

# Genotypic and Phenotypic Characteristics of *Staphylococcus aureus* Prosthetic Joint Infections: Insight on the Pathogenesis and Prognosis of a Multicenter Prospective Cohort

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**Background.** *Staphylococcus aureus* is the leading cause of prosthetic joint infection (PJI). Beyond the antibiogram, little attention has been paid to the influence of deep microbiological characteristics on patient prognosis. Our aim was to investigate whether microbiological genotypic and phenotypic features have a significant influence on infection pathogenesis and patient outcome.

**Methods.** A prospective multicenter study was performed, including all *S. aureus* PJIs (2016–2017). Clinical data and phenotypic (*agr* functionality,  $\beta$ -hemolysis, biofilm formation) and genotypic characteristics of the strains were collected. Biofilm susceptibility to antimicrobials was investigated (minimal biofilm eradication concentration [MBEC] assay).

**Results.** Eighty-eight patients (39.8% men, age 74.7  $\pm$  14.1 years) were included. Forty-five had early postoperative infections (EPIs), 21 had chronic infections (CPIs), and 19 had hematogenous infections (HIs). Twenty (22.7%) were caused by methicillin-resistant *S. aureus*. High genotypic diversity was observed, including 16 clonal complexes (CCs), with CC5 being the most frequent (30.7%). *agr* activity was greater in EPI than CPI (55.6% vs 28.6%;  $P = .041$ ). Strains causing EPI were phenotypically and genotypically similar, regardless of symptom duration. Treatment failure (36.5%) occurred less frequently among cases treated with implant removal. In cases treated with debridement and implant retention, there were fewer failures among those who received combination therapy with rifampin. No genotypic or phenotypic characteristics predicted failure, except vancomycin minimal inhibitory concentration  $\geq 1.5$  mg/L (23.1% failure vs 3.4%;  $P = .044$ ). MBEC<sub>50</sub> was  $>128$  mg/L for all antibiotics tested and showed no association with prognosis.

**Conclusions.** *S. aureus* with different genotypic backgrounds is capable of causing PJI, showing slight differences in clinical presentation and pathogenesis. No major microbiological characteristics were observed to influence the outcome, including MBEC.

**Keywords.** biofilm; bone infections; pathogenesis; prosthetic joint infections; *Staphylococcus aureus*.

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Prosthetic joint infection (PJI) is a serious complication of arthroplasty, resulting in significant morbidity and costs. Although the outcome of PJI is largely dependent on specialized surgery and appropriate antimicrobial treatment, the etiology is also very important for defining the pathogenesis and prognosis.

The ability of bacteria to cause PJI depends on virulence factors that enable attachment, biofilm development, tissue damage, and intracellular invasion, among other complications. The specific bacteria responsible for the infection have

a significant influence on clinical presentation [1]. Common classifications of PJI are based on timing and have proved to be useful for predicting the etiology of infection and taking management decisions. However, specific definitions and time limits are sometimes arbitrary and do not explain why specific bacterial species lead to particular types of PJI [2, 3].

*Staphylococcus aureus*, a leading cause of PJI, is a versatile microorganism, able to cause various types of PJI, mainly acute postoperative and hematogenous, but also chronic [2, 4]. This flexibility is probably due to the large number of virulence factors, which are mainly regulated by the accessory gene regulator (*agr*) [5]. There is little information about differences in genetic background or the molecular mechanisms of *S. aureus* strains according to the clinical presentation of PJI. Moreover, few studies have analyzed the impact of microbiological characteristics and virulence factors on the prognosis of the infection [6, 7].

It is well known that the in vitro activity of antimicrobials, commonly measured as the minimal inhibitory concentration (MIC), correlates poorly with clinical outcome in the setting of biofilm-associated infections. Other approaches based on measuring the minimal biofilm inhibitory concentration (MBIC) and/or the minimal biofilm eradication concentration (MBEC) have been proposed [8]. While these parameters have been studied in strains causing PJI, the correlation with outcome has not been addressed [9].

In this study, we characterized in detail a prospective multicenter cohort of patients with *S. aureus* PJI with the aim of investigating the influence of a wide range of phenotypic and genotypic characteristics of this microorganism on clinical presentation and also on the outcomes of patients.

## METHODS

### Setting and Patients

A prospective observational multicenter pilot study that included every *S. aureus* PJI between May 2016 and September 2017 was conducted at 11 teaching hospitals in Madrid (Spain).

Staphylococcal PJI was defined as  $\geq 1$  surgical, joint aspirate, or blood culture yielding *S. aureus*, along with a compatible clinical presentation [3]. Data collected included clinical data on baseline features, prosthesis characteristics, and clinical presentation, along with information about surgical and medical treatment. Patient management was decided by the treating medical team on an individualized basis and followed current recommendations [1, 3, 10]. Antibiotics were administered according to the antimicrobial susceptibility profile, with a preference for rifampin-based combinations.

### Clinical Definitions: Types of PJIs and Outcomes

PJI was considered acute or chronic depending on whether it began within the first 90 days after prosthesis placement or

later, respectively. Hematogenous infection was defined as acute onset after a clinically suspected or proven bacteremia. Cases that were considered hematogenous were excluded from being classed as early or chronic postoperative infections. Alternatively, PJI could also be caused by the spread of a contiguous suppurative focus and by “positive intraoperative cultures” for cases of prosthesis revision due to presumed aseptic loosening [2].

Failure of therapy was considered in cases of death from any cause within 90 days after surgery, persistent or relapsing signs of staphylococcal infection, and/or the need for salvage therapy due to *S. aureus*, including antimicrobial suppressive therapy and unplanned surgeries (except for extra debridements in the first 30 days after the initial therapeutic surgery). Patient follow-up was carried out until death, failure, or loss to follow-up for at least 1 year.

### Microbiological and Molecular Characterization of *S. aureus* Isolates

**Identification and Antimicrobial Susceptibility Testing** Isolation and identification of *S. aureus* were based on standard microbiological procedures at each laboratory. All isolates were sent to a central laboratory (Hospital 12 de Octubre). Antimicrobial susceptibility testing was performed with the MicroScan Walkway System (Siemens, West Sacramento, CA, USA), and MICs were interpreted according to EUCAST criteria. The E-test macromethod was also performed to screen for heteroresistant vancomycin-intermediate *S. aureus* (h-VISA) phenotypes in isolates with vancomycin MIC  $\geq 1.5$  mg/L (by E-test) [11].

### Antimicrobial Susceptibility Testing in Biofilm: Calgary

**Biofilm Device** The Calgary Biofilm Device (CBD; Innovotech, Edmonton, AB, Canada) was used to study the MBEC and MBIC. Studies involving the CBD were conducted as previously described [8] with minor modifications (Supplementary Data, Supplementary Table 1) on isolates from patients treated with debridement, antibiotics, and implant retention (DAIR) and on antibiotics administered for a significant period of time ( $\geq 14$  days in the first month after DAIR and/or  $\geq 21$  days during the whole treatment period). The antibiotics were oxacillin, daptomycin, levofloxacin, and rifampin, with a concentration range of 0.5–256.0 mg/L. The combination of levofloxacin plus rifampin was also tested: rifampin (in a concentration range of 0.5–256.0 mg/L) with a fixed concentration of levofloxacin (3.0 mg/L), then levofloxacin (in a concentration range of 0.5–256.0 mg/L) with a fixed concentration of rifampin (5.0 mg/L). Fixed concentrations of levofloxacin and rifampin were chosen to approximate those expected in bone (Supplementary Data).

**Phenotypic Characterization** The activity of the *agr* operon was measured by  $\delta$ -hemolysin production [12] and categorized as negative, weak, or strong.  $\beta$ -hemolysin production produced

by  $\alpha$ -hemolysin (Hla) activity was also analyzed [13]. Biofilm formation was assessed in triplicate with the 0.7% crystal violet method on microtiter plates using 33% glacial acetic acid as the discoloring solution [14]. Absorbance was measured at 595 nm, and the results were interpreted in accordance with Stepanovic [15]. *S. aureus* colony phenotype, including small colony variants (SCVs), was also observed.

**Genotypic Characterization** Virulence and antibiotic resistance genes [16] were determined by DNA microarrays based on the ArrayTube platform, in accordance with the manufacturer's instructions (*S. aureus* Genotyping Kit 2.0, Alere, Jena, Germany).

#### Statistical Analysis

As this was an exploratory study, no sample size calculations were made. Continuous variables were compared using the *t* test or Mann-Whitney *U* test, and categorical parameters were compared using the  $\chi^2$  or Fisher's exact test, as appropriate. For the analysis of antimicrobial treatment among patients managed by DAIR, treatment with a specific drug was considered if it was administered for at least 14 days in the first 30 days after surgery and/or for at least 21 days during the entire antimicrobial treatment period [17]. All tests were 2-tailed, and a *P* value <.05 was considered statistically significant. Statistical analyses were carried out using SPSS, version 20.0, and figures were created using GraphPad Prism, version 6.

#### PATIENT CONSENT STATEMENT

The study was designed and performed in accordance with the ethical standards of the Helsinki Declaration. It was evaluated and approved by the Hospital Research Ethics Committee (Expte 16/188). Due to the observational nature of the study, no informed consent was deemed necessary.

#### RESULTS

##### Clinical and Microbiological Description of the Cohort

Eighty-eight patients were included in the study. Forty-five subjects had early postoperative infection (51.1%), 21 were chronic (23.9%), and 19 were hematogenous (21.6%); in addition, 2 patients had an infection as a result of contiguous spread from a suppurative focus, and 1 patient had a positive intraoperative culture.

Clinical features and microbiological characteristics (phenotypic and genotypic) are shown in detail in Table 1 and Supplementary Table 2. Twenty isolates (22.7%) were methicillin-resistant (MRSA), and all harbored the *mecA* gene. All strains were vancomycin-susceptible, 11 (12.5%) showed MICs  $\geq 1.5$  mg/L, and none had an h-VISA phenotype. Most isolates showed  $\beta$ -hemolysis (87.5%, *n* = 77) and biofilm formation (95.5%, *n* = 84). Four strains showed uncommon

phenotypes: 3 were SCVs and 1 showed the mucous phenotype. Molecular epidemiology analysis showed that *S. aureus* isolates belonged to 16 different clonal complexes (CCs), with CC5 being the most frequent (30.7%, *n* = 27) (Table 1; Supplementary Table 2). Of interest, 6 cases (6.8%) with *S. aureus* infection belonged to CC398 (1 MRSA and 5 MSSA).

##### Comparative Analysis According to Methicillin Susceptibility

The clinical characteristics of MRSA and methicillin-susceptible *S. aureus* (MSSA) cases were similar (Table 1; Supplementary Table 2) except for type of infection: MRSA more frequently presented as a chronic PJI. Consequently, PJIs caused by MSSA were more frequently acute and managed with DAIR than those caused by MRSA (72.1% vs 45.0%; *P* = .025).

From a microbiological perspective, MRSA isolates mostly belonged to CC5 (85%), while MSSA strains were distributed in 16 CCs, with CC30 being the most frequent (19.1%). MRSA strains formed more biofilm (OD 595 nm  $0.18 \pm 0.17$  vs  $0.11 \pm 0.07$ ; *P* = .001).

With respect to virulence genes, those with a significantly higher representation in MRSA isolates included the enterotoxins *seg*, *sei*, *sem*, *sen*, *seo*, and *seu*; the leukocidins *lukD*, *lukE*, and *lukY*; the serine proteases *splA* and *splB*; the staphylococcal exotoxin-like proteins *setB2* and *setB3*; capsule type 5 (*cap 5*); the microbial surface component-recognizing adhesive matrix molecules (MSCRAMMs) *fib*, *fnbB*, and *sasG*; and immunodominant surface antigen B (*isaB*). By contrast, the serine protease *splE*, collagen-binding adhesin (*cna*), and chemotaxis-inhibiting protein (*chp*) genes were significantly more frequent among MSSA isolates.

##### Comparative Analysis According to Clinical Presentation

Hematogenous infection occurred more frequently in immunocompromised patients, on knee prostheses, and showed more fever, bacteremia, and higher C-reactive protein (Table 1; Supplementary Table 2). Clonal diversity was also higher in hematogenous infection (19 isolates belonged to 13 different CCs) than in early postoperative (12 CCs for 45 isolates) or chronic infection (8 different CCs for 21 isolates). CC5 was less frequent in HI than in EPI and CPI (10.5%, 33.3%, and 38.1%, respectively). Interestingly, *S. aureus* isolates causing HI were less resistant to penicillin (68.4% vs 95.6%; *P* = .007) and less frequently harbored the *blaZ* (beta-lactamase) gene (73.7% vs 93.3%; *P* = .044).

In the subset of HIs, blood cultures were negative or positive in 10 and 9 cases, respectively. Overall, bacteremic and nonbacteremic cases were similar from a clinical and microbiological perspective (Supplementary Table 3), although nonbacteremic cases occurred relatively sooner (interquartile range) after placement of the prosthesis (1.26 [0.66–7.65] years vs 7.05 [2.91–21.4] years; *P* = .043) and the number of revision prostheses was higher (40% vs 11%; *P* > .05). No significant differences in *agr* functionality were detected, although

**Table 1. Case Series and Microbiological (Phenotypic and Genotypic) Description and Comparative Analysis According to Methicillin Susceptibility and Clinical Presentation of the Prosthetic Joint Infection**

Baseline features	All Cases (n = 88), No. (%)	MRSA (n = 20), No. (%)	MSSA (n = 68), No. (%)	P	Hematogenous Infection (n = 19), <sup>a</sup> No. (%)	Postsurgical <90 d (EPI) (n = 45), <sup>a</sup> No. (%)	Postsurgical >90 d (CPI) (n = 21), <sup>a</sup> No. (%)	P (HI vs EPI)	P (EPI vs CPI)	P (HI vs CPI)
Sex (men)	35 (39.8)	8 (40.0)	27 (39.7)	.981	8 (42.1)	15 (33.3)	11 (52.4)	.504	.140	.516
Age, y <sup>b</sup>	74.7 ± 14.1	79.8 ± 8.8	73.2 ± 15.0	.102	77.4 ± 13.9	74.2 ± 14.1	73.5 ± 15.3	.293	.962	.307
Diabetes mellitus	16 (18.2)	5 (25.0)	11 (16.2)	.509	4 (21.1)	8 (17.8)	3 (14.3)	.739	1.000	.689
Chronic renal impairment	14 (15.9)	3 (15.0)	11 (16.2)	1.000	4 (21.1)	6 (13.3)	4 (19.0)	.466	.714	1.000
Rheumatoid arthritis	7 (8.0)	2 (10.0)	5 (7.4)	.655	2 (10.5)	3 (6.7)	1 (4.8)	.629	1.000	.596
Prosthesis location (knee) <sup>c</sup>	40 (45.5)	8 (40.0)	32 (47.1)	.619	16 (84.2)	18 (40.0)	4 (19.0)	.002*	.160	<.001*
Revision prosthesis	22 (25.0)	4 (20.0)	18 (26.5)	.770	5 (26.3)	9 (20.0)	7 (33.3)	.742	.355	.736
Clinical presentation										
Hematogenous infection <sup>a</sup>	19 (21.6)	2 (10.0)	17 (25.0)	.220	-	-	-	-	-	-
Polymicrobial infection	14 (15.9)	3 (15.0)	11 (16.2)	1.000	0 (0.0)	8 (17.8)	6 (28.6)	.093	.346	.021*
Bacteremia	18 (20.5)	3 (15.0)	15 (22.1)	.753	9 (47.4)	6 (13.3)	2 (9.5)	.008*	1.000	.007*
Temperature >37°C	31 (35.2)	6 (30.0)	25 (36.8)	.578	11 (57.9)	16 (35.6)	3 (14.3)	.098	.075	.004*
Sinus tract	24 (27.3)	8 (40.0)	16 (23.5)	.146	0 (0.0)	7 (15.6)	16 (76.2)	.094	<.001*	<.001*
Leukocytes, ×10 <sup>9</sup> /L <sup>b,d</sup>	10.3 ± 6.3	7.6 ± 6.8	11.1 ± 5.9	.010*	11.4 ± 7.6	10.4 ± 5.4	9.0 ± 6.5	.397	.247	.124
C-reactive protein, mg/L <sup>b,d</sup>	140.4 ± 128.9	121.4 ± 127.1	145.8 ± 129.9	.367	244.8 ± 130.1	128.5 ± 122.3	75.0 ± 89.4	.002*	.096	<.001*
Surgical management										
DAIR <sup>g</sup>	58 (65.9)	9 (45.0)	49 (72.1)	.025*	16 (84.2)	33 (73.3)	8 (38.1)	.521	.006*	.003*
Antimicrobial resistance										
Oxacillin	20 (22.7)	-	-	-	2 (10.5)	9 (20.0)	7 (33.3)	.483	.239	.133
Levofloxacin	21 (23.9)	17 (85.0)	4 (5.9)	<.001*	3 (15.8)	9 (20.0)	8 (38.1)	1.000	.117	.115
Rifampin	3 (3.4)	1 (5.0)	2 (2.9)	.543	0 (0.0)	1 (2.2)	2 (9.5)	1.000	.236	.488
Vancomycin MIC ≥1.5 mg/L	11 (12.5)	6 (30.0)	5 (7.4)	.015*	2 (10.5)	1 (2.2)	7 (33.3)	.208	.001*	.133
Phenotypic characteristics										
<i>agr</i> functionality										
Negative	30 (34.1)	6 (30.0)	24 (35.3)		6 (31.6)	13 (28.9)	9 (42.9)			
Weak	24 (27.3)	4 (20.0)	20 (29.4)	.474	10 (52.6)	7 (15.6)	6 (28.6)	.003*	.117	.335
Strong	34 (38.6)	10 (50.0)	24 (35.3)		3 (15.8)	25 (55.6)	6 (28.6)			
β-hemolysis	77 (87.5)	20 (100)	57 (83.8)	.063	15 (78.9)	42 (93.3)	17 (81.0)	.182	.196	1.000
Biofilm formation, OD 595 nm <sup>h</sup>	0.12 ± 0.11	0.18 ± 0.17	0.11 ± 0.07	.001*	0.09 ± 0.04	0.13 ± 0.14	0.13 ± 0.07	.150	.117	.008*
Molecular epidemiology										
Clonal complex										
CC5	27 (30.7)	17 (85.0)	10 (14.7)		2 (10.5)	15 (33.3)	8 (38.1)			
CC15	7 (8.0)	0 (0.0)	7 (10.3)		1 (5.3)	4 (8.9)	2 (9.5)			
CC30	13 (14.8)	0 (0.0)	13 (19.1)	<.001*	3 (15.8)	5 (11.1)	5 (23.8)	.162	.605	.088
CC45	12 (13.6)	1 (5.0)	11 (16.2)		2 (10.5)	8 (17.8)	2 (9.5)			
Other <sup>e</sup>	29 (33.0)	2 (10.0)	27 (39.7)		11 (57.9)	13 (28.9)	4 (19.0)			

**Table 1. Continued**

agr group	All Cases (n = 88)		MRSA (n = 20)		MSSA (n = 68)		P	Hematogenous Infection (n = 19), <sup>a</sup> No. (%)		Postsurgical <90 d (EPI) (n = 45), <sup>a</sup> No. (%)		Postsurgical >90 d (CPI) (n = 21), <sup>a</sup> No. (%)		P (HI vs EPI)		P (EPI vs CPI)		P (HI vs CPI)		
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
agr I	26 (29.5)	2 (10.0)	24 (35.3)	8 (42.1)	13 (28.9)	4 (19.0)														
agr II	35 (39.8)	17 (85.0)	18 (26.5)	5 (26.3)	18 (40.0)	10 (47.6)	<.001*													.225
agr III	27 (30.7)	1 (5.0)	26 (38.2)	6 (31.6)	14 (31.1)	7 (33.3)														.685

Abbreviations: CC, clonal complex; CPI, chronic postoperative infection; DAIR, debridement, antibiotics, and implant retention; EPI, early postoperative infection; HI, hematogenous infection; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; OD, optical density.

<sup>a</sup>Three cases (2 infections caused by a contiguous suppurative focus and 1 positive intraoperative culture) were excluded from this comparison.

<sup>b</sup>Mean ± SD.

<sup>c</sup>There were 40 knee prostheses and 46 hip prostheses (14 hemiarthroplasties and 32 total hip replacements).

<sup>d</sup>Data obtained at diagnosis, before the performance of surgical treatment (either debridement or prosthesis removal).

<sup>e</sup>Other CCs in all cases (n = 88): 1 (1.1%) CC1, 2 (2.3%) CC6, 4 (4.5%) CC8, 2 (2.3%) CC9, 1 (1.1%) CC10, 5 (5.7%) CC22, 2 (2.3%) CC25, 1 (1.1%) CC188, 6 (6.8%) CC398, 3 (3.4%) CC509, 1 (1.1%) CC707, and 1 (1.1%) CC1021.

\*These results are statistically significant.

biofilm formation was observed to be slightly higher among nonbacteremic cases (OD 595 nm  $0.10 \pm 0.04$  vs  $0.07 \pm 0.01$ ;  $P = .133$ ). In the subset of patients with EPI, we compared the characteristics of patients with onset of symptoms in the first month after prosthesis placement and those with infection appearing between days 30 and 90. As shown in [Table 2](#) and [Supplementary Table 3](#), no clinical or microbiological differences were observed between the 2 groups.

Compared with acute infections (EPI and HI), CPI cases more frequently occurred on hip prostheses, were less inflammatory, and more frequently presented with a sinus tract. CPI isolates showed a higher vancomycin MIC than EPI (geometric mean: 1.01 vs 0.87 mg/L;  $P = .051$ ) and less frequently harbored the *blaZ* gene (71.4% vs 93.3%;  $P = .024$ ). There were fewer isolates in CPI with strong *agr* functionality than in EPI isolates (28.6% vs 55.6%;  $P = .041$ ). In postoperative infections, biofilm formation was similar, but higher than in strains causing HI.

### Comparative Analysis of Clinical and Microbiological Characteristics According to Outcome of Infection

Thirty-one failures (36.5%) were observed in the 85 patients (96.6%) whose outcome we were able to evaluate (follow-up  $1.26 \pm 0.6$  years) ([Table 3](#); [Supplementary Table 4](#)). Most cases with poor outcome were managed with DAIR compared with those with a good outcome (83.9% vs 53.7%;  $P = .005$ ). Among the former, failure was more frequent in patients with CPI and a longer delay to debridement. The use of rifampin and the exchange of removable components were associated with a good prognosis in patients treated with DAIR. A comprehensive comparison of phenotypic and genotypic microbiological characteristics showed no factors associated with failure, with the single exception of vancomycin MIC  $\geq 1.5$  mg/L, which was higher in cases in patients treated with DAIR with a poor outcome (23.1% vs 3.4%;  $P = .044$ ). There was also a trend toward greater presence of *S. aureus* belonging to CC5 (30.8% vs 13.8%;  $P = .192$ ) and *agr* type II (46.2% vs 24.1%;  $P = .099$ ) among the failures.

### Antimicrobial Susceptibility in Biofilm (CBD)

Biofilm susceptibility to antimicrobials was assessed in antibiotics administered for a significant period of time ( $\geq 14$  days in the first 30 days or  $\geq 21$  days over the whole treatment) in cases managed with DAIR. All strains were susceptible to daptomycin oxacillin, levofloxacin, and rifampin by MIC criteria. The MBEC distribution for these antimicrobials is represented in [Figure 1](#) and [Supplementary Table 5](#). MBEC<sub>50/90</sub> values for all antibiotics in monotherapy were  $>256.0$  mg/L, except for oxacillin (MBEC<sub>50</sub> 256.0 mg/L) and rifampin (MBEC<sub>50/90</sub> 128.0 and 256.0 mg/L, respectively). The addition of a fixed concentration of levofloxacin showed a nonsignificant reduction in the MBEC of rifampin ([Figures 1F](#) and [2](#)). Conversely, the addition of a fixed concentration of rifampin led to a nonsignificant

**Table 2. Comparative Analysis of Clinical and Microbiological (Phenotypic and Genotypic) Characteristics Between Prosthetic Joint Infections With Onset of Symptoms <30 Days After Surgery and Prosthetic Joint Infections With Onset of Symptoms in the 30–90 Days After Surgery**

	Postsurgical <30 d (n = 30), No. (%)	Postsurgical 30–90 d (n = 15), No. (%)	P
<b>Baseline features</b>			
Sex (men)	10 (33.3)	5 (33.3)	1.000
Age, mean ± SD, y	76.1 ± 12.8	70.4 ± 16.2	.201
Diabetes mellitus	4 (13.3)	4 (26.7)	.410
Chronic renal impairment	5 (16.7)	1 (6.7)	.647
Rheumatoid arthritis	2 (6.7)	1 (6.7)	1.000
Prosthesis location (knee)	10 (33.3)	8 (53.3)	.197
Prosthesis revision	6 (20.0)	3 (20.0)	1.000
<b>Clinical presentation</b>			
Polymicrobial infection	5 (16.7)	3 (20.0)	1.000
Bacteremia	4 (13.3)	2 (13.3)	1.000
Temperature >37°C	11 (36.7)	5 (33.3)	.826
Sinus tract	4 (13.3)	3 (20.0)	.670
Leukocytes, mean ± SD, <sup>a</sup> ×10 <sup>9</sup> /L	10.2 ± 5.4	10.7 ± 5.5	.779
C-reactive protein, mean ± SD, <sup>a</sup> mg/L	121.7 ± 120.4	140.5 ± 128.9	.864
<b>Surgical management</b>			
DAIR	21 (70.0)	12 (80.0)	.722
<b>Antimicrobial resistance</b>			
Oxacillin	5 (16.7)	4 (26.7)	.454
Levofloxacin	5 (16.7)	4 (26.7)	.454
Rifampin	1 (3.3)	0 (0.0)	1.000
Vancomycin MIC ≥1.5 mg/L	1 (3.3)	0 (0.0)	1.000
<b>Phenotypic characteristics</b>			
<i>agr</i> functionality			
Negative	9 (30.0)	4 (26.7)	
Weak	5 (16.7)	2 (13.3)	1.000
Strong	16 (53.3)	9 (60.0)	
β-hemolysis	27 (90.0)	15 (100.0)	.540
Biofilm formation, mean ± SD, OD 595 nm	0.13 ± 0.15	0.13 ± 0.09	.682
<b>Molecular epidemiology</b>			
<b>Clonal complex</b>			
CC5	9 (30.0)	6 (40.0)	
CC15	2 (6.7)	2 (13.3)	
CC30	5 (16.7)	0 (0.0)	.462
CC45	6 (20.0)	2 (13.3)	
Other	8 (26.7)	5 (33.3)	
<b><i>agr</i> group</b>			
<i>agr I</i>	9 (30.0)	4 (26.7)	
<i>agr II</i>	10 (33.3)	8 (53.3)	.381
<i>agr III</i>	11 (36.7)	3 (20.0)	

Abbreviations: CC, clonal complex; DAIR, debridement, antibiotics, and implant retention; MIC, minimum inhibitory concentration; OD, optical density.

<sup>a</sup>Data obtained at diagnosis, before the performance of surgical treatment (either debridement or prosthesis removal).

increase in the MBEC of levofloxacin (Figure 1D). Overall, we found no association between MBEC values and clinical outcome (Supplementary Table 6).

## DISCUSSION

In this prospective multicenter study, we explored in some detail the relationships between clinical and microbiological (phenotypic and genotypic) features of staphylococcal PJI beyond species and the antibiogram that could lead to a better understanding of pathogenesis and prognosis.

We observed a high clinical, microbiological, and genetic diversity of *S. aureus* causing PJI. Sixteen different staphylococcal CCs were observed in our cohort, with CC5 being the most frequent [6, 18–20]. The frequency of MRSA in our study was similar to other studies [17, 21, 22]. The genetic backgrounds of MSSA and MRSA are known to be different [23], as is reflected in the distribution of genes. Remarkably, MRSA exhibited a higher biofilm-producing ability. Nevertheless, we found few clinical differences between MRSA and MSSA infections, except for a lower frequency of hematogenous acquisition among the former [17]. Indeed, MRSA PJI is fundamentally a

**Table 3. Comparative Analysis of Clinical and Microbiological (Phenotypic and Genotypic) Characteristics According to the Outcome of the Prosthetic Joint Infection**

	All (n = 85)			Managed With DAIR (n = 55)			Managed With Prosthesis Removal (n = 30)		
	Failure (n = 31), No. (%)	Cure (n = 54), No. (%)	P	Fail (n = 26), No. (%)	Cure (n = 29), No. (%)	P	Fail (n = 5), No. (%)	Cure (n = 25), No. (%)	P
<b>Baseline features</b>									
Sex (men)	15 (48.4)	19 (35.2)	.232	10 (38.5)	10 (34.5)	.759	5 (100.0)	9 (36.0)	.014*
Age, mean ± SD, y	76.3 ± 13.1	73.4 ± 14.9	.298	75.6 ± 13.8	73.5 ± 14.3	.320	80.2 ± 8.8	73.4 ± 15.9	.627
Diabetes mellitus	5 (16.1)	10 (18.5)	.781	5 (19.2)	6 (20.7)	.893	0 (0.0)	4 (16.0)	1.000
Chronic renal impairment	5 (16.1)	8 (14.8)	1.000	3 (11.5)	4 (13.8)	1.000	2 (40.0)	4 (16.0)	.254
Rheumatoid arthritis	2 (6.5)	5 (9.3)	1.000	1 (3.8)	1 (3.4)	1.000	1 (20.0)	4 (16.0)	1.000
Prosthesis location (knee)	14 (45.2)	24 (44.4)	1.000	13 (50.0)	18 (62.1)	.422	1 (20.0)	6 (24.0)	1.000
Prosthesis revision	4 (12.9)	18 (33.3)	.043*	4 (15.4)	8 (27.6)	.339	0 (0.0)	10 (40.0)	.140
<b>Clinical presentation</b>									
Hematogenous infection	9 (29.0)	9 (16.7)	.269	8 (30.8)	7 (24.1)	.763	1 (20.0)	2 (8.0)	.433
Polymicrobial infection	3 (9.7)	10 (18.5)	.358	2 (7.7)	6 (20.7)	.257	1 (20.0)	4 (16.0)	1.000
Bacteremia	8 (25.8)	9 (16.7)	.311	7 (26.9)	4 (13.8)	.224	1 (20.0)	5 (20.0)	1.000
Temperature >37°C	13 (41.9)	18 (33.3)	.428	12 (46.2)	13 (44.8)	.921	1 (20.0)	5 (20.0)	1.000
Sinus tract	9 (29.0)	15 (27.8)	.902	6 (23.1)	5 (17.2)	.589	3 (60.0)	10 (40.0)	.628
Leukocytes, mean ± SD, <sup>a</sup> × 10 <sup>9</sup> /L	10.4 ± 5.0	12.3 ± 5.6	.106	10.8 ± 5.3	12.0 ± 5.0	.348	8.8 ± 2.7	12.8 ± 6.2	.162
C-reactive protein, mean ± SD, <sup>a</sup> mg/L	161 ± 139	122 ± 122	.219	152 ± 132	168 ± 148	.957	207 ± 181	71 ± 52	.245
<b>Surgical management</b>									
DAIR	26 (83.9)	29 (53.7)	.005*	-	-	-	-	-	-
Time to debridement, median (IQR), d	-	-	-	10 (6-24)	5 (2-10)	.001*	-	-	-
Polyethylene exchange	-	-	-	15 (57.7)	22 (75.9)	.152	-	-	-
Need for ≥2 debridements	-	-	-	2 (7.7)	6 (20.7)	.257	-	-	-
Rifampin ≥14 d <sup>b</sup>	-	-	-	12 (57.0)	25 (86.0)	.021*	-	-	-
Prosthesis removal	5 (16.1)	25 (46.3)	.005*	-	-	-	-	-	-
Antibiotic-loaded spacer	-	-	-	-	-	-	1/3 (33.3)	11/14 (78.6)	.191
<b>Antimicrobial resistance</b>									
Oxacillin	7 (22.6)	12 (22.2)	.970	4 (15.4)	4 (13.8)	1.000	3 (60.0)	8 (32.0)	.327
Levofloxacin	9 (29.0)	11 (20.4)	.365	5 (19.2)	3 (10.3)	.455	4 (80.0)	8 (32.0)	.128
Rifampin	1 (3.2)	2 (3.7)	1.000	1 (3.8)	0 (0.0)	.473	0 (0.0)	2 (8.0)	1.000
Vancomycin MIC ≥1.5 mg/L	7 (22.6)	4 (7.4)	.089	6 (23.1)	1 (3.4)	.044*	1 (20.0)	3 (12.0)	.538
<b>Phenotypic characteristics</b>									
<i>agr</i> functionality									
Negative	9 (29.0)	20 (37.0)	-	8 (30.8)	11 (37.9)	-	1 (20.0)	9 (36.0)	-
Weak	8 (25.8)	16 (29.6)	.548	7 (26.9)	6 (20.7)	.806	1 (20.0)	10 (40.0)	.392
Strong	14 (45.2)	18 (33.3)	-	11 (42.3)	12 (41.4)	-	3 (60.0)	6 (24.0)	-
β-hemolysis	28 (90.3)	47 (87.0)	.740	23 (88.5)	25 (86.2)	1.000	5 (100.0)	22 (88.0)	1.000
Biofilm formation, mean ± SD, OD 595 nm	0.12 ± 0.07	0.12 ± 0.12	.373	0.13 ± 0.07	0.13 ± 0.16	.231	0.10 ± 0.03	0.12 ± 0.07	.957
<b>Molecular epidemiology</b>									
Clonal complex									
CC5	10 (32.3)	16 (29.6)	-	8 (30.8)	4 (13.8)	-	2 (40.0)	12 (48.0)	-
CC15	4 (12.9)	3 (5.6)	-	3 (11.5)	2 (6.9)	-	1 (20.0)	1 (4.0)	-

**Table 3. Continued**

	All (n = 85)			Managed With DAIR (n = 55)			Managed With Prosthesis Removal (n = 30)		
	Failure (n = 31), No. (%)	Cure (n = 54), No. (%)	P	Fail (n = 26), No. (%)	Cure (n = 29), No. (%)	P	Fail (n = 5), No. (%)	Cure (n = 25), No. (%)	P
CC30	4 (12.9)	8 (14.8)	.574	4 (15.4)	5 (17.2)	.276	0 (0.0)	3 (12.0)	.625
CC45	2 (6.5)	9 (16.7)		1 (3.8)	6 (20.7)		1 (20.0)	3 (12.0)	
Other	11 (35.5)	18 (33.3)		10 (38.5)	12 (41.4)		1 (20.0)	6 (24.0)	
<i>agr</i> group									
<i>agr I</i>	7 (22.6)	18 (33.3)		5 (19.2)	10 (34.5)		2 (40.0)	8 (32.0)	
<i>agr II</i>	15 (48.4)	19 (35.2)	.434	12 (46.2)	7 (24.1)	.196	3 (60.0)	12 (48.0)	.675
<i>agr III</i>	9 (29.0)	17 (31.5)		9 (34.6)	12 (41.4)		0 (0.0)	5 (20.0)	

Three patients had unknown outcomes.

Abbreviations: CC, clonal complex; DAIR, debridement, antibiotics, and implant retention; IQR, interquartile range; MIC, minimum inhibitory concentration; OD, optical density.

<sup>a</sup>Data obtained at diagnosis, before the performance of surgical treatment (either debridement or prosthesis removal).

<sup>b</sup>Calculated for patients not failing in the first 30 days of treatment.

\*These results are statistically significant.

postoperative phenomenon, as is illustrated by its greater CC homogeneity (85% CC5), which is consistent with hospital-related source of acquisition [18].

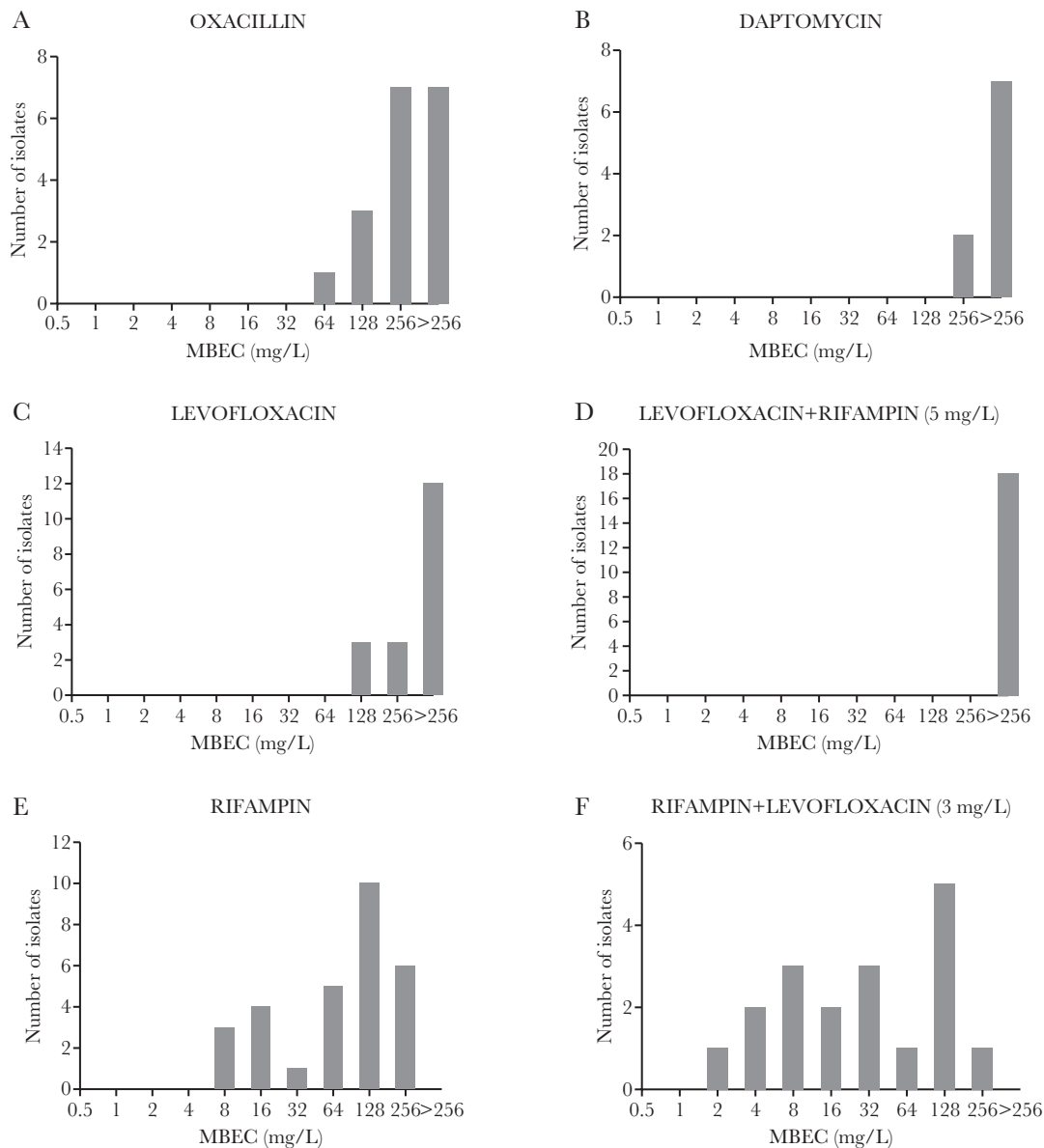
As expected, the clinical presentation of staphylococcal PJI showed a predominance of acute-onset forms (EPI and HI) [1, 2]. Although we were unable to draw firm conclusions, the different types of infection may be partly explained by the different *agr* functionality, which is less functional in CPI cases. In acute presentations, *agr* generally enhances pathogenesis by increasing the expression of aggressive virulence determinants. In contrast, dysfunctional *agr* has a more complex role in chronic infections, leading to biofilm formation [5]. There is also a virulence tradeoff in favor of antibiotic resistance, which was most frequent in our cases of CPI [24].

A comparison of bacteremic and nonbacteremic HI could shed some light on the pathogenesis of this type of PJI. We observed more revision prostheses among nonbacteremic cases (which are at a higher risk of infection during surgery [25]), it took less time to develop infection, and strains had a higher biofilm-forming ability. Although the absence of bacteremia does not rule out a hematogenous route of infection, it may suggest that some of them were the result of reactivation of a long-term latent inoculum of staphylococci that most likely reached the prosthesis at the time of prosthesis placement. Staphylococcal reactivation in bone tissue is indeed a well-described phenomenon in osteomyelitis [26]. In this connection, some authors have suggested the term “late-acute infection” in order to include any type of PJI presenting as an acute infection at some point after prosthesis placement [27].

With respect to pathogenesis, we observed no significant differences in the EPI group between cases with onset of symptoms <30 days after the index surgery and those with symptoms beginning between days 30 and 90. This is worth highlighting because the time limits defining EPI and CPI are sometimes arbitrary and have changed over time [1–3, 28], and labeling patients with 1 type of PJI or another has direct implications for surgical management and the possibility of performing DAIR [1–3, 10]. Our results are consistent with the similar prognosis for cases managed by DAIR reported elsewhere [17].

The failure rate observed was notable (37% overall, 47% for cases managed with DAIR), but comparable to previous reports [17, 22]. In spite of a thorough analysis of multiple genes and phenotypic microbiological features, we did not find any factor that could be related to the patient’s clinical outcome. Although some reports have associated MRSA infection with an increased risk of failure [6, 29, 30], this has been contested by others [17], and we did not observe a worse prognosis among MRSA infections. Overall, PJI is a very complex infection, in which specific microbiological features may be diluted in the sophisticated interplay of host, surgical, foreign body, and therapeutic factors [31]. Nevertheless, we observed a slightly higher vancomycin





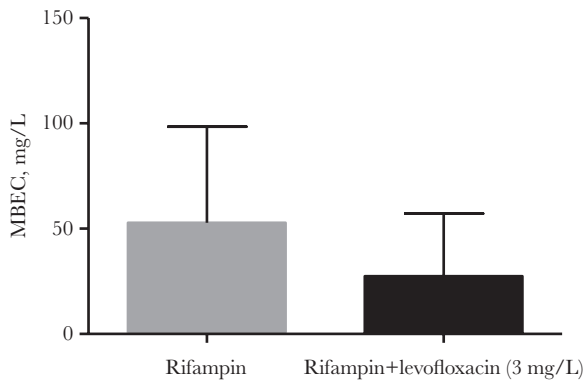
**Figure 1.** Distribution of minimal biofilm eradication concentration (MBEC) for oxacillin (A), daptomycin (B), levofloxacin (monotherapy) (C), levofloxacin in combination with a fixed concentration of 5 mg/L of rifampin (D), rifampin (monotherapy) (E), and rifampin in combination with a fixed concentration of 3 mg/L of levofloxacin (F).

MIC in cases with unfavorable outcomes. Although the performance of the vancomycin E-test can produce significant variability [32], a higher vancomycin MIC has been associated with *agr* dysfunction and biofilm-associated complications [33].

Our results agree with a recent study by Wildeman et al. [34], who observed an association between an antibiotic resistance phenotype, use of non-biofilm-active antimicrobial treatment, and failure, but did not find any association between genetic traits and outcomes of patients with PJIs caused by *S. aureus*.

As previously mentioned, the standard parameters of antibiotic susceptibility such as MIC show a poor correlation with outcome [1, 35]. There is a need for standardized susceptibility

methods for biofilm-associated infections [36–38]. The CBD has been used previously in the setting of PJI [9], although, as far as we know, this is the first study to address the possible correlation between a specific antibiotic MBEC and the clinical results. Overall, the MBECs of oxacillin, daptomycin, and levofloxacin were higher than clinically achievable concentrations and did not show a correlation with patient outcome. As expected, rifampin showed the lowest MBECs, and hence the highest biofilm activity [39], which is consistent with the positive clinical results reported in the literature [17, 22, 28]. Still, the specific rifampin MBEC values were very high and did not show a correlation with outcome. We also explored the MBEC



**Figure 2.** Geometric mean (95% CI) in all cases of rifampin minimal biofilm eradication concentration (MBEC) for rifampin monotherapy and in combination with a fixed concentration of 3 mg/L of levofloxacin.

of combinations by adding fixed clinically relevant concentrations of levofloxacin to rifampin, and vice versa, although the results were no better than the monotherapies. There may be several reasons for the lack of correlation between MBEC and outcome: there may be PK/PD factors that influence bone and biofilm antimicrobial activity, such as the postantibiotic effect or the accumulation of antibiotics intracellularly and in biofilms [37, 40]. More research is needed to standardize the activity of antibiotics against biofilm in this clinical scenario.

Our study has some limitations. First, although the number of cases is not small compared with other series, the sample size and comparisons between different strata do not allow us to draw definitive conclusions. Nor can we rule out that some of the associations observed were caused by chance, as the number of comparisons was many. Second, although our study included a wide range of molecular markers of *S. aureus*, the presence of a given gene does not necessarily imply a specific protein product or cell function. Further phenotypic and transcriptomic studies are needed to achieve a better understanding of the influence of virulence factors of *S. aureus* on the evolution of PJI.

To conclude, despite a thorough clinical, microbiological, and molecular analysis of staphylococcal PJI, we have not found significant phenotypic or genotypic parameters that may account for the clinical presentation or prognosis of the infection, including the MBEC.

### Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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