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Production of biofilm by *Staphylococcus aureus*: Association with infective endocarditis?

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ABSTRACT

Objectives: *Staphylococcus aureus* is a well-known biofilm-producing pathogen that is capable of causing chronic infections owing to its ability to resist antibiotic treatment and obstruct the immune response. However, the possible association between high biofilm production and infective endocarditis (IE) has not been assessed. Our objective was to compare production of biofilm by *S. aureus* strains isolated from patients with bacteremia and IE, catheter-related bloodstream infection (C-RBSI), or non-device associated bacteremia.
Methods: We isolated 260 *S. aureus* strains from the blood of patients with bacteremia who were diagnosed during hospital admission between 2012 and 2015. Patients were divided into 3 groups according to whether they had IE, C-RBSI, or non-device associated bacteremia. Biofilm production was measured in terms of biomass and metabolic activity using the crystal violet and XTT assays, respectively. High biomass and metabolic activity rates (based on tertile ranks classification) were compared between the 3 groups.
Results: The high biomass and metabolic activity rates of each group were 41.9% and 37.2% for IE, 32.5% and 35.0%, for C-RBSI, and 29.0% and 33.3% for non-device associated bacteremia ($p = 0.325$ and $p = 0.885$, respectively).
Conclusions: High biomass and metabolic activity levels for *S. aureus* isolates from IE were similar to those of *S. aureus* isolates from C-RBSI or non-device associated bacteremia.

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Producción de biofilm en *Staphylococcus aureus*: ¿Asociación con endocarditis infecciosa?

Palabras clave:
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Staphylococcus aureus
Bacteriemia relacionada con catéter

RESUMEN

Objetivos: *Staphylococcus aureus* es un conocido microorganismo productor de biofilm, capaz de causar infecciones crónicas debido a su capacidad de resistir el tratamiento antibiótico y dificultar la respuesta inmunitaria. Sin embargo, no se ha evaluado la posible asociación entre una elevada producción de biofilm y la endocarditis infecciosa (EI). Nuestro objetivo fue comparar la producción de biofilm por parte de cepas de *S. aureus* aisladas de pacientes con bacteriemia y EI, bacteriemia relacionada con el catéter (BRC) o bacteriemia no asociada a dispositivos.

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Métodos: Se aislaron 260 cepas de *S. aureus* de sangre de pacientes con bacteriemia que fueron diagnosticados durante su ingreso hospitalario entre 2012 y 2015. Los pacientes se dividieron en tres grupos según tuvieran EI, BRC o bacteriemia no asociada a dispositivos. La producción de biofilm se midió en términos de biomasa y de actividad metabólica utilizando los ensayos de cristal violeta y XTT, respectivamente. Se compararon los índices de alta biomasa y actividad metabólica (basadas en clasificación por terciles) entre los tres grupos.
Resultados: Los índices altos de biomasa y actividad metabólica de cada grupo fueron del 41,9 y del 37,2% para EI, del 32,5 y del 35,0% para BRC, y del 29,0 y del 33,3% para bacteriemia no asociada a dispositivos (p = 0,325 y p = 0,885, respectivamente).
Conclusiones: Los niveles altos de biomasa y actividad metabólica de los aislados de *S. aureus* procedentes de EI fueron similares a los de los aislados de BRC o de bacteriemia no asociada a dispositivos.
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Introduction

Staphylococcus aureus is a colonizing pathogen capable of causing hospital-acquired infections, with an estimated annual cost of almost $450 million in the United States.[1,2]

S. aureus is capable of producing the extracellular matrix that facilitates survival in hostile or extreme environments and enables initial attachment and subsequent biofilm formation.

Biofilm is responsible for chronic infections in indwelling devices (including heart valves, catheters, and joint prosthetics) and in host tissues.[1,3–6]

Infective endocarditis (IE) is a typical biofilm-associated infectious disease frequently caused by commensal staphylococci. It is associated with high morbidity, mortality, and health care costs.[7] The annual incidence of IE in Spain is at least 3.5 cases/100,000 inhabitants, in which 23.6% of the episodes are caused by *S. aureus*.[8] Some studies aimed to demonstrate an association between *S. aureus* biofilm production and patient outcome.[3,9,10] However, to our knowledge, the association between high-biofilm-production *S. aureus* strains and IE has not been assessed.

We hypothesized a possible association between high biofilm production of *S. aureus* strains—in terms of biomass and metabolic activity levels—and IE.

Materials and methods

We retrospectively recovered 260 *S. aureus* strains isolated from the blood of patients with bacteremia (adults >16 years and infants/children 0–16 years) who were diagnosed during admission between 2012 and 2015 in Hospital Gregorio Marañón, a tertiary teaching institution in Madrid, Spain. We included only the first isolates from each episode. Patients did not receive antimicrobial therapy before blood cultures were drawn.

We recorded patient and microbiological characteristics for the analysis. Patients were divided into 3 groups: 1, those who had *S. aureus* bacteremia and IE ($N = 39$); 2, those who had *S. aureus* C-RBSI ($N = 40$); and 3, those with non-device associated *S. aureus* bacteremia ($N = 181$). Only 4 patients had both C-RBSI and IE; these patients were included in the IE group. Patients included in group 3 also meet the criteria for non-recurrent or persistent bacteremia.

Biofilm production

Biofilm production was measured in terms of biomass (using the crystal violet staining assay) and metabolic activity (using the XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] tetrazolium salt assay).

Briefly, a loopful of fresh culture was inoculated in 20 ml of tryptic soy broth and incubated at 30 °C with shaking overnight. After 3 cycles of centrifugation-resuspension with phosphate-buffered saline (PBS), the inoculum was adjusted to 0.5 McFarland (10^8 cfu/ml). One hundred microliters of each suspension was placed in a polypropylene 96-well microtiter plate and incubated at 37 °C for 24 h. Fresh medium was used as a negative control. Plates were washed 3 times with PBS. All strains were tested in triplicate for each biomass and metabolic activity assay.

Biomass production

After plates were completely dry, 200 μl of 99% methanol was added for 10 min at room temperature. Methanol was discarded, and 125 μl of 0.1% crystal violet was added for 10 min at room temperature. Plates were washed with distilled water, and fixed crystal violet was released using 125 μl of 30% acetic acid for 15 min The volume was transferred to a new plate, and absorbance (550 nm) was measured in a spectrophotometer (Biochrom EZ Read 400).

The tertile ranks used to classify the strains according to biomass production were as follows: low, <0.530; moderate, 0.530–1.291; and high, ≥1.292.

Only high biomass production rates were compared between the 3 groups.

Metabolic activity

After plates were completely dry, 100 μl of a premixed solution of 10 ml of XTT (0.5 mg/ml) with 1 μl of menadione (1.72 mg/ml) was added and protected from light. Plates were incubated at 37 °C for 2 h, and absorbance was measured at 492 nm in a spectrophotometer (Biochrom EZ Read 400).

The tertile ranks used to classify the strains according to metabolic activity were as follows: low, <0.214; moderate, 0.214–0.416; high, ≥0.417.

Only high metabolic activity rates were compared between the 3 groups.

Definitions

Catheter-related bloodstream infection: We defined an episode of C-RBSI based on the isolation of the same microorganism both in peripheral blood cultures and on the catheter tip.[11]

Infective endocarditis (IE). IE was defined according to the modified Duke criteria.[12]

Non-device associated bacteremia: *S. aureus* bacteremia without persistent or recurrent bacteremia, and without 30-day crude mortality.

Persistent bacteriemia: Repeated positive blood cultures (two or more different days) 48–72 h after starting appropriate antimicrobial therapy.

Recurrent bacteremia: Positive blood cultures (two or more different days) ≥7 days after starting appropriate antimicrobial therapy and after a period with negative blood cultures.

30-day crude mortality: Mortality resulting 30 days after the episode of *S. aureus* bacteremia.

Statistical analyses

Qualitative variables appear with their frequency distribution. Quantitative variables are expressed as the median and interquartile range (IQR).

Non-normally distributed continuous variables were compared using the Kruskal–Wallis test. Qualitative variables were compared using the ANOVA test or the Fisher exact test (when only 2 variables were analyzed).

Statistical significance was set at $p < 0.05$. All tests were performed using the statistical program SPSS 21.0 (Armonk, NY: IBM Corp).

Results

Patients' demographic characteristics were similar between the study groups. The median age of adults and infants was 71 (58–80) years and 3 (0–16) months. Most patients were male (66.9%), and the main underlying disease was solid organ tumor (28.8%), followed by cardiac disease (23.5%) and abdominal disease (15.4%). Most patients had a non-fatal McCabe score (73.8%), a median Charlson comorbidity index of 3 (2–6), and an APACHE II of 6 (3–6). Patients spent a median of 24 (13–49) days in hospital (Table 1).

Regarding clinical characteristics, both the IE and C-RBSI groups had persistent and recurrent bacteremia compared with the non-device associated *S. aureus* bacteremia group (as the absence of persistent and recurrent bacteremia was implicit in the definition of non-device associated *S. aureus* bacteremia, $p < 0.001$). Rates for persistent and recurrent bacteremia in the IE/C-RBSI groups were, respectively, 10.3%/15.0% and 10.3%/10.0%. Overall 30-day mortality was 8.1%, with the highest rate being found in the in IE group (38.5%). Methicillin-resistant *S. aureus* (MRSA) was detected in 68/260 cases (26.2%), with no difference in rates between the groups. As expected, the duration of antimicrobial therapy was significantly longer in the IE group (22 [14–35] days) than in the C-RBSI and non-device associated *S. aureus* bacteremia groups (15 [9–21] and 15 [8–21], respectively) ($p = 0.001$) (Table 1).

High biomass-producing *S. aureus* strains were distributed as follows: group 1, 18 patients (46.2%); group 2, 13 patients (32.5%); and group 3, 53 patients (29.3%) ($p = 0.325$). High metabolically active *S. aureus* strains were distributed as follows: group 1, 16 patients (41.1%); group 2, 14 patients (35.0%); and group 3, 59 patients (32.6%) ($p = 0.885$) (Table 1).

In the 39 cases of IE, 28 strains (71.8%) were isolated from native valves and 11 (28.2%) from prosthetic valves. In native valve IE, biomass and metabolic activity production were high in 11 and 14 of the 28 cases (39.3% and 50.0%), respectively ($p = 0.332$). In prosthetic valve IE, biomass and metabolic activity production were high in 6 and 2 of the 11 cases (54.5% and 18.2%), respectively ($p = 0.086$).

Discussion

In our study we were not able to find an association between high-biofilm-producing *S. aureus* strains and IE, suggesting that it may be necessary to identify specific biofilm-associated factors to demonstrate the clinical involvement of biofilm.

S. aureus is the most common cause of IE, with high rates of morbidity and mortality.[8] The pathogenicity of *S. aureus* in IE is based on its ability to adhere to, colonize, and persist on native and prosthetic valves within a structured biofilm matrix.[1,4]

Little is known about the impact of *S. aureus* biofilm on the clinical characteristics and outcome of IE. Di Domenico et al. recently demonstrated that microbial biofilm correlates with increased antibiotic tolerance and poor therapeutic outcome in IE. However, the authors only tested 8 isolates from patients with surgically treated IE (only 4 were caused by *S. aureus*) and used a novel method for biofilm quantification, the clinical Biofilm Ring Test.[13] Fernández-Hidalgo et al. showed that phenotype and genotype provided no additional predictive value beyond conventional clinical characteristics in a multicenter study of 213 patients with *S. aureus* IE.[14]

Previous studies failed to demonstrate an association between the possible role of biofilm production and clinical outcome. A previous study by our group with 485 *S. aureus* strains isolated from patients with bacteremia demonstrated that biofilm production (in terms of biomass and metabolic activity) was not associated with poor clinical outcome (IE, 30-day attributable mortality, and persistent/recurrent bacteremia).[10] Moreover, Fernández-Hidalgo et al. only demonstrated an association between biomass production and the clonal linage of 209 *S. aureus* strains causing IE, whereas no other relationship was found between biomass production and other prognostic characteristics.[9] Similar findings were also reported by Naicker et al., who could only demonstrate an association between biofilm formation and the clonal linage of *S. aureus* isolates, but not between biofilm formation and the source of bacteremia.[15] These data correlate with our study findings, which show that neither high biomass production nor high metabolic activity of *S. aureus* strains was associated with IE or C-RBSI. Despite we consider that our data were based on a large sample size ($n = 260$ patients) enough to demonstrate no statistical significance, we could appreciate a slightly difference regarding biofilm production among the 3 groups, in which high biofilm production (both in terms of biomass and metabolic activity) was higher in EI group compared to the non-device associated bacteremia group (46.2% vs. 29.3% and 41.1% vs. 32.6%, respectively).

Furthermore, other clinical aspects of the IE group, such as the 30-day mortality rate (34.9%) or median (IQR) hospital stay (27.00 [18.00–61.00] days), correlated with that previously described by Muñoz et al. (28.9% and 36.00 [21.00–53.00], respectively).[16]

Regardless of biofilm production, the requirements of *S. aureus* for developing tissue infection, such as IE or skin abscess, differ from those necessary for infection on indwelling devices, such as C-RBSI or prosthetic joint infection. The requirements for tissue infections include toxins and superantigens, as well as higher bacterial load (because of vascularization and factors associated with innate immunity, which are absent in device-related infections).[17] We applied this interesting aspect in our study to analyze whether biofilm production was associated with the origin of endocarditis, as we hypothesized that the biomass production and metabolic activity of *S. aureus* strains would be greater in native IE than in prosthetic IE, although we were unable to identify an association. Maybe because of the low sample size, so future studies including more patients would be interesting to see whether differences could be found.

Factors associated with the virulence of *S. aureus* also differ depending on the environment. For example, one of the main factors associated with maturation and disassembly of biofilm in staphylococci is the *agr* quorum-sensing system, which is essential for IE and osteomyelitis but not for infection by a foreign body.[1,3,6,17] In contrast, it has been demonstrated that *ica* operon is an independent mechanism of biofilm formation in *S. aureus* and that its detection plays no useful role in diagnosing isolates associated with biofilm-mediated device-related infection.[18] Moreover, Pericàs et al. recently showed no clear association between genes

Table 1
Patient characteristics in terms of *Staphylococcus aureus* bacteremia.

Variable	Total	IE	C-RBSI	Non-device associated bacteremia	p^*
	N = 260	*N* = 39	*N* = 40	*N* = 181	
Demographic characteristics					
Adults, N = 230					
Median (IQR) age >16 yrs (years)	71 (57–79)	68 (56–78)	65 (54–76)	73 (59–80)	0.055
Infants, N = 30					
Median (IQR) age 0–16 yrs (months)	3 (0–15)	160 (1–160)	1 (0–81)	4 (0–14)	0.505
Sex, N (%)					
Male	174 (66.9)	25 (64.1)	24 (60.0)	125 (69.1)	
Female	86 (33.1)	18 (46.2)	16 (40.0)	52 (28.7)	
McCabe score (nonfatal), N (%)	192 (73.8)	33 (84.6)	28 (70.0)	131 (72.4)	0.780
Median (IQR) Charlson comorbidity index	3.00 (2.00–6.00)	3.00 (2.00–6.00)	3.00 (2.00–6.00)	3.00 (2.00–5.50)	0.864
Median (IQR) APACHE II score	6.00 (3.00–6.00)	6.00 (3.00–9.00)	5.50 (2.00–6.75)	6.00 (3.00–6.00)	0.327
Median (IQR) days of hospital stay	24 (13–49)	27 (18–61)	31 (13–56)	22 (12–47)	0.286
Clinical characteristics					
Persistent bacteremia, *N* (%)	10 (3.8)	4 (10.3)	6 (15.0)	0 (0.0)	<0.001
Recurrent bacteremia, *N* (%)	8 (3.1)	4 (10.3)	4 (10.0)	0 (0.0)	<0.001
30-day attributable mortality, *N* (%)	21 (8.1)	15 (38.5)	6 (15.0)	0 (0.0)	>0.001
MRSA, *N* (%)	68 (26.2)	9 (23.1)	13 (32.5)	46 (25.4)	0.486
Median (IQR) duration of antimicrobial therapy (days)	16 (9–25)	22 (14–35)	15 (9–21)	15 (8–21)	0.001
High biofilm production, N (%)					
High biomass	84 (32.3)	18 (46.2)	13 (32.5)	53 (29.3)	0.325
High metabolic activity	89 (34.2)	16 (41.1)	14 (35.0)	59 (32.6)	0.885

IE, infective endocarditis; C-RBSI, catheter-related bloodstream infection; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation; MRSA, methicillin-resistant *Staphylococcus aureus*.

* Statistically significant at $p < 0.05$.

encoding virulence factors, *agr* type, clonal complexes, mortality, and major embolic events according to vancomycin MIC. They also found that methicillin-susceptible *S. aureus* isolates with higher vancomycin MICs exhibited a poorer ability to form biofilms with and without the presence of vancomycin.[19]

All of the above may explain, in part, why we were unable to find an association between high biofilm production by *S. aureus* and IE. The factors that are closely related to *S. aureus* biofilm formation in IE may not actually be biomass or metabolic activity, but other biofilm-associated virulence factors, associated mainly with genotypic characteristics. Moreover, another aspect to take into account is that discordance between biofilm formation in polystyrene dish assay and virulence in animal models have been described. So, ex vivo biofilm formation on a relevant tissue surface may be warranted to validate results of in vitro assays.[20–22]

In our opinion, determining overall biofilm production by means of the amount of biomass in order to establish a clinical association (i.e., with the development of endocarditis or with patient outcome) is not reliable, because it is an approximate approach that requires too many factors to be taken into account. We consider that analysis of the impact of *S. aureus* biofilm must be based on a study of the implicit characteristics of the biofilm itself, such as virulence factors.

One of the limitations of the study was that our data cannot be extrapolated to other biofilm-forming microorganisms such as streptococci or enterococci, which are also common causative agents in IE and in which biofilm formation is also closely related to invasive colonization.

Despite we could not demonstrate a relationship between high biofilm production of *S. aureus* and IE, future studies are need to further investigate biofilm production in the pathogenesis of IE using different group of patients and large sample size.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

1. de Vor L, Rooijakkers SHM, van Strijp JAG. Staphylococci evade the innate immune response by disarming neutrophils and forming biofilms. FEBS Lett. 2020;594:2556–69.
2. Song X, Perencevich E, Campos J, Short BL, Singh N. Clinical and economic impact of methicillin-resistant *Staphylococcus aureus* colonization or infection on neonates in intensive care units. Infect Control Hosp Epidemiol. 2010;31:177–82.
3. Kwiecinski JM, Jacobsson G, Horswill AR, Josefsson E, Jin T. Biofilm formation by *Staphylococcus aureus* clinical isolates correlates with the infection type. Infect Dis (Lond). 2019;51:446–51.
4. Moormeier DE, Bayles KW. *Staphylococcus aureus* biofilm: a complex developmental organism. Mol Microbiol. 2017;104:365–76.
5. Oliveira WF, Silva PMS, Silva RCS, Silva GMM, Machado G, Coelho L, et al. *Staphylococcus aureus* and *Staphylococcus epidermidis* infections on implants. J Hosp Infect. 2018;98:111–7.
6. Schilcher K, Horswill AR. Staphylococcal biofilm development: structure, regulation, and treatment strategies. Microbiol Mol Biol Rev. 2020;84.
7. Jung CJ, Yeh CY, Shun CT, Hsu RB, Cheng HW, Lin CS, et al. Platelets enhance biofilm formation and resistance of endocarditis-inducing streptococci on the injured heart valve. J Infect Dis. 2012;205:1066–75.
8. Olmos C, Vilacosta I, Fernández-Pérez C, Bernal JL, Ferrera C, García-Arribas D, et al. The evolving nature of infective endocarditis in Spain: a population-based study (2003 to 2014). J Am Coll Cardiol. 2017;70:2795–804.
9. Fernández-Hidalgo N, Basas J, Viedma E, Ribera A, Larrosa N, Pérez-Montarelo D, et al. Association between biomass formation and the prognosis of infective

endocarditis due to *Staphylococcus aureus*. Enferm Infecc Microbiol Clin. 2020;38:263–6.
10. Guembe M, Alonso B, Lucio J, Pérez-Granda MJ, Cruces R, Sánchez-Carrillo C, et al. Biofilm production is not associated with poor clinical outcome in 485 patients with *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect. 2018;24, 659 e1–e3.
11. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;49:1–45.
12. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. 2000;30:633–8.
13. Di Domenico EG, Rimoldi SG, Cavallo I, D'Agosto G, Trento E, Cagnoni G, et al. Microbial biofilm correlates with an increased antibiotic tolerance and poor therapeutic outcome in infective endocarditis. BMC Microbiol. 2019;19:228.
14. Fernández-Hidalgo N, Ribera A, Larrosa MN, Viedma E, Origüen J, de Alarcón A, et al. Impact of *Staphylococcus aureus* phenotype and genotype on the clinical characteristics and outcome of infective endocarditis. A multicentre, longitudinal, prospective, observational study. Clin Microbiol Infect. 2018;24:985–91.
15. Naicker PR, Karayem K, Hoek KG, Harvey J, Wasserman E. Biofilm formation in invasive *Staphylococcus aureus* isolates is associated with the clonal lineage. Microb Pathog. 2016;90:41–9.
16. Muñoz P, Kestler M, De Alarcon A, Miro JM, Bermejo J, Rodríguez-Abella H, et al. Current epidemiology and outcome of infective endocarditis: a multicenter, prospective, cohort study. Medicine (Baltimore). 2015;94:e1816.
17. Paharik AE, Horswill AR. The staphylococcal biofilm: adhesins regulation, and host response. Microbiol Spectr. 2016;4.
18. Fitzpatrick F, Humphreys H, O'Gara JP. The genetics of staphylococcal biofilm formation – will a greater understanding of pathogenesis lead to better management of device-related infection? Clin Microbiol Infect. 2005;11:967–73.
19. Pericàs JM, Cervera C, Garcia-de-la-Mària C, Sharma-Kuinkel BK, Gonzales R, Moreno A, et al. Relationship between vancomycin MIC and virulence gene expression in clonal complexes of methicillin-susceptible *Staphylococcus aureus* strains isolated from left-sided endocarditis. Antimicrob Agents Chemother. 2020;64.
20. Leuck AM, Johnson JR, Dunny GM. A widely used in vitro biofilm assay has questionable clinical significance for enterococcal endocarditis. PLoS ONE. 2014;9:e107282.
21. Frank KL, Guiton PS, Barnes AM, Manias DA, Chuang-Smith ON, Kohler PL, et al. AhrC and Eep are biofilm infection-associated virulence factors in *Enterococcus faecalis*. Infect Immun. 2013;81:1696–708.
22. Lizcano A, Chin T, Sauer K, Tuomanen EI, Orihuela CJ. Early biofilm formation on microtiter plates is not correlated with the invasive disease potential of *Streptococcus pneumoniae*. Microb Pathog. 2010;48:124–30.