Molecular Characterization of an Early Invasive *Staphylococcus epidermidis* Prosthetic Joint Infection

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Historically regarded as a skin commensal, *Staphylococcus epidermidis* has been increasingly implicated in invasive foreign body infections such as catheter-related bloodstream infections, indwelling device infections, and prosthetic joint infections. We report a case of an aggressive, difficult-to-eradicate, invasive prosthetic hip infection occurring early after hardware implant and associated with a high-grade bacteremia and assess its salient molecular characteristics. The clinical and molecular characteristics of this isolate mirror the pathogenesis and persistence commonly seen with invasive methicillin-resistant *S. aureus* and may be attributed to the combination of resistance genes (SCCmec type IV), putative virulence factors (*arcA* and *opp3a*), cytolytic peptide production (α -type phenol-soluble modulins), and biofilm adhesion, interaction, and maturation (*bhp*, *aap*, and β -type phenol-soluble modulins).

Introduction

THE MOST COMMON GRAM-POSITIVE PATHOGENS, accounting for more than half of the deep prosthetic joint infections (PJIs), are methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and *Staphylococcus epidermidis*.¹ Initial four-category classification schemes divided infections into intraoperative positive cultures with revisions, early postoperative infections occurring <1 month postoperatively, late/chronic infections >1 month postoperatively, and acute hematogenous infection.^{5,17} Other classification schemes define early onset as less than 2 years and late onset as greater than 2 years post-hip arthroplasty.¹⁴ Early infections operatively acquired present with fever, joint pain, erythema, effusion, and warmth at the surgical site; the organisms isolated are usually virulent pathogens such as *S. aureus* and Gram-negative bacilli. Late PJIs may be hematogenously acquired at any point or may be caused by slower-growing, less-virulent organisms, which may take longer time to manifest clinical signs, such as joint pain or prosthesis loosening. These are classically attributed to coagulase-negative staphylococci and *Propionibacterium acnes*.²³

Generally viewed as an opportunistic pathogen, *S. epidermidis* has a biofilm-forming capacity, allowing it to elaborate an ecological niche and colonize biomaterials such as indwelling catheters, prosthetic heart valves, and other implanted devices. Adhesion factors, such as biofilm associated-homologous protein (*bhp*) and intercellular adhesin locus (*ica*) composed of the genes *icaA*, *icaD*, *icaB*, and *icaC*, which code for polysaccharide intercellular adhesin (PIA), play a putative role in the ability of *S. epidermidis* to persist, leading to difficulty in its eradication. Activation of the *ica* locus results in PIA synthesis and thick biofilm production. This can be inactivated by insertion sequences, specifically IS256, which reversibly disrupts the *ica* locus, leading to a phenotypic switch from biofilm-positive to -negative organisms.²² In *ica*-negative strains, surface proteins such as the accumulation-associated protein (*aap*) have been shown to play a primary role in biofilm formation.⁴

mecA is harbored on a mobile staphylococcal cassette chromosome (SCC) and encodes for an altered penicillin-binding protein (PBP2') that imparts resistance to methicillin and other β -lactam antibiotics.⁹ Neighboring the SCCmec element on the *S. epidermidis* chromosome is a large operon, the arginine catabolic mobile element (ACME), a virulence

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determinant encoding arginine deaminase pathway genes, including *arcA* and oligopeptide permease (*opp3a*).⁶ The SCCmec-ACME association, previously described in USA300 *S. aureus*, was also noted in *S. epidermidis* and postulated to confer a selective survival advantage.⁶

Additionally, we investigated the expression of phenol-soluble modulins (PSMs), which have been implicated in enhanced *S. aureus* and *S. epidermidis* virulence through leukocyte destruction.^{3,18} *S. epidermidis* produces several PSMs that share similarity with those found in *S. aureus*.³ The β -type PSMs of *S. epidermidis* have been implicated in biofilm maturation and the dissemination of biofilm-associated infection,¹⁹ whereas the frequently cytolytic α -type PSMs, such as δ -toxin, are commonly produced at lower levels in *S. epidermidis* compared with *S. aureus*.³

We hypothesize that a combination of molecular determinants imparts a selective advantage, allowing certain strains of *S. epidermidis* to become important clinical pathogens, as typified by the following case. Using this acute, rapidly progressive early prosthetic hip infection with associated high-grade bacteremia as a representation of an invasive *S. epidermidis* infection, we sought to determine the combination of molecular characteristics of this isolate and further define associated genetic markers of this early invasive and persistent organism, which behaves like an aggressive *Staphylococcus aureus*. In addition to molecular typing methods, including SCCmec analysis, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST), we assessed the presence of adhesion factors and mobile elements *blp*, *icaA*, IS256, IS257, and ACME as well as PSM production and the biofilm formation capacity of this organism.

Case report

A 60-year-old diabetic woman sustained a femoral neck fracture necessitating a left hip hemiarthroplasty. Two weeks postprocedure, she developed purulent drainage from the surgical site, requiring incision and drainage of the wound and treatment for 4 days with an unknown oral antibiotic, presumed to be a fluoroquinolone. She was subsequently hospitalized with fever, chills, increasing pain, erythema, and extensive drainage at the incision at 4 weeks after initial hemiarthroplasty.

On admission, she was febrile with a temperature of 38.2°C and appeared acutely ill. Purulent material was draining from the surgical wound, and erythema, warmth, and tenderness were present over the lateral hip. Laboratory tests revealed a leukocyte count of 14.6×10^9 cells/L, 83% neutrophils, and a serum blood glucose of 368 mg/dl, consistent with infection. Hip radiograph showed a well-fixed cemented hemiarthroplasty.

Extensive surgical debridement and modular component exchange were done. Copious purulent periprosthetic material noted intraoperatively was sent for microbiological culture. Four intraoperative tissue cultures and two sets of blood cultures (four of four bottles) grew methicillin-resistant *S. epidermidis* (MRSE). Intravenous vancomycin and oral rifampin were initiated after cultures were taken. After 8 days, therapy was switched to intravenous linezolid because of worsening renal insufficiency. A transthoracic echocardiogram showed no valvular abnormalities or vegetations, and the patient completed 6 weeks of oral linezolid as an outpa-

tient. Five months after the debridement, she continued to have pain and underwent resection arthroplasty with placement of a temporary high-dose antibiotic-loaded cement spacer. Screening preoperative nasal and axillary cultures grew out methicillin-susceptible *S. epidermidis* and all four intraoperative tissue cultures grew out methicillin-resistant *S. epidermidis*, with the same antibiogram as the original isolates. The patient was subsequently treated with vancomycin, awaiting joint re-implantation.

Materials and Methods

Two sets of blood cultures from admission and four tissue cultures were collected intraoperatively at the time of initial incision and drainage, and four repeat intraoperative cultures were obtained during the revision surgery. As per routine, all microbiology cultures were processed according to Clinical and Laboratory Standards Institute (CLSI) procedures and MICs were determined by Vitek2 automated system (bioMérieux, Durham, NC).

Pulsed-field gel electrophoresis following an established protocol was performed on the four initial incision and drainage cultures, two blood cultures, four revision arthroplasty cultures, and the nasal and axillary swab cultures using *SmaI*.¹³ These strains and the reference strains American Type Culture Collection (ATCC) 35984, that is, RP62A, and ATCC 12228 were analyzed by the MLST protocol as previously published.¹⁶

Biofilm assay was performed according to previously published techniques in a 96-well microtiter plate with the following changes to the protocol.¹⁰ Two blood culture isolates, eight intraoperative cultures, four from the first incision and drainage and four from the revision arthroplasty, and positive (RP62A) and negative controls (trypticase soy broth [TSB]) were grown overnight in a 37°C incubator on 5% Trypticase® Soy Agar with 5% sheep blood (BD, Sparks, MD). One loopful (10^{7-10}) was added to 100 μ l of 900 ml TSB with 6 g yeast extract and 8 g glucose per liter and vortexed gently to suspend. Bacterial suspension was diluted 1:1000, and four duplicate 200 μ l samples were added to a 96-well tissue culture-treated polystyrene plates (Corning Costar, Corning, NY) and incubated at 37°C overnight in air. To stain for biofilm production, nonadherent cells were washed in cold water by immersing the plate in a basin and tapping off loose cells. Two hundred microliters of crystal violet was added to each well for 60 seconds and washed by immersing in cold water 10 times. The crystal violet was solubilized with the addition of 100 μ l ethanol for 10 minutes. After allowing to air dry for 30 minutes, the optical density was measured at OD_{595 nm} (VersaMax; Molecular Devices, Sunnyvale, CA).

SCCmec analysis was performed using our recently developed multiplex molecular beacon assay that distinguishes SCCmec types I-IV.² Multiplex PCR for ACME (*arcA* and *opp3*) and Pantone-Valentine leukocidin (PVL) was performed in 30 μ l reaction mixtures with Denville Taq® reaction mix. DNA amplification was performed on a Biogene automated thermocycler using the following conditions: 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 60 seconds, and 72°C for 90 seconds, and a final elongation step at 72°C for 5 minutes. Primer sequences used are as follows: *arcA*: 5'-GAGCCAGAAGTACGCGAG-3', 5'-CACGTAACCTTGCTAGAACGAG-3'; *opp3A*: 5'-AAAAGC

GATTGGAACAGGTG-3', 5'-TGACGTTGCACTTTTTCAG C-3'); and *lukF*: 5'GCCAGTGTTATCCAGAGG-3', 5'CTATCC AGTTGAAGTTGATCC-3'. The PCR products were run for 40 minutes at 110 mV on a 1% agarose gel and stained with ethidium bromide.

Conventional PCR for *bhp*, IS256, IS257, and 16s RNA for internal positive controls was performed using previously published primer sequences.⁸ Conventional PCR and southern blotting for *ica* were completed according to previously published methods.¹¹ All clinical isolates were compared with biofilm-producing reference strain RP62A (ATCC 35984) and non-biofilm-producing strain ATCC 12228 (Manassas, VA). The PCR for *aap* was performed according to previously published literature.¹²

Cultures in which PSM production has to be determined were inoculated from precultures grown overnight at 1:100 and grown to stationary growth phase (8 hours). One hundred microliters of culture filtrates were used to measure PSMs by reversed-phase high-pressure chromatography/electrospray ion mass spectrometry (HPLC/MS) on an Agilent 1100 system coupled to a Trap SL mass spectrometer using a water/acetonitrile gradient, as previously described.²⁰ PSM production was quantified by calculating extracted ion chromatograms for the major *m/z* peak of each PSM, using Agilent Quant Analysis software. PSM production in strain ATCC12228 was set to 100%.³ Measurements were performed using three independent cultures for each strain.

Results

The PFGE pattern demonstrated that the *S. epidermidis* from intraoperative hip cultures, both from the initial surgery and the revision arthroplasty, were identical to the two

FIG. 1. PFGE of *Staphylococcus Epidermidis*. Lane designation: L: ladder; 1: RP62A; 2: blood isolate; 3: blood isolate; 4-7: tissue isolate from first surgery; 8-11: tissue isolate from second surgery; 12: nares; 13: axilla.

FIG. 2. *S. epidermidis* eBURST (based on repeated sequence types) demonstrating relatedness between ATCC 12228, RP62A, and clinical isolates (accessed February 9, 2011; <http://sepidermidis.mlst.net/eburst/database.asp>).

FIG. 3. Phenol-soluble modulins (PSM) production of RP62A and clinical isolates (1–2: blood isolates; 3–6: tissue isolates) versus ATCC 12228, standardized to 100%; all were statistically significant ($p < 0.05$).

blood isolates using *Sma*I restriction enzymes, but different from the axillary and nasal isolates as well as the reference strain (Fig. 1). Clinical isolates from the hip and bloodstream were susceptible to rifampin (MIC <1), tetracycline (MIC <4), trimethoprim/sulfamethoxazole (MIC $<0.5/9.5$), linezolid (MIC = 2), daptomycin (MIC <0.5), and vancomycin

(MIC <2 mg/L), resistant to nafcillin (MIC >2), amoxicillin/clavulanate (MIC $>4/2$), cefazolin (MIC >8), and macrolides (MIC >4), and intermediate to levofloxacin (MIC = 4).

MLST sequencing identified clinical isolates as sequence type (ST) 5, a double locus variant of the ST10 biofilm-producing RP62A, whereas reference strain ATCC 12228 belonged to ST8 (Fig. 2). SCCmec typing showed that the clinical isolates contained SCCmec IV, compared with RP62A, which contains SCCmec II. The PCR results demonstrated that the invasive clinical isolates carried ACME (*arcA* and *opp3*), but not PVL. Conventional PCR for virulence and persistence factors demonstrated that the clinical isolates and RP62A control possess biofilm-associated homologous protein (*bhp*) and IS257. IS256 and *ica* were present in the biofilm producer RP62A. Interestingly, our clinical strains, although possessing the *bhp* locus, were not biofilm positive in our assay (data not shown). *Aap* was present in RP62A, ATCC 12228, all blood and introperative cultures, and the axilla screen, but was negative in the nasal screen *S. epidermidis* isolate (PCR not shown). The clinical isolates showed an overall stronger production of PSMs when compared with the biofilm-producing strain RP62A and nonbiofilm producer ATCC 12228, as shown for the most strongly produced δ -toxin and β -type PSMs (Fig. 3). Compared with the non-biofilm producer ATCC 12228, the biofilm producer RP62A demonstrated a reduced delta toxin (PSM γ) production (41.9%) as well as a reduction in PSM- β 1 (41.0%) and PSM- β 2 (22.0%) toxins. The clinical isolates (two blood cultures

TABLE 1. PHENOL-SOLUBLE MODULIN PRODUCTION DURING *IN VITRO* GROWTH (8 HOURS) DETERMINED BY HPLC/MS

Comparison of clinical isolates (singly) versus RP62A			
(one-way ANOVA, Dunnett's posttest):			
Clinical samples	β 1	β 2	δ Toxin
All (1–6)	***	***	***
Comparison of clinical isolates (singly) versus ATCC 12228			
(one-way ANOVA, Dunnett's posttest)			
	β 1	β 2	δ Toxin
1	*	ns	ns
2	ns	ns	ns
3	ns	ns	*
4	***	ns	***
5	ns	*	ns
6	**	**	**
Comparison of all clinical isolate data averaged to RP62A and ATCC 12228			
(one-way ANOVA, Bonferroni posttest)			
	β 1	β 2	δ Toxin
RP vs. ATCC	*	**	*
RP vs. clinical	***	***	***
ATCC vs. clinical	*	*	**

Production is shown relative to the strain ATCC 12228. Statistical evaluation was performed on raw data (clinical samples 1–2: blood isolates; 3–6: tissue isolates) as follows: Single clinical isolates were compared with RP62A or ATCC 12228, respectively, by one-way ANOVA and Dunnett's posttest. Averaged data for clinical isolates were compared with RP62A or ATCC 12228 by one-way ANOVA with Bonferroni posttest.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

ns, non-significant.

and primary incision and drainage cultures) demonstrated an increased production of δ -toxin, PSM- β 1, and PSM- β 2 production when compared with RP62A, (Table 1) whereas the production compared with ATCC 12228 was significant only for some isolates and when values for all isolates were averaged (Fig. 3).

Discussion

Adhesion, controlled by polysaccharide intercellular adhesin (PIA), is encoded by the *icaADBC* locus and associated with insertion sequence (IS) 256 through phase variation of biofilm formation, which alters the expression of *ica*.¹¹ This insertion sequence was reported to be a more sensitive molecular marker of invasiveness than *ica*; however, conflicting evidence has been presented as to the role of *ica* in *S. epidermidis*.^{8,11} In strains lacking the *icaADBC* locus, biofilm formation is mediated by the biofilm-associated protein or biofilm homologous protein (*bap*, *bhp*) or the accumulation-associated protein (*aap*).¹⁵ Although our clinical infecting strain contained *bhp* and *aap*, it was not associated with a biofilm-positive phenotype by standard crystal violet assay, raising the speculation that the expression of the biofilm homologous protein is downregulated, favoring detachment and dissemination, consistent with the clinical presentation of our device infection associated with a high-grade bacteremia. The ACME gene cluster, generally associated with MRSA USA300, was identified on the chromosome of *S. epidermidis* ATCC 12228, but not in biofilm-producing RP62A (ATCC 35984).^{6,7} Our non-biofilm-producing clinical isolates, although evolutionarily more closely related to RP62A, contain both *arcA* and *opp3A* genes, suggesting that ACME may act as a key virulence factor in non-biofilm-producing *S. epidermidis* as well.

PSMs are a group of pro-inflammatory and cytolytic peptides with an additional function in biofilm development.^{19,21} Absence of PSMs is believed to result in more pronounced biofilm formation, likely owing to abolished biofilm detachment, which in *S. epidermidis* is facilitated by β -type PSMs. In contrast particularly to the biofilm former RP62A, the clinical strains showed pronounced production of both cytolytic α -type and biofilm-active β -type PSMs, in keeping with our *in vitro* and clinical observations of low biofilm-forming capacity and strong bacteremia caused by those strains.

The clinical presentation of an early PJI described herein, caused by a phenotypically non-biofilm-producing methicillin-resistant ST5 *S. epidermidis*, is evolutionarily related to the classic biofilm producer RP62A. Our ACME *arcA*- and *opp3A*-containing clinical strain caused a clinical syndrome comparable to infections commonly encountered with invasive USA300 methicillin-resistant *S. aureus*. We hypothesize that this combination of virulence determinants, resistance factors, and the appropriate clinical setting allowed for attachment, invasion, and proliferation of an otherwise commensal organism. Although *S. epidermidis* is frequently discounted as an invasive pathogen, our representative case report and molecular characterization demonstrate that with acquisition of mobile genetic elements and under appropriate conditions, *S. epidermidis* can become aggressive early in a prosthetic joint infection, even in the absence of biofilm formation. This enhanced bacterial virulence and persistence superimposed on a background of antimicrobial resistance and medication intolerance not only limited anti-infective treatment op-

tions, but also initiated an infection difficult to eradicate with invasive intervention. As seen with this clinical example, a heightened clinical suspicion and aggressive treatment course may be warranted when encountering an otherwise "low-virulence" organism.

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Disclosure Statement

No competing financial interests exist.

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