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Molecular analysis of the *ica* adhesion gene in *Staphylococcus aureus* strains, isolated from inert surfaces in clinical and hospital areas

J.D. Tola[1]*, P.P. Orellana[2], C.F. Andrade[2], J.E. Torracchi[3], D.A. Delgado[4], A.C. Pavón[5] and D.G. Carchi[6]

[1]Faculty of Dentistry, Universidad Católica de Cuenca. Cuenca, Ecuador.

[2]Faculty of Dentistry, Laboratorio de Biología Molecular y Genética del Centro de Investigación, Innovación y Transferencia de Tecnología at the Universidad Católica de Cuenca. Cuenca, Ecuador.

[3]Faculty of Dentistry, Centro de Investigación, Innovación y Transferencia de Tecnología at the Universidad Católica de Cuenca. Cuenca, Ecuador.

[4]Student of the Universidad Católica de Cuenca.

[5]Faculty of Dentistry, Universidad Católica de Cuenca. Cuenca, Ecuador.

[6]Regional Economics Research Group (GIER) - Department of Economics, Business and Sustainable Development, Faculty of Economics and Administrative Sciences, University of Cuenca. Cuenca, Ecuador.

Corresponding author: Juan Daniel Tola Rueda
E-mail: jtolarueda@gmail.com

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ABSTRACT. *Staphylococcus aureus* is a ubiquitous bacterium that has managed to inhabit various inert surfaces. The ability of *S. aureus* to form biofilms is considered an important virulence factor that influences its survival and persistence in the environment. Biofilm formation in *S. aureus* is determined by a production mechanism called Polysaccharide Intracellular Adhesin (PIA). The *ica* (intercellular adhesion) operon comprises four genes that encode the proteins IcaA, IcaB, IcaC, and IcaD, which aim to produce PIA. To detect the frequency of genes that regulate adhesion in *Staphylococcus aureus* isolated strains from different inert surfaces. Fifty-nine positive samples of *Staphylococcus aureus* were used,

from which the alkaline lysis process obtained DNA. The endpoint PCR assay allowed amplification of the genes, and the amplicons were separated by electrophoresis on agarose gels and observed on a UV transilluminator. Among the 59 *S. aureus* strains, 39% were positive for *icaA*, 86.4% for *icaB*, 84.7% for *icaC*, and all strains carried *icaD*. This study found no statistically significant relationship between the presence of *icaA, icaB, icaC,* and *icaD* genes and methicillin-resistant *Staphylococcus aureus* (MRSA). It is evident that there is a statistically significant relationship between the gene *icaA* and the different inert surfaces in hospital areas and dental offices.

Key words: Virulence; Biofilm; genes, cell adhesion

INTRODUCTION

Staphylococcus aureus (S. aureus) is a gram-positive, facultative anaerobic pathogenic microorganism that is commensal to humans due to its adaptive capacity (Sanguano, 2021). The development of biofilms produced by mechanisms present in *S. aureus* represents one of the main and important virulence factors (O'Gara, 2007), and its prevalence in hospital and clinical environments is of concern for its ability to adhere and persist on hospital devices and surrounding surfaces (Vestby et al., 2020). *S. aureus* can cause infections such as: endocarditis, pneumonia, bacteremia and infections related to soft tissues and skin (Khatoon et al., 2018). These infections present a high degree of complexity with respect to their treatment, since the presence of biofilms increases resistance to antibacterial agents (Lee, 2020).

S. aureus is a ubiquitous bacterium that has managed to inhabit a great diversity of surfaces such as: the mucous membranes and skin of health staff, operating rooms, emergency rooms, intensive care wards, etc. A relevant characteristic is the great capacity to remain viable for weeks and even several months on inert surfaces, making it the main nosocomial microorganism (Gharsa, 2016; Hernández, 2017).

The capacity of this bacteria to form biofilms is considered an important virulence factor that influences its survival and persistence in the environment, as well as in the host (Torlak et al., 2017). Biofilm formation in *S. aureus* is determined by a production mechanism called Polysaccharide Intercellular Adhesin (PIA), (Cramton et al., 1999). The *ica* operon is composed by four genes, these encode the proteins: *IcaA, IcaB, IcaC* and *IcaD*; whose objective is the production of PIA (Muñoz 2017). Within this mechanism, the *icaA* and *icaD* genes work together for the regulation of biofilm formation, due to *icaA* encodes an enzyme called N-Acetylamino-glucosamine transferase (transmembrane protein) (Peng et al., 2023). The *icaC* gene is a transmembrane protein involved in the export of residues to the cell surface. For its part, the *icaB* gene is responsible for the deacetylation process of the PIA molecule that synthesizes the cell surface and gives an important positive charge to the polymer so that adhesion to the cell surface and intercellular adhesion can occur (Vuong et al., 2004).

It is now known that contaminated surfaces contribute significantly to the transmission of clinically relevant pathogens and therefore involve the acquisition of healthcare-associated infections (HAIs). *S. aureus*, one of the most common pathogens in this type of infections, possesses genes that encode several virulence factors, including biofilm formation adhesion (Torlak et al., 2017), among the last ones are the *icaA, icaB, icaC* and *icaD* genes. Therefore, the objective of this study was to detect the frequency of genes that regulate *ica* adhesion *(A, B, C, D)* in *Staphylococcus aureus* strains

isolated from different inert surfaces. This research was carried out at the Laboratorio de Biología Molecular y Genética del Centro de Investigación, Innovación y Transferencia de Tecnología of Universidad Católica de Cuenca.

MATERIALS AND METHODS

Bacterial Isolation and Identification

The present study is part of a macro research project (PICCIITT19-40) that seeks to isolate *Staphylococcus aureus* from inert surfaces, with a quantitative approach, by means of a cross-sectional, observational, field research. For this purpose, *Staphylococcus aureus* strains were studied, which were kept in the strain-room of the Laboratorio de Biología Molecular y Genética of Universidad Católica de Cuenca (13). The samples were kept frozen at -80°C and were isolated as shown in Table 1. *Staphylococcus aureus* strains were identified by microbiological tests (mannitol, coagulase, catalase, DNase and Gram-type staining) and the *nucA* and *femB* genes were detected (Vallejo et al., 2022). In addition, the strains were identified as Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA), which will allow statistical tables to be drawn up.

Molecular Detection of *ica* Genes (A, D, B,C)

Bacterial DNA was obtained through alkaline lysis (Vallejo et al., 2022) using Sodium Dodecylsulfate (SDS) with a concentration of 1% in NaOH at 0.25N. For the identification of the adhesion genes (*icaA, icaB, icaC, icaD*), the Polymerase Chain Reaction (PCR) technique was used, for which we worked with: Green GoTaq 2x master mix from Promega (10 µl), nuclease-free water (5µl), primers for each gene Forward (1.5 µl), reverse (1.5 µl) and with the addition of sample DNA (2µl), obtaining a final volume of 20µl (Sánchez et al., 2022). The reaction tubes were placed inside a thermal cycler "Thermal Block" from Bioneer brand and model "All In One Cycler" as shown in the protocol which shows Table 2 (Sánchez et al., 2022). For visualization of amplicons, the horizontal electrophoresis technique was used in 1.5% P/V agarose gel with the use of 2µl SYBR Safe in 50 ml of gel at 90 Volts with power supply (Bio-Rad, Model Powerpack Basic, USA) for 1 hour.

Table 1. Sampling of *S. aureus* on inert surfaces.

Surfaces	Total of Samples	Positive Samples for *S. aureus*	Reference
Dentistry students' cellphone screens. (CE)	99 samples	16	Laica et al., 2021
Cellphone screens, Cuenca Hospital. (HM)	47 samples	7	To be published
Odontologists' cellphone screens. (CO)	100 samples	12	To be published
Inert surfaces in Cuenca Clinic. (EC)	200 samples	2	Sánchez et al., 2022
Dentistry student multipurpose boxes. (CJ)	139 samples	8	Orellana et al., 2022
Veterinarians' cellphone screens. (CV)	90 samples	6	To be published
Inert surfaces of a Cuenca Hospital. (SHM)	200 samples	5	Sanmartín et al., 2021
Odontologists' cellphone screens. (PCO)	30 samples	3	Orellana, 2021

Statistical Analysis

The Stata 17 program was used for the statistical analysis, and the presence of the *icaA, icaB, icaC* and *icaD* genes in the *S. aureus* strains isolated from inert surfaces in the hospital areas and dental offices was analyzed by means of contingency tables. Chi-square was used to establish the significant statistical relationship between adhesion genes and inert surfaces. In the same way, contingency tables were performed to obtain the relationship between adhesion genes and methicillin resistance.

RESULTS

Typification *ica*

On different inert surfaces (Table 1) 59 strains of *S. aureus* were isolated, each of these strains was analyzed for the presence of *icaA, icaB, icaC* and *icaD* genes, the results are shown in Table 3 and Table 4.

Table 2. Protocol for amplification of *icaA, icaB, icaC, icaD* genes.

Gene (Product size) **5′- 3′ Initiator sequence**	**Condition of PCR**	**Reference**
***icaA:* 1200pb** Forward: GGTAGGTAAAGAAATTGCAAT Reverse: AGCGTTGGGTATTCCCTCTGTCT	Initial Denaturalization 94°C 5 min 94°C 30sec 55°C 1min 72°C 1,5min (30cycles) Final extension 72°C 5 min	Kiem et al., 2004
***icaB:* 900pb** Forward: AGAATCGTGAAGTATAGAAAATT Reverse: TCTAATCTTTTTCATGGAATCCGT	Initial Denaturalization 94°C 5 min 94°C 30sec 52°C 1min 72°C 1,5min (30cycles) Final extension 72°C 5 min	Kiem et al., 2004
***icaC:* 1100pb** Forward: ATGGGACGGATTCCATGAAAAAGA Reverse: TAATAAGCATTAATGTTCAATT	Initial Denaturalization 94°C 5 min 94°C 30sec 50°C 1min 72°C 1,5min (30cycles) Final extension 72°C 5 min	Kiem et al., 2004
***icaD:* 198pb** Forward: ATGGTCAAGCCCAGACAGAG Reverse: CGTGTTTTCAACATTTAATGCAA	Initial Denaturalization 94°C 5 min 94°C 30 sec 55,5°C 30 sec 72°C 30 sec (50cycles) Final extension 72°C 1 min	Mahmood et al., 2022

Table 3. Frequency of *S. aureus* isolated adhesion genes on inert surfaces of hospital areas and dental offices.

Surface	*icaA*		*icaB*		*icaC*		*icaD*		Total	
	N	%	N	%	N	%	N	%	N	%
CE	2	100.0	2	100.0	2	100.0	2	100.0	2	100.0
CJ	5	62.5	8	100.0	8	100.0	8	100.0	8	100.0
CO	3	25.0	12	100.0	12	100.0	12	100.0	12	100.0
CV	6	100.0	6	100.0	5	83.3	6	100.0	6	100.0
EC	6	37.5	12	75.0	12	75.0	16	100.0	16	100.0
HM	1	14.3	6	85.7	6	85.7	7	100.0	7	100.0
PCO	0	0.0	2	66.7	2	66.7	3	100.0	3	100.0
SHM	0	0.0	3	60.0	3	60.0	5	100.0	5	100.0
Total	23	39.0	51	86.4	50	84.7	59	100.0	59	100.0
Test Statistic (X2)	22.289		10.165		8.277		n/a		…	
P-value	**0.002**		0.179		0.309		n/a		…	

* CE = dental students' cellular phones.
* CJ = multi-purpose boxes of dental students.
* CO = cell phones of personnel working in dental offices of an educational institution.
* CV = cellular telephones of personnel working in veterinary clinics.
* HM = cellular phone screens of health personnel working in a public hospital.
* PCO = cellular phones of dentists working in private practice.
* SHM = inert surfaces of a public hospital.
* EC = inert surfaces of a private clinic.

Table 4. Frequency of adherence and determinant genes between MSSA and MRSA isolates in *S. aureus*.

Type	Gene	MSSA (%)	MRSA (%)	X^2	P value
Adherence	*icaA*	38.98	6.78	2.398	0.122
	icaB	6.78	22.03	2.025	0.155
	icaC	8.47	22.03	1.265	0.261
	icaD	0.00	28.81	n/a	n/a

P-value associated with the chi-square statistic. * Statistically significant at 5%. Methicillin Sensitive *Staphylococcus aureus* (MSSA). Methicillin Resistant *Staphylococcus aureus* (MRSA).

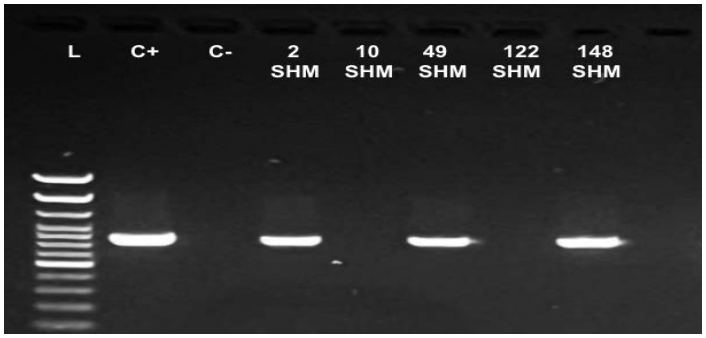

Figure 1. PCR product for the *icaB* gene (900 bp) in *Staphylococcus aureus* strains isolated from inert surfaces of a public hospital (SHM), first lane Ladder (L), second lane positive control (C+) strain *Staphylococcus aureus* ATCC 11632; third lane negative control (C-) strain *Streptococcus pyogenes* ATCC; positive samples: 2SHM, 49SHM and 148SHM.

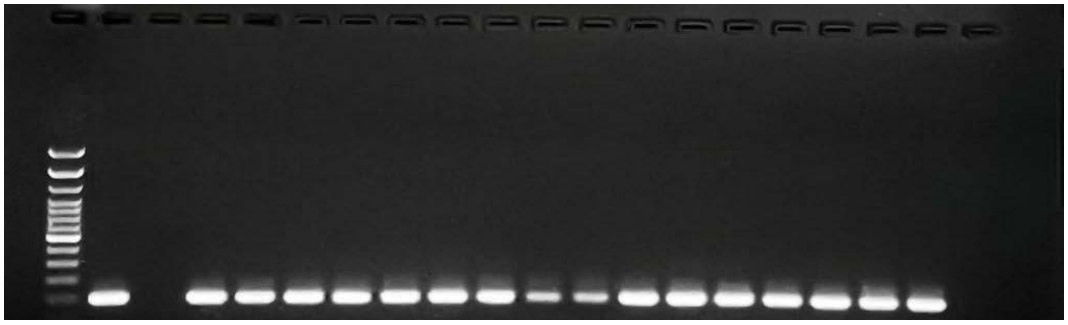

Figure 2. PCR product for the *icaD* gene (198 bp) in *Staphylococcus aureus* strains isolated from dental student cell phones (CE), first lane Ladder (L), second lane positive control (C+) *Staphylococcus aureus* strain ATCC 11632; third lane negative control (C-) *Streptococcus pyogenes* strain ATCC; positive samples: 2CE, 3CE, 4CE,13CE, 34CE, 40CE, 41CE, 42CE, 50CE, 56CE, 61CE, 62CE, 62CE, 73CE, 79CE, 80CE and 83CE.

Figures 1 and 2 Show some of the PCR amplifications used to detect the adhesion genes in S. aureus strains isolated from samples obtained from inert surfaces.

DISCUSSION

The ability of *S. aureus* to form biofilms is considered an important virulence factor that influences its survival and permanence both in the environment and in the host, biofilm formation is associated with the production of intercellular adhesion (Torlak et al. 2017).

Intercellular adhesion in *Staphylococcus aureus* is a complex and dynamic process that is being studied to better understand how this bacterium adheres to cells, host tissues and inert surfaces, which could lead to the development of new therapeutic strategies to fight infections and prevent biofilm formation.

Staphylococcus aureus is the main pathogenic species in its gender, causing diverse infections of community and hospital origin, this bacterium can be isolated from inert surfaces and hospital devices, becoming a source of contamination, besides causing a wide range of infections, from mild cutaneous to severe systemic diseases (Inweregbu et al., 2005).

In the present study the results indicate the frequency of genes, *icaA* 39%, *icaB* 86.4%, *icaC* 84.7% and finally *icaD* 100%, significant results regarding the presence of genes that form biofilms, also in the present study it is recorded that the frequencies reached by *icaA* was 6.78%, *icaB* and *icaC*, reached 22.03%; while *icaD* reached 28.81% with respect to MRSA; however, the significant value of relationship between the type of gene and MRSA, may vary due to the number of strains resistant to methicillin.

In agreement with our study (Torlak et al., 2017) this author studied 243 samples from inert objects (similar to our research) that were previously exposed to dental office, a total of 32 (13.2%) were positive for *S. aureus*. The highest positive contamination rate was observed in samples taken from surfaces of dental chair pushers (25.9 %), containers (22.2 %), where all 32 isolates of *S. aureus* possessed the *icaA* and *icaD* genes, these results are similar with respect to *icaD*, but differ in *icaA* from our investigation, these dissimilarities in the results may be due to the number of samples analyzed and geographical location. It is interesting to observe that there is a high prevalence of *icaA* genes among *S. aureus* strains isolated from different surfaces.

Another study performed on clinical samples by (Ebrahimzadeh et al., 2013) with 60 *S. aureus* strains isolated from: blood catheter (40%), urine catheter (27.5%), sputum (8.3%), injuries (10%) and tracheal tube (14.2%), performed the detection of the *icaD* gene by PCR method and this gene was present in 100% of *S. aureus* strains, these results are important because they indicate that there is no relationship between the presence of *icaD* and the origin of the isolation of *S. aureus*, and we have similar data in isolates from inanimate surfaces and clinical samples.

Regarding the study by (Karki et al., 2019), obtained clinical samples from 570 patients (345 pus aspirates and 225 wound swabs), it was obtained that 19.3% (110/570) were positive for *S. aureus*, where their genomic analysis presented that 77.2% (85/110) of isolates were positive for the presence of *icaD*, data which is less with our study which may be due to the reasons mentioned above.

Another research (Nourbakhsh & Ebrahimzadeh, 2016) collected strains from three hospitals where *icaC* (69.3%) had the highest frequency with respect to other adherence genes, *icaD* presented 54.8%, results that are different from our study and even from studies previously done to these authors, it would be important in our case to perform other investigations with different clinical and inert samples to obtain conclusive results.

Another result that differs from this research was performed by (Ghaioumy et al., 2021), through a molecular study of the *ica* operon, with 46 isolates of *S. aureus*, in tissues with adenoid hypertrophy, by means of PCR test, 6.3% carried *icaA* and 59.4% *icaD*, while *icaC* and *icaB* were not detected.

Few studies have been carried out in Ecuador for the detection of ica operon genes, one of them was performed by (Sanguano et al., 2021) isolated *S. aureus* in different clinical samples from three third level hospitals in the cities of Quito and Puyo (ocular, vaginal, tracheal, pharyngeal secretion, abscesses, blood, spinal fluid, cerebrospinal fluid, wounds), the PCR test was used, where it was determined that the isolated strains carried the *icaA* and *icaD* genes in 87.18%; so we can indicate that the values are not close to those studied in our research, especially with the *icaA* gene, it would be important to conduct further studies where the origin of the sample and the relationship with *icaA* are analyzed, to obtain conclusive data.

Martinez et al., 2016, indicates that biofilm formation is an important virulence factor for *S. aureus*, both MSSA and MRSA employ different mechanisms for this. MSSA strains predominantly form biofilms dependent on the *icaADBC* operon and PIA production, while MRSA strains form biofilms independent of PIA. (O'Neill, 2007; O'Neill, 2008; Moghadam, 2014; McCarthy, 2015; Cruz, 2016)

In our study It is evident that there is a statistically significant relationship between the virulence gene *icaA* and the different inert surfaces in hospital areas and dental offices. But there is no statistically significant relationship between MSSA-MRSA and *ica* genes, this may be due to the number of samples analyzed, so it is recommended that for future research it is performed with a greater number of these samples.

It should be emphasized that, in Ecuador, very few studies have been carried out on the frequency of *ica* genes on inert surfaces, so we recommend starting to investigate this type of genes, especially on inanimate surfaces such as: catheters, probes, implants, turbines, surgery rooms, cell phones, in which the adherence factor plays a fundamental role in the permanence of *S. aureus* and in cross-contamination with patients. The present study shows that there is a significant statistical relationship between the virulence gene *icaA* and the different inert surfaces of the hospital areas

and dental offices; therefore, it would be important to analyze this gene in inanimate surfaces that are directly related to the patients and thus avoid cross-contamination.

In order to have conclusive data on the relationship between MRSA strains and adherence genes, it is important to work with a greater number of samples.

The analysis of the frequency of the *icaA, B, C, D* genes is determinant for biofilm formation, especially in *S. aureus* strains isolated from inanimate surfaces directly related to patients to avoid cross-contamination and trigger serious chronic infections.

CONCLUSIONS

The present study shows that there is a significant statistical relationship between the gene *icaA* and the different inert surfaces of the hospital areas and dental offices; therefore, it would be important to analyze this gene in inanimate surfaces that are directly related to the patients and thus avoid cross-contamination.

In order to have conclusive data on the relationship between MRSA strains and adherence genes, it is important to work with a greater number of samples. The analysis of the frequency of the *icaA, B, C, D* genes is determinant for biofilm formation, especially in *S. aureus* stains isolated from inanimate surfaces directly related to patients to avoid cross-contamination and trigger serious chronic infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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