icaA Is Not a Useful Diagnostic Marker for Prosthetic Joint Infection

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A collection of 99 staphylococcal isolates associated with prosthetic joint infection and 23 coagulase-negative staphylococci isolated from noninfected arthroplasty-associated specimens were screened in order to determine whether the presence of icaA could be used to distinguish between pathogens and nonpathogens. All Staphylococcus aureus prosthetic joint infection isolates (n = 55) were icaA positive. A total of 46% (20 out of 44) of coagulase-negative staphylococcal prosthetic joint infection isolates were icaA positive, and 30% (7 out of 23) of arthroplasty-associated non-prosthetic joint infection-associated coagulase-negative staphylococcal isolates were icaA positive (P = 0.23). Certain coagulase-negative Staphylococcus species appeared more likely to be isolated as either arthroplasty-associated non-prosthetic joint infection-associated isolates (e.g., Staphylococcus warneri and Staphylococcus hominis) or pathogens (e.g., Staphylococcus lugdunensis). The presence of icaA in a coagulase-negative staphylococcal isolate associated with an arthroplasty is not a useful diagnostic indicator of pathogenicity.

Despite the overwhelmingly high surgical success rate of prosthetic joint implantation, prosthetic joint infection (PJI) contributes significantly to arthroplasty failure (30). Prosthesis colonization may occur either at the time of surgery or postoperatively; in the former case, implantation provides an opportunity to introduce opportunistic skin commensals, particularly coagulase-negative staphylococci (CNS), to the joint (30). Not surprisingly, staphylococci are the most frequently isolated etiologic agents of PJI (8, 10, 30). Unfortunately, the microbiologic diagnosis of PJI is frequently confounded by contamination of joint tissue and/or fluid specimens with normal skin flora (often CNS) at the time of specimen collection or during laboratory processing. A marker to distinguish CNS isolated as contaminants from those isolated as pathogens from joint tissue and/or fluid associated with arthroplasties would improve the accuracy of diagnosis of PJI, directing the surgical approach and the administration (or not) of antimicrobial therapy.

The pathogenesis of staphylococcal PJI is hypothesized to depend on the ability of the infecting organism to adhere to and form biofilm on indwelling medical devices (11). Biofilm formation occurs upon initial rapid attachment of staphylococci to the surface of a device, followed by multilayered cellular proliferation and intercellular adhesion in an extracellular polysaccharide matrix excreted by the bacteria (19). Cell adhesion between staphylococci is mediated by polysaccharide intercellular adhesin (PIA), a linear homopolymer of β-1,6-linked N-acetylglucosamine residues (25). The icaA operon, which has been well characterized in Staphylococcus epidermidis (12, 18, 21) and S. aureus (13) and has been preliminarily identified in several other CNS (1, 13, 26), encodes biosynthetic products responsible for the generation of PIA. These genes are required for in vitro biofilm formation by S. aureus and S. epidermidis (13, 21), suggesting a role for products of the ica locus as virulence factors in medical device-related staphylococcal infections.

Two reports that investigated prosthesis-related infections caused by either S. epidermidis (16) or S. epidermidis and S. aureus (7) found that the ica operon may be used to discriminate pathogenic strains from normal human flora isolates. However, the prevalence of icaA among staphylococci isolated as contaminants from joint specimens, the issue of clinical relevance, has not been examined. We used a PCR assay to assess the frequency with which icaA could be detected in a collection of staphylococcal isolates associated with PJI, as well as non-PJI CNS isolated from the site of prosthetic hip or knee joints.

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Bacterial isolates were collected from explanted prostheses or either synovial tissue or fluid associated with total knee or hip arthroplasties from 1999 to 2003. PJI was defined, using previously established criteria (10, 30), as the presence of one or more of the following criteria at the time of revision or resection arthroplasty: two or more joint aspirate or intraoperative tissue specimen cultures yielding the same organism, purulence surrounding the prosthesis, acute inflammation on histopathologic examination, and sinus tract communication with the prosthesis. All isolates from cases not meeting this definition of PJI were classified as arthroplasty-associated non-PJI-associated isolates. The associated organism was deemed the causative agent of PJI only if cultured from two or more specimens (i.e., synovial fluid, synovial tissues, or the explanted prosthesis itself). Polymicrobial cases of PJI and cases of PJI where a microorganism was isolated from a single specimen were excluded.

Fifty-five S. aureus and 44 coagulase-negative staphylococcal PJI isolates and 23 arthroplasty-associated non-PJI-associated CNS isolates were studied. Staphylococci were identified using...
overnight using a modified alkaline wash protocol (20). Briefly, cells were pelleted, resuspended in 500 μl of calf serum solution (0.05 M sodium citrate, 0.5 M NaOH), and incubated at room temperature for 20 min. Tubes were spun at 14,000 × g for 1 min, and pellets were washed with 500 μl of 0.5 M Tris-HCl, pH 8.0, and subsequently resuspended in 100 μl of sterile water. Tubes were placed in boiling water for 10 min and spun at 14,000 × g for 5 min. Five microliters of each supernatant was used in 50-μl reactions with oligonucleotides icaAF (5’T-GATGGMAGTCTCGATAATAC-3’) and icaAR (5’-CTCCTGTGCGGCTTGACC-3’), which were designed to anneal to regions of high homology found among the icaA sequences of S. aureus (GenBank accession number AP004831 [9]), S. epidermidis (GenBank accession number AJ13859), and S. caprae (GenBank accession number AF246926 [1]) and which amplify a ~980-bp region of icaA.

Primers KFicaAF (5’-GATGGGAAGTTGCGATAATAC-3’) and icaAR were also used to amplify icaA in S. pasteurii and S. saprophyticus. Thermocycling conditions were as follows: 95°C for 10 min, followed by 40 cycles of 94°C for 1 min, 48 or 50°C for 1 min, and 72°C for 1 min. S. aureus IDRL-2581, determined as icaA positive in preliminary experiments, and Escherichia coli IDRL-290 were used as positive and negative controls, respectively. Several amplified products were selected and prepared for sequencing as previously described (28) using primer icaAF, KFicaAF, or icaAR. Sequencing was performed on an ABI Prism 377 DNA sequencer with an ABI Prism Big Dye Terminator cycle sequencing ready reaction kit (Perkin-Elmer Applied Biosciences, Foster City, Calif.).

PJII was most commonly caused by S. aureus, S. epidermidis, and S. lugdunensis (Table 1). S. aureus comprised the majority (56% [55 out of 99]) of the PJII-associated isolates investigated in this study, and all were found to be icaA positive, substantiating past findings that the ica operon is common to all strains of S. aureus (13, 14, 22). A total of 55% (17 out of 31) of S. epidermidis PJII isolates were icaA positive. icaA was also detected in PJII-causing S. caprae/capitis isolates and in S. saprophyticus. The S. saprophyticus icaA PCR product was sequenced, and an 856-nucleotide region of the gene was found to be 98% identical to S. aureus icaA (GenBank accession numbers AP003138 [24], AP003366 [24], and AP004831 [9]) and 99% identical to S. saprophyticus strain N2.1A icaA (GenBank accession number AF500264 [26]) with a BLASTN 2.2.8 search.

All arthroplasty-associated non-PJI isolates were CNS, with S. epidermidis, S. hominis, and S. warnerii occurring most frequently (Table 1). Of these, five of nine S. epidermidis isolates yielded positive results for icaA, whereas icaA was not detected in either S. hominis or S. warnerii. Other icaA-positive non-PJI arthroplasty-associated isolates included S. caprae/capitis, which is consistent with previous data showing that all S. caprae/capitis isolates carry the ica locus (1, 2, 26), and S. pasteurii. The presence of icaA in S. pasteurii was previously suggested only in low-stringency hybridization experiments in which an S. aureus icaA probe was used (13), so an 815-bp region of the S. pasteurii icaA PCR product was sequenced. A TBLASTX 2.2.8 search revealed that the predicted protein encoded by this region of S. pasteurii icaA is 62% identical to the predicted protein encoded by S. aureus icaA (GenBank accession numbers AP003138 [24], AP003366 [24], and AP004831 [9]).

While our assay was able to detect icaA in S. epidermidis, S. caprae/capitis, S. pasteurii, and S. saprophyticus, we did not observe icaA in any other coagulase-negative staphylococcal species. It has been reported that icaA could be detected under low-stringency hybridization conditions in S. lugdunensis, whereas no icaA hybridization signals were detected under similar conditions in several other CNS, including S. hominis, S. saprophyticus, S. simulans, and S. warnerii (13). Recently, icaA sequences were shown to be present in S. saprophyticus and S. simulans isolated from food processing environments (26). We suspect that our failure to identify icaA in S. lugdunensis (and possibly in other species) was due to our PCR primer design being based on icaA sequences from a limited number of Staphylococcus species.

The difference in the frequency of detection of icaA in coagulase-negative staphylococcal PJII isolates (46% [20 out of 44]) compared to arthroplasty-associated non-PJI-associated coagulase-negative staphylococcal isolates (30% [7 out of 23]) was not statistically significant (P = 0.23, chi-squared test) (Table 1). Additionally, the occurrence of icaA in S. epidermidis PJII isolates (55% [17 out of 31]) compared to S. epidermidis arthroplasty-associated non-PJI-associated isolates (56% [5 out of 9]) was statistically insignificant (P = 1.00, chi-squared test).

A study conducted by Galdhart et al. examined the prevalence of the ica operon in 54 S. epidermidis isolates from 14 patients with PJII and 23 S. epidermidis isolates from hand skin
swabs of eight healthy humans (16). The presence of the ica operon was reported in 82% of pathogens but in only 17% of skin flora strains. A later study (7), wherein the authors tested S. epidermidis isolates for icaA and icaD by PCR, found that 9 out of 15 S. epidermidis isolates from orthopedic prosthesis infections, but 0 out of 10 S. epidermidis strains from healthy human skin or mucosa, contained the ica locus. In contrast, we found icaA present at nearly equal rates in S. epidermidis PJI isolates (55%) and arthroplasty-associated non-PJI-associated isolates (56%). The discrepancy between the earlier reports and the data we present here is likely due to the source of the nonpathogenic isolates studied in each case. As the noninfecting isolates in our study were obtained directly from explanted prostheses or fluid or tissue from the site of arthroplasties, this collection is representative of staphylococci relevant to the clinical context of differentiating pathogens from contaminants. Our finding that approximately half of the S. epidermidis arthroplasty-associated non-PJI-associated isolates are icaA positive is in accord with results of a recent study (31), which reported icaA in 15 out of 29 S. epidermidis skin isolates from healthy subjects.

We observed that S. epidermidis and S. lugdunensis were most frequently isolated as pathogens in PJI, whereas S. warneri and S. hominis were most frequently found as arthroplasty-associated non-PJI isolates. Specifically, 78% (95% confidence interval, 62 to 89%) of S. epidermidis isolates (n = 40) and 100% (95% confidence interval, 54 to 100%) of S. lugdunensis isolates (n = 6) studied were associated with PJI. Reciprocally, 86% (95% confidence interval, 42 to 100%) of S. warneri isolates (n = 7) and 100% (95% confidence interval, 48 to 100%) of S. hominis isolates (n = 5) were arthroplasty-associated non-PJI isolates.

The isolation of several staphylococci primarily as pathogens (S. aureus, S. lugdunensis, S. saprophyticus, and S. simulans) or arthroplasty-associated non-PJI CNS (S. hominis and S. pasteuri) suggests that laboratory identification of CNS to the species level when isolated from joint specimen cultures from hip and knee arthroplasties may aid in determining the likelihood of specific species as the causative agents of PJI. The exclusive association of S. lugdunensis as a pathogen is not surprising in view of its previously described propensity to cause native-valve endocarditis (27). Further studies addressing apparent differences in the pathogenicities of various coagulase-negative Staphylococcus species isolated from the site of arthroplasty are warranted.

Staphylococci are both frequent causes of PJI and, in the case of CNS, frequent contaminants isolated from the site of an arthroplasty. For this reason, a straightforward test to differentiate pathogens from non-infection-associated site-specific staphylococci could enhance the laboratory diagnosis of PJI. We addressed whether the presence of icaA, as a marker for the ica operon, could be detected with increased frequency in PJI-associated staphylococci compared to arthroplasty-associated non-PJI-associated staphylococci using isolates obtained from the site of a knee or hip arthroplasty. Our results reveal no statistically significant difference in the occurrence of icaA in pathogens versus non-PJI arthroplasty-associated isolates (Table 1).

Interestingly, a high percentage (45% [14 out of 31]) of the S. epidermidis isolates identified as causing PJI in our study lacked icaA by our detection method. Previous studies have also reported icaA-negative infection-associated S. epidermidis strains associated with PJI (5, 6, 7, 16), catheter-related infection (3, 4), and medical device-related infection (15, 33). Arciola et al. recently published studies in which icaA was detected by PCR in only 56% (69 out of 123) (5) and 43% (51 out of 120) (6) of PJI-associated isolates of S. epidermidis. While it is possible that sequence heterogeneity in the icaA operon is responsible for false-negative PCR results, icaA may truly be absent from these organisms, implying that PIA may not be produced by some PJI-associated S. epidermidis isolates. The ica operon is not present in a recently sequenced non-biofilm-forming S. epidermidis strain (ATCC 12228) commonly used for diagnostic tests in food products (32). Biofilm-forming strains of S. epidermidis that lack the ica locus, however, have been noted (3, 4, 17, 29, 33), suggesting the existence of alternative mechanisms for biofilm formation in S. epidermidis. Assuming that the icaA-negative S. epidermidis PJI-associated isolates in our collection do not contain the ica locus (i.e., our findings are not secondary to false-negative PCR results), this finding would suggest that icaA is not required for S. epidermidis PJI.

In conclusion, the frequency of detection of icaA in coagulase-negative staphylococcal PJI isolates compared to arthroplasty-associated non-PJI coagulase-negative staphylococcal isolates shows no statistically significant difference, indicating that the presence of icaA in a staphylococcal isolate associated with an arthroplasty is not a reliable marker for PJI. On the other hand, certain coagulase-negative Staphylococcus species, such as S. hominis and S. warneri, occurred more frequently as arthroplasty-associated non-PJI isolates, whereas other species of CNS, such as S. epidermidis and S. lugdunensis, were more commonly isolated as pathogens from the site of an arthroplasty, an important distinction when considering the diagnosis of staphylococcal PJI.

Nucleotide sequence accession numbers. Partial sequences for S. pasteurii and S. saprophyticus icaA were deposited in GenBank with accession numbers AY512962 and AY512963, respectively.

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