Introduction

Infection is the major limiting factor in the use of intravascular catheters and other prosthetic medical devices such as artificial heart valves and prosthetic joints. The most common aetiologic agent of these infections is Staphylococcus epidermidis.1–4 Bacterial adherence to biomaterials is generally believed to be the pivotal event in the pathogenesis of prosthetic device-centred infections. The process of bacterial adherence can be subdivided into early stages, namely attraction and adhesion, and later ones, aggregation.5 Unfortunately, preoperative skin preparation and prophylactic antibiotics do not eradicate S. epidermidis from their sanctuary sites in the sebaceous glands and apocrine pits on the human skin.6 When prosthetic devices are put in place, small numbers of these organisms are introduced into the surgical wound. The bacteria adhere to the biomaterial and proliferate, eventually causing a clinically evident infection. In the case of intravascular catheters, following insertion, the organisms can move down the outer surface of the catheter and thence into the bloodstream. It appears that adherence of S. epidermidis is a complex, multi-stage process that is mediated at the various steps by different adhesins.7–11

Antibiotics of a variety of classes may influence bacterial adherence. Antibiotics decrease the adherence of bacteria to eukaryotic cells, capsule formation and biofilm production. However, results from studies involving coagulase-negative staphylococci (CoNS) have primarily examined the effect of antibiotics on biofilm formation and have not directly measured their effect on adherence.17–20 In general, these studies revealed a great deal of strain-to-strain variability and no consistent effect has been observed.

The purpose of this study was to determine the effect of subinhibitory concentrations of vancomycin, cefazolin, ofloxacin, L-ofloxacin and D-ofloxacin on adherence to intravascular catheters and biofilm formation by Staphylococcus epidermidis.

Mark E. Rupp* and Kathryn E. Hamer

Department of Internal Medicine, The University of Nebraska Medical Center, 600 S. 42nd Street, Omaha, NE 68198-5400, USA

Staphylococcus epidermidis is the preeminent cause of nosocomial bacteraemia and infection of prosthetic medical devices. Bacterial adherence to biomaterial is a crucial early event in the pathogenesis of these infections. Antibiotics affect bacterial adherence to eukaryotic cells, capsule formation and biofilm production. However, results from studies involving coagulase-negative staphylococci are equivocal. In this study, the in-vitro adherence of radiolabelled bacteria was assayed to determine the effect of sublethal concentrations of a number of antibiotics on the attachment of four strains of S. epidermidis, with well-characterized adherence profiles, to intravascular catheters. The effect of antibiotics on biofilm production by S. epidermidis was assayed using a quantitative spectrophotometric assay. Although there was some strain-to-strain variability, none of the tested antibiotics affected bacterial attachment. However, treatment with cefazolin or vancomycin resulted in a significant decrease in biofilm elaboration. These data suggest that bacterial attachment by S. epidermidis, the initiating event associated with prosthetic device infection, cannot be prevented by subtherapeutic levels of fluoroquinolone, glycopeptide or β-lactam antibiotics. However, later aggregative stages of adherence, associated with biofilm production, may be influenced by cell wall-active agents such as cefazolin and vancomycin.
ofloxacin and its two isomers, as well as two commonly used antistaphylococcal antibiotics, cefazolin and vancomycin, on the ability of S. epidermidis to attach to intravascular catheters and to elaborate biofilm.

**Materials and methods**

**Bacteria**

The following well-characterized strains of S. epidermidis were used in the adherence and biofilm assays: (i) RP62A: slime-associated antigen (SAA)-positive, biofilm-positive; received from G. Christensen, Columbia, MO, USA; (ii) M 187: polysaccharide adhesin (PSA)-positive, biofilm-positive; received from G. Pier, Boston, MA, USA; (iii) 1457: polysaccharide intercellular adhesin (PIA)-positive, biofilm-positive; received from D. Mack, Hamburg, Germany; (iv) SE5: haemagglutinin (HA)-positive, biofilm-positive.

**Antibiotics**

Ofloxacin, L-ofloxacin (or levofloxacin, the active isomer of ofloxacin), and D-ofloxacin (the inactive isomer of ofloxacin) were supplied by R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ, USA. Cefazolin was supplied by SmithKline Beecham Pharmaceuticals, Philadelphia, PA, USA. Vancomycin was purchased from Sigma Chemical Co., St Louis, MO, USA. Teflon intravascular catheters (Quik-Cath 20-gauge; Baxter, Deerfield, IL, USA) were used in the bacterial adherence assay.

**Intravascular catheters**

Teflon intravascular catheters (Quik-Cath 20-gauge; Baxter, Deerfield, IL, USA) were used in the bacterial adherence assay.

**Antimicrobial susceptibility**

MICs of the antibiotics for the four strains of S. epidermidis were determined by a microbroth dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations.

**Adherence assay**

An in-vitro radiolabelled adherence assay was used to assess bacterial attachment to the intravascular catheter segments. Briefly, bacteria were radiolabelled by overnight incubation in trypticase soy broth (TSB; Becton Dickinson, Cockeysville, MD, USA) containing 10 μCi/mL [³H]thymidine (A mersham, A rlington Heights, IL, USA) and 0.5 × MIC of the test antibiotic. Negative-control bacteria were prepared in an identical fashion but were not exposed to the antibiotic. The cells were harvested by centrifugation and resuspended in phosphate buffered saline (PBS) to a standard inoculum (OD₆₀₀ = 0.7) corresponding to approximately 5.0 × 10⁸ cfu/mL. Quantitative plate counts were performed to confirm inoculum size. The radiolabelled bacterial suspension was incubated with 1 cm segments of intravascular catheter for 15 min. Nonadherent bacteria were removed by repeated washing on a mechanical shaker (48 oscillations per min, rotating from -45° to +45°; wash fluid changes at 2, 5 and 10 min). A adherent bacteria were quantified by scintillation counting (Tri-carb 4530, Packard Instrument Co.). Scintillation counts were converted to cfu. To correct for small differences in the inoculum size, values were normalized to the standard inoculum size of 5 × 10⁸ cfu/mL. A II tests were performed in triplicate and repeated three times.

**Biofilm assay**

Biofilm elaboration was measured in 96-well microtitre plates, as previously described. Minor modifications to the procedure were made. Briefly, 1 × 10⁵ cfu of bacteria were inoculated into wells containing 200 μL of TSB, with and without the test antibiotic. The plates were incubated for 24 h at 37°C without shaking. The broth supernatant was discarded and the wells were washed three times with PBS. The adherent biofilm was fixed with Bouin’s fixative, stained with Crystal violet and air-dried, and the optical density was measured at 570 nm using a micro-ELISA reader (2550 E IA R eader; B io-R ad, H ercules, C A , U SA ). The test was performed in quadruplicate and the values were averaged. A clean microtitre plate well, treated with medium, fixative and stain as described above, was used as a zero point. For purposes of statistical analysis, optical density readings of >2.0 were adjusted to 2.01.

**Statistical analysis**

The adherence of antibiotic-treated S. epidermidis to intravascular catheters was compared with that of untreated controls using a paired t-test. Biofilm elaboration of antibiotic-treated S. epidermidis was compared with that by untreated control strains by one-way analysis of variance (ANOVA). Pairwise comparisons were performed using Dunnet’s multiple comparison test.

**Results**

Effect of antibiotics on S. epidermidis attachment to intravascular catheters

The effects of ofloxacin and cefazolin are illustrated in Figure 1, panels a and b, respectively, and the results of the paired t-test analysis for all antibiotics in the Table. Although there was some minor strain-to-strain variability, there was no consistent statistically significant effect on attachment for any of the antibiotics on the tested strains of S. epidermidis.
Effect of subinhibitory antibiotics on adherence

The effect of the antibiotics on biofilm production by strains SE5 and RP62A is shown in Figure 2 panels a and b, respectively. A one-way ANOVA for the effect of the tested antibiotics on the ability of each strain to elaborate biofilm demonstrated significance (P < 0.0001). Cefazolin, and to a lesser extent, vancomycin, consistently significantly decreased the production of biofilm. Although there was some strain-to-strain variation, the fluoroquinolones did not significantly affect the elaboration of biofilm. The q, P and 95% CI for difference obtained from Dunnett’s multiple comparison test were as follows:

**Strain SE5.**
- Ofloxacin: q = 1.44, P > 0.05, CI -0.62 to 1.99;
- D-ofloxacin: q = 2.57, P > 0.05, CI -0.09 to 2.53;
- L-ofloxacin: q = 1.45, P > 0.05, CI -0.62 to 1.99;
- Cefazolin: q = 13.69, P < 0.01, CI 5.18 to 7.79;
- Vancomycin: q = 10.9, P < 0.01, CI 3.85 to 6.47.

**Strain RP62A.**
- Ofloxacin: q = 1.69, P > 0.05, CI -0.55 to 2.53;
- D-ofloxacin: q = 2.40, P > 0.05, CI -0.25 to 2.95;
- L-ofloxacin: q = 1.45, P > 0.05, CI -0.62 to 1.99;
- Cefazolin: q = 13.69, P < 0.01, CI 5.18 to 7.79;
- Vancomycin: q = 10.9, P < 0.01, CI 3.85 to 6.47.

**Table.** Statistical analysis of the effect of antibiotics on attachment of the tested strains of S. epidermidis to intravascular catheters

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Strain</th>
<th>t</th>
<th>P</th>
<th>95% CI for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>SE5</td>
<td>0.72</td>
<td>0.50</td>
<td>-923,771 to 1,695,199</td>
</tr>
<tr>
<td></td>
<td>RP62A</td>
<td>1.00</td>
<td>0.36</td>
<td>-2,115,890 to 9,318,904</td>
</tr>
<tr>
<td></td>
<td>M187</td>
<td>0.57</td>
<td>0.59</td>
<td>-1,507,337 to 935,909</td>
</tr>
<tr>
<td></td>
<td>1457</td>
<td>0.19</td>
<td>0.85</td>
<td>-3,336,734 to 3,908,162</td>
</tr>
<tr>
<td>L-Ofloxacin</td>
<td>SE5</td>
<td>0.74</td>
<td>0.49</td>
<td>-496,597 to 946,597</td>
</tr>
<tr>
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<td>RP62A</td>
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<td>0.16</td>
<td>-222,203 to 1,097,203</td>
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<tr>
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<td>M187</td>
<td>0.84</td>
<td>0.44</td>
<td>-1,695,801 to 862,468</td>
</tr>
<tr>
<td></td>
<td>1457</td>
<td>0.71</td>
<td>0.50</td>
<td>-103,394 to 1,957,838</td>
</tr>
<tr>
<td>D-Ofloxacin</td>
<td>SE5</td>
<td>1.26</td>
<td>0.25</td>
<td>-640,521 to 2,090,521</td>
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<tr>
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<td>1.17</td>
<td>0.28</td>
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</tr>
<tr>
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<td>1.40</td>
<td>0.11</td>
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<td>1457</td>
<td>0.86</td>
<td>0.42</td>
<td>-1,778,419 to 828,419</td>
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<tr>
<td>Cefazolin</td>
<td>SE5</td>
<td>0.85</td>
<td>0.42</td>
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<tr>
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<td>0.06</td>
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</tr>
<tr>
<td></td>
<td>M187</td>
<td>0.66</td>
<td>0.53</td>
<td>-127,523 to 2,286,234</td>
</tr>
<tr>
<td></td>
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<td>1.03</td>
<td>0.33</td>
<td>-223,456 to 584,586</td>
</tr>
<tr>
<td>Vancomycin</td>
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<td>0.57</td>
<td>-417,933 to 706,822</td>
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<tr>
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<td>0.35</td>
<td>-2,233,514 to 933,660</td>
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<tr>
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<td>M187</td>
<td>0.76</td>
<td>0.47</td>
<td>-999,235 to 744,626</td>
</tr>
<tr>
<td></td>
<td>1457</td>
<td>0.76</td>
<td>0.47</td>
<td>-999,235 to 1,981,457</td>
</tr>
</tbody>
</table>
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2.3; D- ofloxacin: \( q = 1.74, P < 0.05, CI -0.53 \) to 2.32; L- ofloxacin: \( q = 0.03, P > 0.05, CI -1.41 \) to 1.44; cefazolin: \( q = 10.88, P < 0.01, CI 4.19 \) to 7.04; vancomycin: \( q = 8.47, P < 0.01, CI 4.19 \) to 7.04.

Strain M187. Ofloxacin: \( q = 8.55, P < 0.01, CI 2.01 \) to 3.92; D- ofloxacin: \( q = 6.0, P < 0.01, CI 1.12 \) to 3.04; L- ofloxacin: \( q = 1.75, P > 0.05, CI -1.56 \) to 0.35; cefazolin: \( q = 14.35, P < 0.01, CI 4.02 \) to 5.93; vancomycin: \( q = 12.26, P < 0.01, CI 3.3 \) to 5.21.

Strain 1457. Ofloxacin: \( q = 0.98, P > 0.05, CI -1.51 \) to 3.17; D- ofloxacin: \( q = 1.6, P > 0.05, CI -0.99 \) to 3.7; L- ofloxacin: \( q = 0.004, P > 0.05, CI -2.34 \) to 2.34; cefazolin: \( q = 7.74, P < 0.01, CI 4.22 \) to 8.9; vancomycin: \( q = 4.86, P < 0.01, CI 1.78 \) to 6.46.

Discussion

The Centers for Disease Control and Prevention National Nosocomial Infection Surveillance System reported increases in the incidence of *S. epidermidis* bacteraemia from 1980 to 1989 of 161–654%, depending on the type of hospital studied. These infections lead to significant morbidity and mortality and, thus, efforts to develop means to prevent or treat them are warranted.

The pathogenesis of prosthetic device infection and catheter-related bacteraemia is complex. A adherence is a complex multistep process that can arbitrarily be subdivided into the stages of attraction, adhesion and aggregation. These stages of adherence are mediated by a variety of nonspecific factors, such as hydrophobicity and electrostatic charge, and specific factors, such as bacterial adhesins. Increasing experimental evidence points to the importance of bacterial carbohydrate adhesins which act at various stages of the adherence process.

Because of the importance of bacterial adherence, a number of investigators have studied the effect of antibiotics on this process. A variety of antibiotics have been demonstrated to affect the adherence of Gram-negative bacilli and fungi to eukaryotic cells and biomaterials. Of particular interest, due to the similar polysaccharide biofilms of *S. epidermidis*, is the effect of fluoroquinolones on biofilm production and adherence by *Pseudomonas aeruginosa*. Studies regarding the effect of antibiotics on adherence and biofilm production by *S. epidermidis* are less numerous and the conclusions less clear-cut. All of these studies are limited by one or more of the following factors: (i) the use of strains of *S. epidermidis* with poorly characterized adherence profiles; (ii) the assumption that biofilm formation and adherence are analogous phenomena; and (iii) the use of prolonged biomaterial bacterial incubation times which do not reflect the short contact times that CoNS may have in physiological conditions. In an attempt to avoid these limitations, we initiated the following measures: (i) four strains of well-characterized *S. epidermidis* were used. Each of the clinical isolates came from a patient with a well-defined infection and serves as a prototypical strain expressing an identified adhesin. RP62A is known to produce biofilm and express SAA, an antigen associated with adherence and biofilm production. Strain 1457 also elaborates biofilm and produces PIA which is important in the formation of multi-layer macrocolonies seen in the later stages of adherence. Lastly, SE5 is a biofilm-producing strain that produces a haemagglutinin, which appears to be important in the initial stages of adherence. (ii) Separate assays were used to define attachment and biofilm formation. Attachment to intravascular catheters was measured directly by detecting attachment of radiolabelled bacteria. Biofilm formation was measured using a previously validated spectrophotometric assay. (iii) A short bacteria–biomaterial incubation period (15 min) was used in the adherence
also surgically implanted prosthetic devices are at greatest risk for bacterial colonization at the time of implantation, which is generally brief. Shortly after implantation, prosthetic materials are coated by a host-derived conditioning film, consisting of a variety of proteins and glycoproteins, which alter the bacteria–biomaterial interaction. Consequently, using longer incubation times, as some investigators have done, without addition of these substances, is not analogous to physiological conditions. However, the effect of the host-derived conditioning film on biomaterials is less important in the adherence of S. epidermidis than in Staphylococcus aureus, which is known to have specific binding proteins for a variety of serum components and connective tissue moieties.

The study antibiotics were chosen for a number of reasons. The active fluoroquinolones ofloxacin and l- ofloxacin were chosen because of the interesting activity of fluoroquinolones on biofilm elaboration and adherence by Gram-negative bacilli. Also, there are limited data suggesting that fluoroquinolones decrease biofilm production by CoNS. The bacteriologically inactive d-isomer of ofloxacin was studied to ascertain whether an effect on biofilm production or adherence was due to properties of the drug other than its antibacterial properties, such as effects on hydrophobicity, electrostatic interactions or other nonspecific forces. Lastly, because first-generation cephalosporins and glycopeptide antibiotics are frequently used to treat patients with staphylococcal infections and as prophylactic agents during the implantation of prosthetic devices, cefazolin and vancomycin were chosen for study.

Our data demonstrate that biofilm formation and adherence are distinct phenomena. Overall, there was no significant effect of the antibiotics on the early phase of adherence. Conversely, cefazolin and, to a somewhat lesser extent, vancomycin, significantly decreased biofilm elaboration. These observations support the morphological studies of Peters et al., in which staphylococci adherent to catheters were devoid of demonstrable biofilm 12 h after adherence. Our results support the belief that S. epidermidis biofilm formation is important in the later, aggregative phases of adherence and may protect the staphylococcal colony from a hostile environment (host defence cells, antibiotics) or improve the local nutritional environment. Although cefazolin and vancomycin do not affect the initial phases of adherence, they do affect biofilm elaboration and, thus, may play a role in treating or preventing the evolution and maturation of the staphylococcal macrocolony on a biomaterial.

In conclusion, these studies indicate that adherence and biofilm production are distinct processes. Sublethal concentrations of the tested fluoroquinolones, glycopeptides and cephalosporins have no effect on the initial adherence events. Although there was some minor strain-to-strain variation, sublethal concentrations of the fluoroquinolones do not influence biofilm elaboration, while cefazolin and to a lesser extent, vancomycin, greatly reduce biofilm elaboration by S. epidermidis. This may prevent the final aggregative stages of S. epidermidis adherence and may have clinical utility in this role.

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