formation, and for *bbp*, *cna*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *clfA*, and *clfB* genes that encode microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). The biofilm formation assay was performed in 96-well microtiter polystyrene plates. The presence of genes was determined by polymerase chain reaction (PCR).

Results: An association was found between *icaD* gene detection and biofilm formation; 100% of the strains harbored *icaD* and produced biofilm. None of the isolates harbored *bap* or *bbp*. Furthermore, 96.23% isolates were positive for *fnbA*, 60.37% for *eno*, 43.39% for *clfA* and *clfB*, 11.32% for *cna*, 9.34% for *ebpS*, 5.66% for *fib*, and 1.89% for *fnbA*.

Conclusions: Our findings showed that the MRSA carriage in Marrakech children was high. The genetic variations of adhesion genes require further investigation.

Key words: adhesion genes; MSCRAMMs; methicillin-resistant *Staphylococcus aureus*; biofilms.

J Infect Dev Ctries 2016; 10(8):863-869. doi:10.3855/jidc.8361

(Received 09 March 2016 – Accepted 30 July 2016)

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the most prominent of all bacterial pathogens. It is a commensal inhabitant of a significant proportion of the healthy population, but it also has the capacity to cause a diverse array of infections, ranging from relatively superficial skin infections to serious, life-threatening infections, including endocarditis, pneumonia, and osteomyelitis. Many forms of staphylococcal infection are associated with the formation of a bacterial biofilm [1,2]. Biofilm is a microbial-derived sessile community characterized by cells that are irreversibly attached to a substratum of interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibiting an altered phenotype with respect to growth rate and gene transcription [3]. Two steps appear to be involved in the molecular mechanisms implicated in host colonization by *Staphylococcus aureus*. The first step is bacterial adhesion to host extracellular matrix and plasma proteins mediated by different proteins of the family of microbial surface components recognizing adhesive matrix molecules. The second step is growth-dependent accumulation of bacteria in multilayered cell clusters where genes involved in biofilm formation come into play [4,5]. The microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)

are protein components of the microbial surface that are able to interact with and bind to a variety of extracellular proteins. Among adhesins, the fibronectin-binding protein, the collagen-binding protein, the elastin-binding protein, and the bone sialoprotein-binding protein have been proven to significantly contribute to tissue colonization in various pathological conditions. Among the *ica* genes, *icaD* have a significant role in slime production in *Staphylococcus aureus* and *Staphylococcus epidermidis* [6]. Biofilm formation protects microorganisms from opsonophagocytosis and antimicrobial agents [7].

In human *Staphylococcus aureus* strains, several of these virulence factors play a role in life-threatening infections [8]; for this reason, considerable effort has been expended to define the specific staphylococcal factors that promote biofilm formation and/or persistence within a biofilm. The goal of the present study was to evaluate the presence of genes encoding adhesion factors and biofilm forming capacity in MRSA. We further evaluated the correlation between biofilm production by clinical isolates and the presence of *icaD* genes.

Methodology

Clinical strains

In this study, 53 isolates of MRSA, 41 strains of blood culture origin and 12 staphylococcal strains of all origins, were recovered from December 2010 to May 2014 in a mother and child hospital, CHU Mohamed VI in Marrakech, Morocco.

Identification

All isolates were identified by classic microbiological methods including colony morphology, mannitol fermentation, Gram staining, catalase test, coagulase test, and the API Staph test (bioMérieux, Marcy l'Etoile - France). Methicillin resistance was confirmed using a cefoxitin disk (30 µg) on Mueller-Hinton agar plates (Bio-Rad, Marnes-la Coquette, France) as recommended by the French Society for Microbiology (FSM, 2013), and polymerase chain reaction (PCR) was used to confirm the presence of the *mecA* gene.

Biofilm formation assay

The biofilm formation assay was performed in 96-well microtiter polystyrene plates as described by O'Toole *et al* [9]. At first, cells were grown on liquid Luria Bertani (Bio-Rad, Marnes-la-Coquette, France) in 96-well microplates. After incubation at 37°C, detached cells were gently rinsed three times with sterile water, and the bacteria that attached to the surface were stained with crystal violet 0.1% for 20 minutes. The excess crystal violet dye was washed by rinsing the wells with water. The amount of biofilm formed was estimated by solubilization of the dye with 95% ethanol, and the optical density (OD) was determined at 540 nm. The absorbance was recorded by microplate reader. The biofilm formation were divided into three categories in this study: the strains with OD 550 < 0.38, 0.38 > OD 550 < 0.70, and OD 550 > 0.70 were defined as biofilm formers of weak level, moderate level, and strong level, respectively, based on the ODs [10]. *Pseudomonas aeruginosa* ATCC 27853 was used as a positive control.

Genomic DNA extraction

DNA templates for the PCR were generated by suspending five colonies in 500 µL of DNase and RNase-free water (Bio-Rad, Marnes-la-Coquette, France). The suspension was boiled at 100°C for 10 minutes in a thermal block (Polystat 5, Illkirch-Graffenstaden, France) and immediately frozen at 0°C for 5 minutes, then centrifuged at 15,000 rpm for 5 minutes. After centrifugation, 300 µL of supernatant was recovered. Supernatant containing DNA was stored at -20°C until further use as a DNA template for PCR [11].

PCR assay on MSCRAMM and biofilm genes

The presence MSCRAMM and biofilm genes were detected by PCR using forward and reverse primers to detect the presence of *icaD* (intercellular adhesion gene D), *bap* (encoding biofilm-associated protein), *bbp* (encoding bone sialoprotein-binding protein), *clfA* and *clfB* (encoding clumping factors A and B), *cna* (encoding collagen-binding protein), *ebpS* (encoding elastin-binding protein), *eno* (encoding laminin-binding protein), *fnbA* (encoding fibronectin-binding protein A), *fnbB* (encoding fibronectin-binding protein B), and *fib* (encoding fibrinogen-binding protein) in all isolates of MRSA.

The presence of *icaD* DNA was detected by PCR using forward and reverse primers. The primer sequences for *icaD* were forward (nucleotides 1963–1982), 5'-ATG GTC AAG CCC AGA CAG AG- 3'; reverse (nucleotides 2138–2160), 5'-CGT GTT TTC AAC ATT TAA TGC AA-3'. The PCR reaction volume was in 25 µL, containing the above-mentioned primers (1 µM each), 5 µL of the extracted DNA, 100 µM each of dATP, dCTP, dGTP, and dTTP, 1U of Taq DNA polymerase, and buffer (10 mM Tris-HCl [12], 50 mM KCl, 0.1% Triton X-100, and 2.5 mM MgCl2). The

PCR amplification protocol for *icaD* was as follows: incubation at 94°C for 5 minutes, followed by 50 cycles at 94°C for 30 seconds (denaturation), 55.5°C for 30 seconds (annealing), 72°C for 30 seconds (extension), and 72°C for 1 minute after the conclusion of the 50 cycles. After the first 30 cycles, a further 1 U of Taq DNA polymerase was added.

The detection of *bap*, *ebpS*, and *fnbA* was done by means of simplex PCR tests using the primers reported by Vancraeynest *et al*. [7]. Each 50 µL PCR mixture contained 1.5 mM MgCl2, 2.5 U Taq DNA polymerase, 200 µM of each dNTP, 100 pmol of both primers, and 5 µL DNA sample. Cycling conditions for the amplification of *bap* included an initial denaturation step (30 seconds at 94°C) followed by 30 cycles of amplification (denaturation for 45 seconds at 94°C, annealing for 1 minute at 62°C, and elongation for 1 minute at 72°C), terminated with a 7-minute incubation step at 72°C. *EbpS* and *fnbA* were amplified using an initial denaturation step of 1 minute at 94°C followed by 30 cycles of amplification (denaturation for 45 seconds at 94°C, annealing for 45 seconds at 50°C, and elongation for 1 minute at 72°C) and a termination step of 7 minutes at 72°C.

For the detection of *bbp*, *cna*, *eno* and *clfA*, *clfB*, *fib*, and *fnbB*, two multiplex PCR assays were performed. Each 50 µL PCR mixture contained 3 mM MgCl2, 1.5 U Taq DNA polymerase, 40 µM of each dNTP, 100 pmol of both primers, and 5 µL DNA sample. The thermal cycling conditions for the two multiplex PCRs included an initial denaturation step (5 minutes at 94°C) followed by 25 cycles of amplification (denaturation for 1 minute at 94°C, annealing for 1 minute at 55°C, and extension for 1 minute at 72°C). The reaction was terminated with a 10-minute incubation step at 72°C. The primers and annealing temperatures used in the PCR assays are shown in Table 1.

After amplification, 8 µL of amplicon was mixed with 2 µL of loading buffer and electrophoresis was performed at 100 V for 45 minutes. After electrophoresis, gels were visualized under UV light and photographed, and the 100 bp DNA ladder was used as a DNA size marker.

Statistical analysis

Data analysis was performed using SPSS software, version 10.0 (IBM, Armonk, USA). Statistical significance was assessed using Fisher's exact test.

Results

Detection of biofilm formation

Phenotypic production of biofilm by all strains under study was assessed using the microtiter plate test. All of the strains were biofilm positive; 21 of the 53 MRSA were high virulence, 20 strains were moderate, and 12 strains were low virulence within 24 hours.

PCR assay on MSCRAMM and biofilm genes

The PCR technique was applied to the 53 MRSA strains. The *icaD* gene was present in 100% of both

Table 1. Primers used in this study.

Gene targeted	Primer sequence	PCR annealing temperature (°C)	Amplicon size (bp)	Reference
bap	5'CCCTATATCGAAGGTGTAGAATTG'3 5'GCTGTTGAAGTTAATACTGTACCTGC'3	62	971	Cucarella *et al*. [41]
bbp	AACTACATCTAGTACTCAACAACAG ATGTGCTTGAATAACACCATCATCT	55	575	Tristan *et al.* [39]
cna	GTCAAGCAGTTATTAACACCAGAC AATCAGTAATTGCACTTTGTCCACTG	55	423	Tristan *et al*. [39]
ebpS	AGAATGCTTTTGCAATGGAT AATATCGCTAATGCACCGAT	50	652	Vancraeynest D *et al.* [7]
eno	ACGTGCAGCAGCTGACT CAACAGCATYCTTCAGTACCTTC	55	302	Tristan *et al*. [39]
fnbA	CATAAATTGGGAGCAGCATCA ATCAGCAGCTGAATTCCCATT	50	127	Vancraeynest D *et al.* [7]
fnbB	GTAACAGCTAATGGTCGAATTGATACT CAAGTTCGATAGGAGTACTATGTTC	55	524	Tristan *et al*. [39]
fib	CTACAACTACAATTGCCGTCAACAG GCTCTTGTAAGACCATTTTCTTCAC	55	404	Tristan *et al*. [39]
clfA	ATTGGCGTGGCTTCAGTGCT CGTTTCTTCCGTAGTTGCATTTG	55	292	Tristan *et al*. [39]
clfB	ACATCAGTAATAGTAGGGGGCAAC TTCGCACTGTTTGTGTTTGCAC	55	205	Tristan *et al*. [39]

The nucleotide sequences of *ebpS* (encoding elastin binding protein), *eno* (laminin binding protein), *cna* (collagen binding protein), *fnbA* and *fnbB* (fibronectin binding proteins A and B), *fib* (fibrinogen binding protein), *clfA* and *clfB* (clumping factors A and B), and *bbp* (bone sialoprotein binding protein), *bap* (encoding biofilm associated protein); PCR: polymerase chain reaction.

Table 2. Biofilm-forming capacity of 53 methicillin-resistant *Staphylococcus aureus* (MRSA), and percentage of adhesin genes.

Genes		*icaD*	*ebps*	*fnbA*	*eno*	*cna*	*clfA*	*clfB*	*fib*	*fnbB*
Total MRSA 3	Strong biofilm (n = 21; 39.62%)	100%	9.52%	95.24%	42.9%	14.28%	52.4%	52.4%	9.52%	4.7%
	Moderate biofilm (n = 20; 37.74%)	100%	15%	100%	35%	10%	35%	35%	5%	0%
	Weak biofilm (n = 12; 22.64%)	100%	0%	100%	50%	8.33%	41.7%	41.7%	0%	0%
	P value	1	0.34	0.99	0.67	1	0.56	0.56	0.78	0.99

high-virulence strains and low-virulence strains, giving a 198 base pair band. All of the strains produced slime, so there was a correlation between biofilm production and presence of *icaD* gene in all MRSA isolates. None of the isolates harbored *bap* or *bbp*. Furthermore, 96.23% isolates were positive for *fnbA*, 60.37% for *eno*, 43.39% for *clfA* and *clfB*, 11.32% for *cna*, 9.34% for *ebpS*, 5.66% for *fib*, and 1.89% for *fnbA* (Table 2).

Discussion

The ability to form biofilm is a trait that is closely associated with bacterial persistence and virulence, and many chronic bacterial infections are now believed to be linked to the formation of biofilms [13]. It has been demonstrated that strains having an ability to form biofilm additionally cause chronic polymer-associated infection [14,15]. In this regard, adhesion formation is considered as one of the major virulence factors in MRSA. Providing the phenotypic and genotypic characterization of the *icaD*/biofilm gene may allow a better understanding of the complex process of infections caused by biofilms [16]. Indeed, an increasing number of different *Staphylococcus aureus* adhesion molecules have been found [17]. In this study, a microtiter polystyrene plate was chosen to detect biofilm formation. The presence of adherences genes should be confirmed by genotypic characterization methods. The biofilm development process requires polysaccharidic intercellular adhesin, which is synthesized by the enzymes encoded by the intercellular adhesion cluster (*ica*) [18]. *Ica* expression is regulated by multiple genes such as *sigB*, *sar*, and accessory gene regulator. They may interact each other and regulate biofilm formation.

In the present study, an association was found between *icaD* gene detection and biofilm formation, with 100% of the strains harboring *icaD* and producing biofilm, which is in agreement with the findings of Liberto *et al.* [19]. There was no difference in the distribution of the *icaD* gene in high- and low-virulence strains, which is in agreement with the findings of Rohde *et al*. [20]. Our results showed high rates of biofilm formation, meaning that hospital environments may be more suitable settings for biofilm formation. Various healthcare-associated risk factors have been suggested to affect biofilm formation. The presence of *ica* adhesion genes may explain the role of the various adhesion mechanisms in the pathogenesis of infection. However, some studies found no association between *icaD* detection and biofilm formation; the presence of *icaA*/*D* genes was not always associated with *in vitro* formation of biofilm [7]. Ruzicka *et al.* demonstrated that 20% of strains with *ica* genes did not express phenotype [21]. The lack of biofilm, despite the presence of *ica*, could be due to several reasons, such as the inactivation of the *ica* operon by the action of the *icaR* repressor [22], or the post-transcriptional regulation [23]. Biofilm is a perfect medium for the exchange of resistance plasmids [24].

None of the strains harbored the *bap* gene. This is in agreement with the study of Arciola *et al.* [25]. MRSA expresses many surface proteins of the MSCRAMMs family, which specifically recognize and bind to the extracellular matrix components of the host.

The *fnbA* and *fnbB* genes contribute to the invasion and adhesion of bacteria. In the present study, a low percentage of occurrences of the *fnbB* gene was observed (1.89%). Taneike *et al.* reported that all MRSA isolated from nosocomial outbreaks in Japan lacked *fnbB* [26]. A high occurrence of this gene (99.5%) was reported in the study of Arciola *et al.* [27]; this could partly be ascribed to the different technique of gene detection utilized and also to the different region of the locus analyzed by the couple of primers [28]. The presence of the *fnbB* gene may be correlated with biofilm-forming ability [29], and appears to be a very relevant, almost essential, trait for virulence action in human hosts.

However, the gene encoding the *fnbA* gene was detected in 96.23% of isolates, similar to what was observed by Ikawaty *et al*. [28], and with other more general reports on isolates of *Staphylococcus aureus* from nosocomial infections.

Both the *clfA* and *clfB* genes had a prevalence of 43.39%. We observed, in our study, that the strains from blood origin had a high percentage (86.95%) of both *clfAB* genes, which is consistent with the fact that these genes have been reported to play a determinant role in bacterial virulence [30].

Fibronectin is a high molecular weight glycoprotein, and a component of the extracellular matrix that binds to cell membrane proteins termed integrins, but can also bind to fibrin and collagen.

Fibrinogen is a glycoprotein found in blood plasma. Fibrinogen plays an important role in the blood coagulation process, in which fibrinogen is transformed into its insoluble form, fibrin. The fibrinogen-binding protein is an important virulence factor in infections caused by *Staphylococcus aureus*, as it not only binds to fibrinogen, but can also interfere with the aggregation of platelets and the complement cascade within the host [31-33]. As for the *fib* gene, we detected it at a rate of 5.66% in our study. Elastin is the major component of elastic fibers, which are proteins that provide strength and flexibility to connective tissue [32]; we verified the presence of *ebpS* gene in 9.34% of our isolates.

Collagen is the most abundant group of proteins in the body and is the major component of connective tissue. The *cna* gene is considered an important virulence factor in staphylococcal infections [34]. The incidence of *cna* was 11.32% in our isolates. A high occurrence of this gene was reported by Montanaro *et al.* (29%) [35], Nashev *et al.* (46.7%) [36], and Arciola *et al.* (46%) in Italy and Bulgaria [27], respectively. The difference in the prevalence of the *cna* gene in this study is probably due to different techniques in PCR detection or differences in the distribution of *Staphylococcus aureus* genotype in different countries.

In the present study, the *bbp* gene, associated with osteomyelitis and arthritis in humans [37], was absent in all strains, which is in agreement with a study by Vancraeynest *et al*. [38], where the *bbp* gene were not detected in all isolates.

In this study, 15.09% of isolates were positive for *icaD* and five MSCRAMM genes, which is in agreement with the findings of Tristan *et al*. [39], The presence of the combination of these genes, could mean that they may have a selective advantage, such as better host colonization and good genetic capacity for adherence.

However, there were no significant differences between MSCRAMM gene distribution and biofilm forming capacity ($p > 0.05$ for each gene comparison with exact Fisher's exact test) (Table 2).

The severity of the MRSA infections observed in the pediatric patients might be related to the high frequency of virulence factors carried by the isolates. In this study, a number of genes were associated with the development of invasive disease, contributing independently to the virulence of MRSA. Bacterial adhesion has long been considered as a virulence factor contributing to infections associated with catheters and other indwelling medical devices. In our study, 9.43% of strains were from catheters, showing a strong or moderate biofilm. The interaction of bacteria with biomaterials has been suggested to have a crucial role in conditioning the progress of these severe nosocomial infections [40]. For staphylococcal species, two possible explanations of the ability to colonize artificial materials are the bacterial production of polysaccharide slime and the presence of adhesins for the host matrix proteins that, *in vivo*, are adsorbed onto the biomaterial surface [11].

Results of a comparison between the infection types showed that the isolates from pus infections produced a strong biofilm.

Conclusions

Our findings showed the significant role of *ica* genes as virulence markers for staphylococcal isolates. Their association with biofilm-forming strains strongly suggests that expressions of *icaD* genes play a role in the pathogenic mechanisms of infections, and showed that MRSA carriage in Marrakech children was high. Most of them contained the adhesin genes. From this data, school sanitation and hygiene education programs are needed to minimize transmission of MRSA. In addition, the genetic variation of adhesion genes requires further investigation.

References

1. Lewis K (2001) Riddle of biofilm resistance. Antimicrob agents chemother. 45: 999-1007.
2. Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K (2004) Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett 230: 13-18.
3. Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15: 167-193.
4. Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Götz F (1996) Molecular basis of intercellular adhesion in the biofilm forming *Staphylococcus epidermidis*. Mol Microbiol 20: 1083-1091.
5. Clarke SR, Foster SJ (2006) Surface adhesins of *Staphylococcus aureus*. Adv Microb Physiol 51: 187-224.
6. Arciola CR, Baldassarri L, Montanaro L (2001) Presence of *icaA* and *icaD* Genes and slime production in a collection of

Staphylococcal strains from catheter-associated infections. J Clin Microbiol 39: 2151-2156.
7. Vancraeynest D, Hermans K, Haesebrouck F (2004) Genotypic and phenotypic screening of high and low virulence *Staphylococcus aureus* isolates from rabbits for biofilm formation and MSCRAMMs. Vet Microbiol 103: 241-247.
8. Tangchaisuriya U, Yotpanya W, Kitti T, Sitthisak S (2014) distribution among Thai children of *methicillin resistant staphylococcus aureus*. Southeast Asian J Trop Med Public Health. 45: 149-156.
9. O'Toole GA, Pratt LA, Watnick PI, Newman DK, Weaver VB, Kolter R (1999) Genetic approaches to study of biofilms. Methods Enzymol 310: 91-109.
10. Mliji EM (2009) Study of bacterial adhesion and biofilm formation on surfaces of food industry: Pseudomonas Case on drinking water pipeline and Salmonella Spp on stainless steel. National Thesis, Sultan Moulay Slimane University, Faculty of Science and Technology, Beni Mellal Nb16/2009.
11. Oufrid S, Ghazlane Z, Jamali L, El Otmani F, Talmi M, Elmdaghri N, Zerouali K, Timinouni M (2015) Correlation between staphylococcal biofilm formation in vitro and potential for catheter-related infections. J Infect Dev Ctries 9: 368-372. doi:10.3855/jidc.4839.
12. Lepelletier D, Corvec S, Caillon J, Reynaud A, Rozé JC, Gras-Leguen C (2009) Eradication of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit: Which measures for which success? Am J Infect Control 37: 195-200.
13. Mohamed JA, Huang DB (2007) Biofilm formation by enterococci. J Med Microbiol 56: 1581-1588.
14. Götz F (2004) Staphylococci in colonization and disease: prospective targets for drugs and vaccines. Curr Opin Microbiol 7: 477-487.
15. Götz F (2002) Staphylococcus and biofilms. Mol Microbiol 43: 1367-1378.
16. Vasudevan P, Nair MKM, Annamalai T, Venkitanarayanan KS (2003) Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet Microbiol 92: 179-185.
17. Rooijakkers SH, Van Kessel KP, Van Strijp JA (2005) Staphylococcal innate immune evasion. Trends Microbiol 13: 596-601.
18. Martín-López JV, Pérez-Roth E, Claverie-Martín F, Gil OD, Batista N, Morales M, Méndez-Álvarez S (2002) Detection of *Staphylococcus aureus* clinical isolates harboring the *ica* gene cluster needed for biofilm establishment. J Clin Microbiol 40: 1569-1570.
19. Liberto MC, Matera G, Quirino A, Lamberti AG, Capicotto R G, Puccio GS, Barreca E, Foca A, Cascio (2009) Phenotypic and genotypic evaluation of slime production by conventional and molecular microbiological techniques. Microbiol Res 164: 522-528.
20. Rohde H, Knobloch JK, Horstkotte MA, Mack D (2001) Correlation of *Staphylococcus aureus icaADBC*genotype and biofilm expression phenotype. J Clin Microbiol 39: 4595-4596.
21. Růžička F, Holá V, Votava M, Tejkalová R, Horvát R, Heroldová M, Woznicová V (2004) Biofilm detection and the clinical significance of *Staphylococcus epidermidis* isolates. Folia Microbiol 49: 596-600.
22. Conlon KM, Humphreys H, O'Gara JP (2002) *icaR* encodes a transcriptional repressor involved in environmental regulation of ica operon expression and biofilm formation in *Staphylococcus epidermidis*. J Bacteriol 184: 4400-4408.
23. Dobinsky S, Kiel K, Rohde H, Bartscht K, Knobloch JK-M, Horstkotte MA, Mack D (2003) Glucose-related dissociation between *icaADBC* transcription and biofilm expression by *Staphylococcus epidermidis*: evidence for an additional factor required for polysaccharide intercellular adhesin synthesis. J Bacteriol 185: 2879-2886.
24. Touati A, Achour W, Abbassi M, Hassen AB (2007) Detection of *ica* genes and slime production in a collection of *Staphylococcus epidermidis* strains from catheter-related infections in neutropenic patients. Pathol Biol 277-282.
25. Arciola CR, Campoccia D, Gamberini S, Donati ME, Baldassarri L, Montanaro L (2003) Occurrence of *ica* genes for slime synthesis in a collection of *Staphylococcus epidermidis* strains from orthopedic prosthesis infections. Acta Orthop Scand 74: 617-621.
26. Taneike I, Otsuka T, Dohmae S, Saito K, Ozaki K, Takano M, Higuchi W, Takano T, Yamamoto T (2006) Molecular nature of methicillin-resistant *Staphylococcus aureus* derived from explosive nosocomial outbreaks of the 1980s in Japan. FEBS Lett. 9: 23-34
27. Arciola CR, Campoccia D, Gamberini S, Baldassarri L, Montanaro L (2005) Prevalence of *cna, fnbA and fnbB* adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant. FEMS Microbiol Lett 246: 81-86.
28. Ikawaty R, Brouwer E, Van Duijkeren E, Mevius D, Verhoef J, Fluit A (2010) Virulence factors of genotyped bovine mastitis *Staphylococcus aureus* isolates in the Netherlands. Int J Dairy Sci 5: 60-70.
29. Cha JO, Yoo JI, Yoo JS, Chung HS, Park SH, Kim HS, Lee YS, Chung GT (2013) Investigation of Biofilm Formation and its Association with the Molecular and Clinical Characteristics of Methicillin-resistant *Staphylococcus aureus*. Osong Public Health Res Perspect 4: 225-232.
30. Moreillon PJ, Entenza M, Francioli P, McDevitt D, Foster TJ, Francois P, Vaudaux P (1995) Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. Infect Immun 63: 4738-4743.
31. Shannon O, Flock JI (2004) Extracellular fibrinogen binding protein, Efb, from *Staphylococcus aureus* binds to platelets and inhibits platelet aggregation. Thromb Haemost 91: 779-789.
32. Kumar R, Yadav B, Anand S, Singh R (2011) Prevalence of adhesin and toxin genes among isolates of *Staphylococcus aureus* obtained from mastitic cattle. World J Microbiol Biotechnol 27: 513-521.
33. Singh R, Kumar R, Yadav B (2011) Distribution of pathogenic factors in *Staphylococcus aureus* strains isolated from intra-mammary infections in cattle and buffaloes. Indian J Biotechnol 10: 410-416.
34. Klein RC, Fabres-Klein MH, Brito MAVP, Fietto LG, Ribon Ade O (2012) *Staphylococcus aureus* of bovine origin: Genetic diversity, prevalence and the expression of adhesin-encoding genes. Vet Microbiol 160: 183-188.
35. Montanaro L, Arciola CR, Baldassarri L, Borsetti E (1999) Presence and expression of collagen adhesin gene *(cna*) and slime production in *Staphylococcus aureus* strains from orthopaedic prosthesis infections. Biomaterials 20: 1945-1949.
36. Nashev D, Toshkova K, Salasia SI, Hassan AA, Lammler C, Zschock M (2004) Distribution of virulence genes of *Staphylococcus aureus* isolated from stable nasal carriers. FEMS Microbiol Lett 233: 45-52.
37. Tung HS, Guss B, Hellman U, Persson L, Rubin K, Ryden C (2000) A bone sialoprotein-binding protein from

Staphylococcus aureus: a member of the staphylococcal Sdr family. Biochem J 345: 611-619.
38. Vancraeynest D, Hermans K, Haesebrouck F (2006) Prevalence of genes encoding exfoliative toxins, leucotoxins and superantigens among high and low virulence rabbit *Staphylococcus aureus* strains. Vet Microbiol 117: 211-218.
39. Tristan AL, Ying MBes, Etienne J, Vandenesch J, Lina G (2003) Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. J Clin Microbiol 41: 4465-4467.
40. Francois P, Vaudaux P, Nurdin N, Mathieu H, Descouts P, Lew DP (1996) Physical and biological effects of a surface coating procedure on polyurethane catheters. Biomaterials 17: 667-678.
41. Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penadés JR (2001) Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. J Bacteriol. 9:2888-96.

Corresponding author

Mohammed Timinouni
Molecular Bacteriology Laboratory, Institut Pasteur du Maroc
1 Place Louis Pasteur
Casablanca, 20360, Morocco
Phone: + (212) 05 22 43 44 50
Fax: + (212) 05 22 26 09 57
Email: mohammed.timinouni@pasteur.ma

Conflict of interests: No conflict of interests is declared.