

Characterization of the Importance of *Staphylococcus epidermidis* Autolysin and Polysaccharide Intercellular Adhesin in the Pathogenesis of Intravascular Catheter–Associated Infection in a Rat Model

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A rat central venous catheter (CVC) infection model was used to assess the importance of the proteinacious autolysin (AtlE) and the polysaccharide intercellular adhesin (PIA) in the pathogenesis of *Staphylococcus epidermidis* CVC-associated infection. Wild-type (wt) *S. epidermidis* O-47 was significantly more likely to cause a CVC infection than was either of the isogenic mutant strains (AtlE-negative [O-47mut1] or PIA-negative [O-47mut2]). Bacteria were retrieved from the explanted catheters of 87.5% of rats inoculated with *S. epidermidis* O-47, compared with 25% of rats challenged with either *S. epidermidis* O-47mut1 or O-47mut2 ($P = .007$). Peripheral bacteremia was documented in 75% of rats challenged with *S. epidermidis* O-47, compared with 12.5% and 25% challenged with O-47mut1 and O-47mut2, respectively ($P = .009$). Metastatic disease was more common in rats inoculated with wt *S. epidermidis*, compared with AtlE- or PIA-deficient mutants. These results confirm the importance of initial adherence, associated with AtlE, and biofilm production, mediated by PIA, in the pathogenesis of *S. epidermidis* experimental CVC infection.

Staphylococcus epidermidis is a commensal inhabitant of human skin that rarely causes disease in healthy persons. In recent years, however, largely because of the increased use of intravascular catheters and other indwelling prosthetic devices, *S. epidermidis* has emerged as a major nosocomial pathogen [1, 2]. According to the Centers for Disease Control and Prevention National Nosocomial Infection Surveillance System, during the 1990s, coagulase-negative staphylococci accounted for 37.3% of nosocomial bloodstream infections [3]. Unfortunately, these infections are responsible for significant morbidity and mortality [4–6].

The propensity for *S. epidermidis* to cause intravascular catheter–associated infection can be explained largely by its ability to adhere and proliferate on polymer surfaces. The adherence process for *S. epidermidis* appears to be a 2-step process that requires adhesion of the bacteria to the biomaterial surface, followed by cell-to-cell accumulation and biofilm formation.

In previous studies, Heilmann et al. [7, 8] demonstrated that

a proteinacious autolysin (AtlE) is associated with initial attachment of *S. epidermidis* to plastic. Mack et al. [9–11] described a β -1,6 linked glucosaminoglycan intercellular adhesin (polysaccharide intercellular adhesin [PIA]), synthesized by gene products of the *ica* locus, that mediates cell-to-cell adherence and biofilm accumulation. We demonstrated that PIA also mediates hemagglutination and is essential in the pathogenesis of foreign body infections in 2 in vivo models [12–14]. In the present study, we evaluated the relative contribution of PIA and AtlE in an isogenic background in the pathogenesis of intravascular catheter–associated infection in a rat model.

Materials and Methods

Bacteria. *Staphylococcus epidermidis* O-47 is a biofilm-positive clinical isolate recovered from a patient at the Institute Für Medizinische Mikrobiologie und Hygiene, Universität zu Köln (Köln, Germany) [7]. *S. epidermidis* O-47mut1 is a Tn917 insertional mutant that is deficient in AtlE production [8]. *S. epidermidis* O-47mut2 is a Tn917 insertional mutant that is deficient in PIA production because of interruption of the *ica* locus [15].

Rat central venous catheter (CVC)–associated infection model. A rat CVC-associated infection model was used to compare the virulence of *S. epidermidis* O-47 with its isogenic mutants *S. epidermidis* O-47mut1 and *S. epidermidis* O-47mut2. Twenty-four male Sprague Dawley rats (Charles River) underwent catheterization, as described elsewhere [14, 16]. In brief, a silastic catheter was inserted into the jugular vein and was advanced into the superior vena cava. The proximal portion of the catheter was tunneled subcutaneously to exit in the midscapular space. A rodent restraint jacket was used to protect the catheter and to allow access to it. Twenty-four hours after CVC placement, blood cultures were obtained to ensure sterility,

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the rats were divided into groups of 8, and 10^7 cfu of *S. epidermidis* O-47 or one of the isogenic mutants was injected into the CVC. The catheters were flushed daily with a heparin solution. On day 8, the rats were killed. Peripheral blood was obtained and was cultured quantitatively by directly plating 0.1 mL of blood on trypticase soy agar (TSA; Remel). The location of the distal tip of the CVC in the superior vena cava was confirmed, the CVC and surrounding venous tissues were removed aseptically and were vigorously vortex washed in PBS, after which the wash fluid was quantitatively cultured. Previous studies, in which quantitative culture results were confirmed by electron microscopy, documented complete removal of adherent organisms by this procedure [13]. In addition, to ascertain the extent of metastatic disease, the heart, lungs, liver, and kidneys were aseptically harvested, were weighed, were homogenized in 1.0 mL of PBS with a mechanical tissue homogenizer (PowerGen 700D; Fisher) and were cultured quantitatively by plating 0.1 mL of tissue homogenate on TSA. Bacteria recovered from the catheter, blood, or tissues were identified to species level.

To limit the number of experimental rats used to the minimum that would reveal a significant difference in pathogenicity, inoculum size experiments were performed. Before conducting the comparative studies described above, the smallest inoculum that reliably resulted in a CVC-associated infection was determined in dose-ranging inoculum studies. CVCs were placed as described above. Three rats each were challenged with an inoculum of either 10^3 , 10^4 , 10^5 , 10^6 , or 10^7 cfu of *S. epidermidis* O-47. After inoculation, the rats were evaluated for the presence of infection, as described above.

To document the importance of the intravascular catheter in the pathogenesis of infection, 4 rats, without indwelling CVCs, were inoculated via the tail vein with the minimal infectious dose of *S. epidermidis* O-47, as determined above. After inoculation, the rats were evaluated for evidence of infection, as described above.

Statistical analysis. Nonparametric methods were employed to compare the bacterial burden among the groups of experimental rats inoculated with either *S. epidermidis* O-47 or one of the isogenic mutants, mut 1 or mut 2. The bacterial counts obtained from explanted CVCs, peripheral blood, and peripheral tissues for the 3 groups of rats were compared by using the Kruskal-Wallis test, which was performed using the SAS system (SAS Institute).

Results

Inoculation studies. The inoculation studies showed that rats challenged with 10^3 cfu exhibited no sign of CVC-associated infection at the time of death. One of 3 rats challenged with 10^4 or 10^5 cfu of *S. epidermidis* O-47 exhibited evidence of infection at the time of death, compared with 2 of 3 and 3 of 3 rats challenged with 10^6 and 10^7 cfu, respectively. Therefore, the 10^7 cfu inoculum size, the lowest level that reproducibly caused CVC-associated infection and metastatic disease, was used in the larger comparative trial. None of the 4 rats inoculated with 10^7 cfu of *S. epidermidis* O-47 via the tail vein had evidence of bacteremia or metastatic disease at the time of death.

Comparative studies. Seven (87.5%) of 8 rats inoculated with *S. epidermidis* O-47 had bacteria recovered from the explanted CVC, compared with 2 of 8 rats challenged with either

S. epidermidis O-47mut1 or *S. epidermidis* O-47mut2. The number of bacteria recovered from the explanted catheters was significantly different among the different groups of rats ($P = .007$). The number of bacteria recovered from the catheters is shown in figure 1.

Six (75%) of 8 rats challenged with the wild-type (wt) strain *S. epidermidis* O-47 had bacteria recovered from the peripheral blood at the time of death, compared with 1 of 8 rats challenged with *S. epidermidis* O-47mut1 and 2 of 8 rats challenged with *S. epidermidis* O-47mut2. The number of bacteria recovered from the peripheral blood was significantly different among the different groups of rats ($P = .009$). In no case were peripheral blood cultures positive without concordant recovery of organisms from the CVC. In 2 rats, the CVC culture was positive, and the peripheral blood culture was negative. In both instances, the number of bacteria recovered from the CVC was ≤ 10 cfu. The number of bacteria recovered in the peripheral blood is shown in figure 2.

Table 1 summarizes results from studies defining the burden of metastatic disease in the rats challenged with the various strains of *S. epidermidis*. For all organ systems examined, there were more rats with metastatic disease in the group inoculated with wt *S. epidermidis* O-47 than in the groups inoculated with either adhesin-deficient strain. In addition, for all organ systems

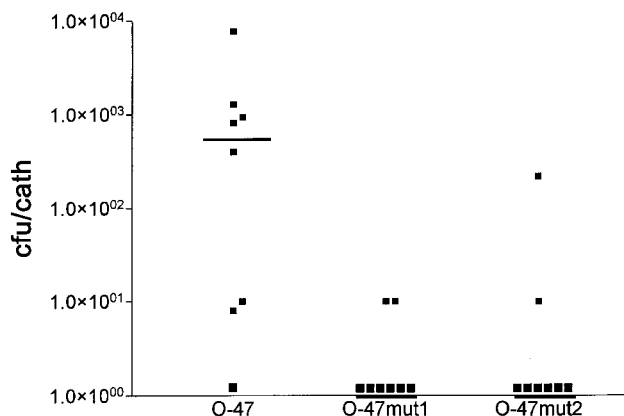


Figure 1. Recovery of *Staphylococcus epidermidis* O-47 or its isogenic autolysin-deficient mutant (mut1) or polysaccharide intercellular adhesin (PIA)-deficient mutant (mut2) from explanted central venous catheters (CVCs) in the rat CVC-associated infection model. Median number of organisms (colony-forming units [cfu]/catheter [cath]) recovered from the CVC of rats inoculated with wild-type *S. epidermidis* O-47 was 6.1×10^2 cfu (range, $0-7.8 \times 10^3$ cfu; lower/upper quartiles, $9-1.1 \times 10^3$ cfu), compared with a median of 0 cfu (range, 0–10 cfu; lower/upper quartiles, 0–5 cfu) for the autolysin-deficient mutant and a median of 0 cfu (range, 0–220 cfu; lower/upper quartiles, 0–5 cfu) for the PIA-deficient mutant ($P = .007$, Kruskal-Wallis test; $P = .027$, corrected P value for *S. epidermidis* O-47 vs. *S. epidermidis* O-47mut1; $P = .045$, *S. epidermidis* O-47 vs. *S. epidermidis* O-47mut2). There was no significant difference between the mut1 and mut2 mutant strains ($P = .50$). Each data point represents cfu/cath for a single experimental animal. Horizontal line, median value.

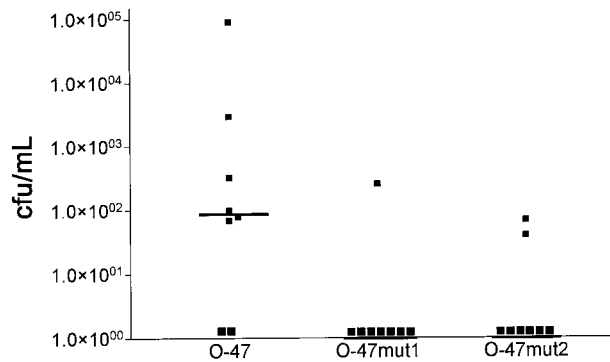


Figure 2. Recovery of *Staphylococcus epidermidis* O-47 or its isogenic autolysin-deficient mutant (mut1) or polysaccharide intercellular adhesin (PIA)-deficient mutant (mut2) from the peripheral bloodstream in the rat central venous catheter (CVC)-associated infection model. Median number of organisms (colony-forming units [cfu]/mL) recovered from the bloodstream of rats inoculated with wild-type *S. epidermidis* O-47 was 90 cfu (range, $0-9.2 \times 10^4$ cfu; lower/upper quartiles, $35-1.7 \times 10^3$ cfu), compared with a median of 0 cfu (range, $0-2.6 \times 10^2$ cfu; lower/upper quartiles, 0–0 cfu) for the autolysin-deficient mutant and a median of 0 cfu (range, 0–70 cfu; lower/upper quartiles, 0–20 cfu) for the PIA-deficient mutant ($P = .009$, Kruskal-Wallis test; $P = .053$, corrected P value for *S. epidermidis* O-47 vs. *S. epidermidis* O-47mut1; $P = .057$, *S. epidermidis* O-47 vs. *S. epidermidis* O-47mut2). There was no significant difference between the mut1 and mut2 mutant strains ($P = 1.0$). Each data point represents cfu/mL blood for a single experimental animal. Horizontal line, median value.

there was a greater microbial burden of metastatic disease (as documented by quantitative cultures) in the rats inoculated with the wt strain than in the rats inoculated with the autolysin- or PIA-deficient mutant strains. In general, the rats inoculated with the wt parent strain had 10–100-fold greater numbers of organisms recovered from peripheral organs than did rats inoculated with the adhesin-deficient mutants. There were no significant differences between the groups of rats inoculated with the *S. epidermidis* O-47mut1 or *S. epidermidis* O-47mut2 mutant strains.

Discussion

Coagulase-negative staphylococci, predominantly *Staphylococcus epidermidis*, are the most common cause of infections of

prosthetic medical devices [2, 17]. It has long been thought that the ability of *S. epidermidis* to attach to biomaterials and to elaborate biofilm explains its propensity to cause biomaterial-based infections. Bacterial adherence to biomaterials is a complex multistep process that Gristina [18] conveniently subdivided into the stages of attachment, adhesion, and aggregation. Electron microscopic studies have shown that bacteria first attach to the surface and later form colonies and multilayered cell clusters embedded in a thick slime matrix known as biofilm [19].

Attachment is thought to be due to nonspecific factors, such as hydrophobicity and surface charge. Adhesion appears to be secondary to more specific factors. Several investigators have described specific molecular entities that are involved in adhesion. Heilmann et al. [7] described Tn917 insertional mutants of *S. epidermidis* deficient in their ability to adhere to polystyrene. The phenotype of the mutant was restored in complementation experiments that revealed a 60-kDa protein as essential for adhesion [7]. Further genetic analysis revealed that the protein is encoded by the gene *atlE*, an autolysin [8]. In addition to mediating adhesion to plastic, AtlE also binds to vitronectin [8]. Polysaccharide adhesin (PSA), described by Pier et al. [20], appears to be important in adherence to plastics. Antibody directed against PSA is protective in animal models of infection, and mutants deficient in PSA are less virulent [21, 22]. McKenney et al. [23] reported data indicating that the *ica* locus plays a role in the production of PSA and that PSA mediates both initial attachment and aggregation. Timmerman et al. [24, 25] described a 220-kDa cell wall-associated protein (Ssp1) that appears to be functional in adhesion and is organized in a fimbria-like structure.

The aggregative phase of adherence, characterized by cell-to-cell adherence and the elaboration of biofilm, is mediated by PIA, as described initially by Mack et al. [11]. The expression of PIA is dependent on glucose in the growth media [11]. Iso-genic PIA-negative, Tn917 mutants are capable of initial adhesion but are unable to form microcolonies [10]. Hussain et al. [26] described a 140-kDa protein, termed accumulation-associated protein, that appears to be important in the aggregative stage of adherence.

Bacterial adherence to biomaterials, however, is only one component of a complex process that involves interactions be-

Table 1. Metastatic disease caused by *Staphylococcus epidermidis* O-47, *S. epidermidis* O-47mut1, or *S. epidermidis* O-47mut2 in the rat central venous catheter-associated infection model.

| Organ | <i>S. epidermidis</i> O-47 | | | <i>S. epidermidis</i> O-47mut1 | | | <i>S. epidermidis</i> O-47mut2 | | |
|--------|----------------------------|--|------------------------------------|--------------------------------|--|------------------------------------|--------------------------------|---|------------------------------------|
| | No. rats infected/total | Median (range) cfu/g tissue | Lower/upper quartiles cfu/g tissue | No. rats infected/total | Median (range) cfu/g tissue | Lower/upper quartiles cfu/g tissue | No. rats infected/total | Median (range) cfu/g tissue | Lower/upper quartiles cfu/g tissue |
| Kidney | 7/8 | 1.49×10^3 (0– 3.7×10^5) | $1.4 \times 10^2/9.6 \times 10^4$ | 4/8 | 2.34×10^2 (0– 3.1×10^3) | $0/1.7 \times 10^2$ | 3/8 | 0 (0– 2.4×10^2) | $0/1.7 \times 10^{2a}$ |
| Liver | 7/8 | 2.7×10^4 (0– 1.73×10^5) | $8.6 \times 10^3/5.6 \times 10^3$ | 3/8 | 0 (0– 1.1×10^4) ^a | $0/6.7 \times 10^{2a}$ | 3/8 | 0 (0– 2.5×10^4) ^a | $0/1.1 \times 10^{4a}$ |
| Heart | 5/8 | 1.6×10^2 (0– 9.3×10^2) | $0/1.3 \times 10^4$ | 3/8 | 0 (8.6×10^2) | $0/8.6 \times 10^2$ | 3/8 | 0 (0–75) | 0/54 |
| Lung | 7/8 | 1.4×10^4 (2.7×10^5) | $6.8 \times 10^2/1.8 \times 10^5$ | 4/8 | 5.1×10^3 (0– 4.3×10^4) | $0/1.7 \times 10^4$ | 4/8 | 8.4×10^3 (0– 4.7×10^4) | $0/2.5 \times 10^4$ |

^a $P < .05$, compared with *S. epidermidis* O-47 (Kruskal-Wallis test).

tween the host, the biomaterial, and the microbe. Therefore, it is desirable to test questions regarding pathogenesis in models that reflect its complexity. We examined the relative contribution of the autolysin, AtlE, and PIA in an isogenic background strain *S. epidermidis* O-47, in the pathogenesis of intravascular device infections in the rat model of CVC-associated infection. In this study, we added additional support for the multistep model of bacterial adherence and demonstrate the crucial role of adherence in biomaterial-based infection. We observed that a strain of *S. epidermidis* lacking either the ability to adhere initially to a catheter or the ability to form cell clusters is less virulent than the wt parent strain. It is interesting that we also found that the background strain *S. epidermidis* O-47 produces less biofilm, as evidenced by hemagglutination titer and biofilm assay (data not shown), than did other *S. epidermidis* strains we have previously studied (*S. epidermidis* SE5 and *S. epidermidis* 1457) and was less virulent in the rat model of CVC-associated infection, as evidenced by the need for a higher infective dose for *S. epidermidis* O-47 (10^7 cfu) than *S. epidermidis* 1457 (10^4 cfu) [14]. Therefore, not only the presence of adhesins but their relative amount may explain differences in pathogenicity. Further studies should be directed at discerning the interaction, relative importance, and regulation of the adhesins of *S. epidermidis*.

We documented that *S. epidermidis* O-47 was more likely to cause a CVC-associated infection, manifested by bacteremia and organisms recovered from the explanted catheter (primary end points in CVC-associated infection model), than were the adhesin-deficient mutants. Although the burden of metastatic disease (secondary end points in the CVC-associated infection model) tended to be greater in rats infected with the wt strain, in general, these differences were not statistically significant; however, observations regarding metastatic seeding are limited by the relatively small sample size. Metastatic seeding of organs may be governed by undefined, nonspecific, or tissue-specific adhesins. Perdreau-Remington et al. [27] noted that a chemically induced mutant deficient in biofilm formation showed no decreased virulence in a rabbit model of endocarditis.

In conclusion, in an isogenic background strain of *S. epidermidis*, we demonstrated that adhesion and aggregation are essentially equally important in the pathogenesis of intravascular catheter-associated infection in the rat model. Strains lacking in the autolysin, AtlE, or PIA were less likely to cause CVC-associated infection manifest by bacteria recovered from catheter tips, blood, or peripheral organs. These findings may suggest novel means to prevent or treat prosthetic device infections.

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