Biomaterial-associated infection: peri-implant tissue is an important niche for Staphylococcus epidermidis survival
Broekhuizen, C.A.N.

Citation for published version (APA):
Broekhuizen, C. A. N. (2009). Biomaterial-associated infection: peri-implant tissue is an important niche for Staphylococcus epidermidis survival

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER

Tissue around catheters is a niche for bacteria associated with medical device infection
Tissue around catheters is a niche for bacteria associated with medical device infection

Corine A. N. Broekhuizen¹, Marcus J. Schultz²,³, Allard C. van der Wal⁴, Laura Boszhard¹, Leonie de Boer¹, Christina M. J. E. Vandenbroucke-Grauls¹,⁵, Sebastian A. J. Zaat¹

¹ Department of Medical Microbiology, Center of Infection and Immunity Amsterdam (CINIMA),

² Department of Intensive Care Medicine,

³ Laboratory for Experimental Intensive Care and Anesthesiology,

⁴ Department of Pathology, Academic Medical Center,

⁵ Department of Medical Microbiology and Infectious Diseases, VU University Medical Center, Amsterdam, The Netherlands

Critical Care Medicine 2008; 36:2395–2402
Tissue around catheters is a niche for bacteria

ABSTRACT

Objective: To investigate whether peri-catheter tissue is an additional niche for bacteria potentially causing catheter-associated infections in humans.  
Design: Postmortem patient study.  
Setting: Intensive care unit, autopsy room, and microbiological laboratory in a university hospital.  
Patients: Eighteen deceased patients from whom 35 catheters plus surrounding tissues were collected.  
Interventions: Under axenic conditions catheters and surrounding tissue were excised from deceased intensive care unit patients. The excised parts of the catheters and samples of surrounding tissue were quantitatively cultured and bacteria identified, and tissue histology/immunohistochemistry was performed.  
Measurements and main results: Nine of the 35 (26%) peri-catheter tissue samples tested were highly culture positive. The corresponding catheter segments were culture negative or yielded only low numbers of bacteria. Bacteria cultured from different sites of the catheter and surrounding tissues almost all were coagulase-negative staphylococci (predominantly Staphylococcus epidermidis) and Enterococcus faecalis. In histology, bacteria were seen in tissue, intercellularly and associated with host phagocytes.  
Conclusions: Tissue surrounding biomedical devices forms a niche for bacteria. This is an as yet nonrecognized element in the pathogenesis of catheter-associated infections, with possible consequences for strategies of prevention and treatment of these infections.

INTRODUCTION

In modern medicine biomaterial-associated infections, such as infections of prosthetic devices and catheters, have become a significant problem, with incidence of infection ranging between 3% and 7% for central venous catheters \(^1\). These infections are mostly caused by coagulase-negative staphylococci, in particular by Staphylococcus epidermidis \(^2-5\). They often require extensive antibiotic treatment and in a considerable number of cases removal of the catheter.
The major hypothesis for the pathogenesis of these infections is that the bacteria adhere to the biomaterial, produce large amounts of exopolysaccharides, and thereby form a biofilm on the surface of the material. Bacteria within these biofilms are not reached by host phagocytes and are not effectively cleared by antibiotics. In *S. epidermidis* experimental biomaterial-associated infection (BAI) in mice, however, only low numbers of bacteria are recovered from the implants themselves, but high numbers are present in the tissue surrounding the implant (peri-implant tissue). This is the case in different mouse strains, infected with different *S. epidermidis* strains, and around biomaterials with different physicochemical characteristics.

Similarly, *S. epidermidis* is recovered from peri-implant tissue in rabbit experimental BAI.

To investigate whether tissue around implanted biomaterials might be an as yet unrecognized niche for bacteria with the capacity to cause biomaterial-associated infection in humans, we investigated whether bacteria were present in tissues surrounding central venous catheter and arterial catheters with no apparent signs of infection. To this purpose, central venous catheters and/or arterial catheters and surrounding tissues excised at autopsy from patients who died in the intensive care unit were analyzed by culture and histology.

**PATIENTS AND METHODS**

**Patients and catheters.** The central venous catheter and surrounding tissue (peri-catheter tissue) were excised from deceased adult patients from the intensive care unit of the Academic Medical Center in Amsterdam, The Netherlands, who were elected for autopsy. The need for review by the Institutional Review Board of studies involving deceased patients was waived by the Dutch Central Committee involving Human Subjects (CCMO), provided that informed consent for autopsy and removal and preserving of tissues for scientific research was given by the next of kin, in accordance with Dutch law. Such informed consent was given by the next of kin. Patients diagnosed with human immunodeficiency virus/acquired immunodeficiency syndrome were excluded. Tissue samples and catheters were excised as soon as possible after the patient had died, before the actual autopsy. Excised catheters were all nontunneled catheters of the following types: chlorohexidine acetate and silver sulfadiazinecoated trilumen and quad-lumen catheters (both...
polyurethane) from Arrow International (Reading PA), and non-coated arterial sheet (polyurethane; Arrow), arterial lines (polyvinylchloride [PVC] and polycarbonate; Edwards Lifesciences, Irvine CA), continuous veno-venous hemofiltration catheters (CVVH, PVC; Edwards Lifesciences, Harleysville PA, USA), Swan-Ganz catheters (PVC; Edwards Lifesciences, Irvine CA), and an intra-aortic balloon pump sheet (IABP) (polyurethane; Arrow).

**Controlled excision procedure.** Peripheral blood was collected from the vena femoralis after careful skin disinfection with iodine, to assess bacteremia. An open blood agar plate was placed on the abdomen of the deceased patient for the entire duration of the excision procedure, as a control for contamination from the environment. Under axenic conditions the catheter dressing was removed, and the skin under the dressing was swabbed for culture before and after disinfection with iodine solution. The skin around the catheter was removed using a sterile scalpel and placed on ice. A swab was taken from the subcutaneous tissue now exposed, avoiding contact with the remaining skin. The wound was spread with a sterile self-retaining skin retractor and the catheter and surrounding tissue were excised with a new sterile scalpel. The tissue biopsy excised (approximately 2–3 cm in diameter and 4–8 cm in length) contained subcutaneous and deeper tissue surrounding the catheter. The blood vessel was not part of the biopsy. When possible, the entire catheter was retracted together with the excised tissue, as shown in Figure 1. All collected materials and swabs were placed in sterile containers and kept on ice to be processed and cultured shortly after the excision procedure. After the excision the defect was closed.

**Sample processing and culture.** All steps in the sample processing were performed in a laminar flow cabinet. Sterile gloves were used throughout. About 50 µl of peripheral blood was streaked on blood agar plates, and 50 µl was cultured in Brewer Tween broth (BT), containing 3% (w/v) thioglycolate, 0.03% (w/v) polyanetholesulfonic acid, and 0.5% Tween 80, adjusted to pH 7.6 with 1M NaOH. Swabs were streaked on blood agar plates for culture. The biopsies were cut in slices of 1 cm perpendicular to the skin surface. Alternating slices were cultured to assess bacterial colonization in spatially separated samples, i.e., the 2\textsuperscript{nd}, 4\textsuperscript{th}, and 6\textsuperscript{th} level slices were not cultured (Figure 1). The 2\textsuperscript{nd} level slices were used for histologic examination (see below). For
culture, the slices were cut in pieces of approximately 1 cm³. The catheter segments were carefully separated from the tissue and rolled on blood agar plates, according to the roll-plate method. Subsequently, they were placed in 1.5 ml vials containing 500 µl saline and sonicated for 30 seconds. The sonicate and 10-fold serial dilutions in saline were cultured on blood agar plates (quantitative culture). The segment itself was placed in Brewer Tween. The Tween in the Brewer Tween broth lyses cells, allowing intracellular bacteria to grow.

Figure 1: Culture results from the nine catheters of eight patients with culture-positive catheter segments and biopsies of the surrounding tissue. Before excision of the catheter and the surrounding tissue, the skin was removed and cultured separately. The excised tissue biopsy (approximately 2–3 cm in diameter and 4–8 cm in length) contained subcutaneous and deeper tissue surrounding the catheter, but did not include the catheterized blood vessel. Alternating biopsy levels were cultured, in order to avoid culturing of contacting samples. The numbers 1 or 3 indicate the levels of the samples in the biopsies referred to as 1st or 3rd level in the text. Bacteria retrieved from 1st and 3rd level biopsies were further analyzed (Table 1). For patient 1, the 1st level cultured is one layer deeper in the tissue compared with the other patients. The
Tissue around catheters is a niche for bacteria

2nd biopsy level tissue (not indicated) was subjected to histologic examination. FL, left arteria femoralis or left vena femoralis; FR, right vena femoralis. Samples with ≥ 5 colony-forming units (cfu) were defined as culture positive (see text).

The tissue samples were weighed (weights ranged from 100 to 900 mg) and a volume of saline corresponding to two times the weight was added. Then tissue samples were homogenized (Tissue Tearor, model 985–370, Biospec products, Bartlesville, UK) and cultured quantitatively on blood agar plates. About 50 µl of homogenate was cultured in Brewer Tween liquid broth. All cultures were incubated at 37°C until growth was visible, in most cases after 1 or 2 days; if no growth occurred, they were discarded after 14 days.

Identification of microorganisms. Microbiological identification was performed by routine methods. In addition 16S rDNA sequencing was performed with primers pf515 and p13b [17]. rDNA sequences were analyzed using the BLAST algorithm [18] [at www.ncbi.nlm.nih.gov/blast and http://rdp.cme.msu.edu]. For further identification of selected coagulase-negative staphylococci and enterococci, API Staph and API Strep tests (BioMerieux, Lyon, France), respectively, were used.

Histology and immunohistochemistry. For histologic confirmation of the microbiological findings, the 2nd level slices of the biopsies (see above) were fixed in formaldehyde and embedded in plastic (methy1methacrylate / buthylmethacrylate; Merck Schuchart, Hohenbrunn, Germany). The samples from six representative biopsies were selected, and 3-5 µm sections were cut at different depths. These sections were stained with hematoxylin-eosin (H-E) and examined by light microscopy.

Immunohistochemical staining to detect *S. epidermidis* and other gram-positive bacteria was performed on selected slides using monoclonal antibodies against *Staphylococcus epidermidis* lipoteichoic acid (anti-LTA; QED, Bioscience Ltd. San Diego, CA) [12]. This antibody recognizes *S. epidermidis, Staphylococcus aureus,* and *Enterococcus faecalis* in tissue sections (not shown). Sections were deplastified, fixed by incubation in 2% paraformaldehyde for 15 mins, rinsed with excess demi water, and cooked in an autoclave pan for 20 mins in 10 mM Tris EDTA buffer. For light microscopic immunohistology, the sections were incubated with 3.5 µg/mL anti-LTA.
for 60 min at room temperature, rinsed three times with phosphate buffered saline, and incubated for 30 min with an IgG1-specific horseradish peroxidase-conjugated secondary antibody (ImmunoVision Technologies, Brisbane, CA). Peroxidase activity was detected using 3,3-diamino benzidine tetrachloride staining. The slides were counterstained with hematoxylin. For fluorescence microscopy, the section was deplastified, fixed and cooked as described above, incubated in sodiumborohydride solution (1 mg/mL) to reduce autofluorescence, and rinsed with phosphate buffered saline. The anti-LTA antibody was labeled using a Zenon-alexa-488 fluor mouse IgG1 labeling kit (Invitrogen-Molecular Probes, Breda, The Netherlands) according to the manufacturer’s protocol and applied to the sections at a concentration of 2.5 µg/mL. After 60 min incubation and rinsing in phosphate buffered saline, sections were inspected with a Leica DMRA Fluorescence microscope (Leica, Rijswijk, The Netherlands).

Statistics. Differences in numbers of colony-forming units (cfu) were analyzed with the Mann-Whitney test. p < 0.05 was considered significant.

RESULTS

Patients and catheters. A total of 35 catheters were excised from 18 patients, 12 male and 6 female. The patients were cooled at 4°C as soon as possible after death (median 2 h 55 min, range 2–6 h) and prepared for autopsy within a mean period of 22 h (median 18 h, range 4–63 h) after death (Table 1). With the exception of one patient, all patients were sampled within 42 h after death, and all before opening of the chest for autopsy. The femoral vein was the preferred location for the catheters (66%), followed by the subclavian vein (31%) and the jugular vein (3%). Thirteen tri-lumen, 1 quatro-lumen, 1 arterial sheet, 5 arterial lines, 11 catheters for venovenous hemofiltration, 2 Swan Ganz catheters, and 2 intra-aortic balloon pump catheters had been used. These catheters had been in place for a mean period of 133 h (median 127, range 4–432 h) at the time of death.
<table>
<thead>
<tr>
<th>Pt</th>
<th>Time to 4°C</th>
<th>Time to sampling</th>
<th>Cath site</th>
<th>Cath type</th>
<th>Days in place</th>
<th>Total no. of cfu</th>
<th>Subcut. swab</th>
<th>1st level biopsy</th>
<th>2nd level biopsy</th>
<th>Roll tip (Pos/Neg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2h30m</td>
<td>14h45m</td>
<td>FL</td>
<td>Arsenal sheath</td>
<td>&gt;16d</td>
<td>4x10⁹</td>
<td></td>
<td>S. epidermidis; E. faecalis</td>
<td>1x10⁹</td>
<td>2x10⁹</td>
</tr>
<tr>
<td>2</td>
<td>2h30m</td>
<td>14h45m</td>
<td>FR</td>
<td>CVVIH</td>
<td>&gt;6d</td>
<td>5x10⁸</td>
<td></td>
<td>S. epidermidis; S. faecalis</td>
<td>4x10⁹</td>
<td>5x10³</td>
</tr>
<tr>
<td>3</td>
<td>3h05m</td>
<td>34h33m</td>
<td>SR</td>
<td>Swan Giant</td>
<td>167h</td>
<td>8x10⁹</td>
<td></td>
<td>Proteus mirabilis; E. faecalis</td>
<td>3x10⁹</td>
<td>10¹</td>
</tr>
<tr>
<td>4</td>
<td>2h55m</td>
<td>10h25m</td>
<td>FR</td>
<td>CVVIH</td>
<td>608h</td>
<td>3x10⁹</td>
<td></td>
<td>3x10⁹</td>
<td></td>
<td>10¹</td>
</tr>
<tr>
<td>5</td>
<td>2h00m</td>
<td>40h32m</td>
<td>FL</td>
<td>CVVIH</td>
<td>646h</td>
<td>6x10⁹</td>
<td></td>
<td>S. epidermidis</td>
<td>2x10⁸</td>
<td>1x10⁹</td>
</tr>
<tr>
<td>6</td>
<td>6h00m</td>
<td>63h30m</td>
<td>FR</td>
<td>Trimamen</td>
<td>60h</td>
<td>3x10⁹</td>
<td></td>
<td>S. epidermidis; S. aureus</td>
<td>1x10⁴</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4h35m</td>
<td>17h54m</td>
<td>FR</td>
<td>CVVIH</td>
<td>&gt;8d</td>
<td>10³</td>
<td></td>
<td></td>
<td>2x10³</td>
<td>2x10³</td>
</tr>
<tr>
<td>8</td>
<td>2h20m</td>
<td>38h30m</td>
<td>FL</td>
<td>Trimamen</td>
<td>46h</td>
<td>2x10³</td>
<td></td>
<td>4x10⁸</td>
<td>S. epidermidis; E. faecalis</td>
<td>2x10³</td>
</tr>
</tbody>
</table>

Table 1

Bacterial species and numbers of CFU cultured from skin biopsy, subcut. swab, and tissue from catheter segment (class segment) and tissue surrounding tissue.

Tissue around catheters is a niche for bacteria.
Table 1 (p.119): Summary of the cultures of samples from the 8 patients with culture positive tissue biopsies and catheter segments. Pt, patient; time to 4°C, period between death and cooling of the corps to 4°C; time to sampling: period between death and excision of catheter and surrounding tissue; Cath. site, catheter site; FL, left arteria femoralis or left vena femoralis; FR, right vena femoralis; SL, left vena subclavia; SR, right vena subclavia; ND, not done; CVVH, continuous venovenous hemofiltration catheters; IABP, intra-aortic balloon pump sheet, cfu, colony-forming units; Pos, positive; Neg, negative. Peripheral blood cultures were all negative (< 5 cfu), as were ante-mortem blood cultures at 1 week before death.

Controlled excision procedure. Table 2 summarizes the culture results for the samples obtained by the excision procedure. The control agar plate placed on the abdomen during the excision procedure never yielded more than 5 cfu of the same colony type. Therefore, 5 cfu of the same colony type was set as the cut-off for culture-positivity for all samples. The peripheral blood culture was positive in one patient, but the cultures of catheters and tissues of this patient were negative. No positive antemortem blood cultures had been reported for any of the patients. Before disinfection, the skin under the catheter dressings was culture-positive in 47% of cases, and disinfection was effective for all but one catheter site. Forty-one percent of the homogenates of skin biopsies were culture positive. The swabs of the subcutaneous tissue yielded growth in five patients, whereas in two patients the swab was negative while the skin and underlying tissue yielded growth (Tables 1 and 2). Culture positive tissue of the 1st as well as of the 3rd level in the biopsies contained higher numbers of cfu than the corresponding catheter segments (Table 1, Figure 2).

Culture positive catheters and peri-catheter tissue. Femoral and subclavian catheters had been in place for a mean period of 141 h (median 110, range 26-432 h) and 127 h (median 127, range 4–283 h), respectively (not significant [NS]). Eight of 23 (35%) femoral vein catheters or surrounding tissues were colonized, versus 1 (9%) of 11 subclavian catheters (NS). Thirteen of the 35 catheters were antimicrobially coated, being five femoral and eight subclavian catheters. Two of these 13 catheters and their surrounding tissues (15%) yielded positive cultures, versus 8 of 22 (36%) of the noncoated catheters and 9 of 22 (41%) of their surrounding tissues (NS).
Tissue around catheters is a niche for bacteria

Table 2: Culture results of samples from all 18 patients. Numbers of the different samples analyzed are not identical since not all samples were available from each patient.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of positive cultures / number analysed (%) (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open plate on abdomen</td>
<td>0/14 (0)</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>1/16 (6)</td>
</tr>
<tr>
<td>Insertion site prior to disinfection</td>
<td>14/30 (47)</td>
</tr>
<tr>
<td>Insertion site after disinfection</td>
<td>1/30 (3)</td>
</tr>
<tr>
<td>Skin biopsy</td>
<td>12/29 (41)</td>
</tr>
<tr>
<td>Subcutaneous tissue swab</td>
<td>5/30 (17)</td>
</tr>
<tr>
<td>First catheter segment</td>
<td>8/35 (23)</td>
</tr>
<tr>
<td>First tissue biopsy</td>
<td>9/35 (26)</td>
</tr>
<tr>
<td>Third catheter segment</td>
<td>7/35 (20)</td>
</tr>
<tr>
<td>Third tissue biopsy</td>
<td>9/35 (26)</td>
</tr>
<tr>
<td>Catheter tip</td>
<td>3/28 (11)</td>
</tr>
</tbody>
</table>

**Culture positive catheters and peri-catheter tissue.** Femoral and subclavian catheters had been in place for a mean period of 141 h (median 110, range 26–432 h) and 127 h (median 127, range 4–283 h), respectively (not significant [NS]). Eight of 23 (35%) femoral vein catheters or surrounding tissues were colonized, versus 1 (9%) of 11 subclavian catheters (NS). Thirteen of the 35 catheters were antimicrobially coated, being five femoral and eight subclavian catheters. Two of these 13 catheters and their surrounding tissues (15%) yielded positive cultures, versus 8 of 22 (36%) of the non-coated catheters and 9 of 22 (41%) of their surrounding tissues (NS).

**Bacterial species retrieved from catheters and surrounding tissue.** Bacteria retrieved from different locations (skin biopsy, subcutaneous swab, peri-catheter tissue, or catheter) almost all were coagulase-negative staphylococci (predominantly *S. epidermidis*) or *E. faecalis* (Table 1). Five explanted tissue / catheter biopsies from four patients yielded both *S. epidermidis* and *E. faecalis*, in the case of the femoral catheter of patient two combined with *Proteus mirabilis*. Patients 5, 6, and 7 mainly had *S. epidermidis* in the tissue biopsies and on their catheter segments. Two patients had *S. epidermidis* on the catheter segments but enterococci in the surrounding tissue.

**Localization of bacteria.** Bacteria were retrieved from the tissues of nine catheter sites of eight patients. In six of these patients additional catheters were present, from
which all samples were negative, indicating that bacterial colonization was restricted to only one site. In general, large numbers of bacteria were cultured from tissue biopsies and only low numbers, if any, from the corresponding catheter segments (Figures 1, 2, and Table 1). Bacteria were also cultured from parts of the biopsies not bordering the catheters (Figure 1). Tip cultures of three patients were positive (Table 1, Figure 2).

The skin biopsies of patients 1, 2, 3, 5, 7, and 8 contained large numbers of bacteria (≥ 10^3 cfu) of the same species as cultured from the subcutaneous tissue, predominantly *S. epidermidis* and *E. faecalis* (Table 1). The subcutaneous tissue swabs yielded no bacteria (patient 4 and 6) or only relatively low numbers of bacteria (patients 2, 3, 5, and 8). In patient 7, the subcutaneous swab yielded > 10^3 bacteria, but the subcutaneous tissue samples, including those from the deeper tissue (3rd level biopsy, Table 1, Figure 1), contained very high numbers of bacteria. Apparently, in this patient both the skin and the subcutaneous tissue were highly colonized. Patient 1 had two femoral lines. The 1st and 3rd level tissue biopsies and catheter segments of the left femoral catheter (patient 1) yielded *S. epidermidis* and *E. faecalis*. Even though no blood cultures and no skin biopsy cultures were available from this patient, the high numbers of bacteria cultured from the tissue strongly suggest that the tissue must have been colonized. The right femoral catheter of this patient yielded *S. aureus* and *E. faecalis* in the 1st level tissue biopsy and *S. epidermidis* and *E. faecalis* in the 3rd level. From the catheter segments, *S. epidermidis* and *E. faecalis* were retrieved, with *S. epidermidis* also being present on the catheter tip.

Bacteria were cultured from tissue samples bordering, but also in large numbers from samples not bordering, the catheters (Figure 1). Of the 1st level biopsies, most samples with positive tissue cultures also had culture positive catheters. For the 3rd level biopsies, in two of nine cases, only the tissue was culture positive (Figure 2; ≥ 5 cfu), and in another three cases, the numbers of cfu cultured from the catheter segments was between 5 and 15 cfu. In seven of nine cases, the subcutaneous tissue from the 3rd level biopsy contained ≥ 10^3 cfu (Table 1).

**Histology.** Sections of the tissue surrounding catheters showed fibrin depositions directly adjacent to the catheter, extravasations of erythrocytes indicating small hematomas and a mild inflammatory response characterized by the presence of sparse neutrophils (aspecific acute inflammatory response; see Figure 3A).
Tissue around catheters is a niche for bacteria

In several cases there was an additional early reparative response of ingrowth of young (swollen) fibroblasts. Immunolabeling with anti-LTA antibodies showed positive staining of coccoid structures with the size of bacteria (1–2 µm diameter) either intracytoplasm in inflammatory cells or in the interstitium (Figure 3B–D). This was confirmed by immunofluorescence microscopy using fluorescently labeled anti-LTA antibodies (Figure 3E–H). Of the inspected samples from patients 1 (right), 3, 5, 6, 7, and 8 (Figure 1), each of one or two inspected sections were positive in immunofluorescence.

Figure 2: Numbers of colony-forming units (cfu) cultured from catheters (cfu / segment) and tissue biopsies (cfu / surrounding tissue). The horizontal lines represent the median values. The dotted line indicates the cut-off for culture positivity (5 cfu). *p < 0.05.
DISCUSSION

We describe a study in which we removed both catheters and surrounding subcutaneous tissues of deceased intensive care unit patients. Nine of 35 catheters and surrounding tissue were culture-positive, and the tissue was more highly colonized than the catheters themselves. Bacteria cultured were identified as coagulase-negative staphylococci, predominantly *S. epidermidis*, and enterococci. In histology and immunohistochemistry, we detected bacteria within the tissue of the patients, in several cases associated with host phagocytic cells.

Three catheter tip cultures yielded growth, but there had been no clinical
signs of catheter-related bloodstream infections before death, and antemortem blood cultures had been negative. Such culture positivity of catheter tips in the absence of clinical infection has been reported before 19-21. Five peri-catheter tissue cultures were positive, whereas the catheter tip cultures were negative, indicating that catheter tip culture results did not correlate with presence of bacteria within the subcutaneous tissue.

Less than half of the cultures of the skin surrounding the catheter insertion site after removal of the wound dressing and before disinfection were positive. This is not different from what is described by Bouza et al. 22 and indicates that skin colonization was not unusually high in our group of deceased patients.

The number of samples we retrieved did not allow stratification for catheter type, implantation site, or duration of catheterization. Despite this, a few remarks can be made. Studies on the efficacy of antimicrobially coated catheters to prevent catheter-related bloodstream infection show conflicting results 23-27. Thirty-seven percent of the catheters in our study were antimicrobially coated. The non-coated catheters and their surrounding tissue were more often culture-positive than the coated catheters (36% versus 15%, NS). This suggests a possible contribution of the antimicrobial coating of the catheters to reduction of colonization, although it cannot be ruled out that the antiseptics of the coating might have suppressed the growth of bacteria in the roll-plate assay 28.

In line with other reports 29,30 femoral catheters were more often culture positive (35%) than subclavian catheters (9%). No difference in levels of catheter or tissue colonization was found for catheters that had been in situ for different periods.

Our excision procedure involved cutting through skin that may have contained bacteria. We therefore applied a highly stringent procedure of first removing the skin, spreading the wound, and subsequently excising the subcutaneous tissue sample with a new sterile scalpel, carefully avoiding to contact the remaining skin. This way, only a very small proportion of the numbers of bacteria present within the skin were expected to be introduced into the underlying tissue. Indeed, the swabs taken after removal of the skin and before excision of the biopsy yielded low numbers of bacteria in all but one case. In this latter case, both the skin and the underlying tissue contained high numbers of bacteria. The biopsies yielded higher numbers of bacteria than cultured from the entire skin tissue sample, implying that these bacteria must
have been present within the subcutaneous tissue before excision, and were not introduced during the sampling. We did not perform control excisions from areas of the body without inserted catheters in all patients. However, all tissue samples from control excisions from the groin area of three patients, sites where no catheter had been inserted, were culture negative (not shown).

Immunohistochemistry with anti-LTA antibodies that recognize the gram-positive bacterial species cultured from the patients showed bacteria within the tissue, proving that these bacteria had not been introduced during sampling. This was observed in all of the six patient samples analyzed. Moreover, bacteria were also seen in association with host phagocytes, indicating that these bacteria must have been present antemortem, and that the host immune system had responded to their presence in the tissue.

The bacterial colonization of a patient may change after death. The value of postmortem microbiological findings has been evaluated in the expert review “Cumitech Postmortem Microbiology” of the American Society for Microbiology, which aims to provide consensus recommendations regarding this specific area of clinical microbiology. According to this Cumitech, the risk of obtaining false-positive postmortem culture results is low, provided that the body temperature is lowered to 4°C within the first hours after death, and if autopsy is performed within 48 h and samples are obtained before manipulation of the gastrointestinal tract. For all our patients except one (autopsy at 63 h postmortem, but cooled within 6 h), these criteria were met. Moreover, in addition to the culture-positive catheter / surrounding tissue, all except patient 1 had at least one catheter / surrounding tissue that was culture-negative, indicating that no generalized postmortem bacterial growth had occurred.

Bacterial species cultured from the skin, subcutaneous tissue, and catheter segments were predominantly *S. epidermidis* and *E. faecalis*. *E. faecalis* is reported as the second most prevalent bacterial species in catheter and other medical device-related infections, after *S. epidermidis* and other coagulase-negative staphylococci. Mixed populations of both species simultaneously adhering to catheters have been reported previously, but colonization of surrounding tissues has to the best of our knowledge never been reported before.

Bacterial growth in tissue surrounding inserted or implanted medical devices has not been the subject of many studies yet. Virden *et al.* analyzed
Tissue around catheters is a niche for bacteria

tissues surrounding breast implants, which had been removed because of infectious complications. *S. epidermidis* was the species most often cultured and was also cultured from tissue surrounding a culture-negative implant. These data are in line with our observations in mice \textsuperscript{11-14} and with our present study. Apparently, bacteria such as *S. epidermidis* and *E. faecalis* are capable of colonizing tissue in the vicinity of an inserted or implanted foreign body, presumably due to local impairment of the host immune function \textsuperscript{11,13,42,43}. Survival in tissue may also depend on virulence (genes) of the colonizing bacterial species. The virulence gene regulator system *agr* (accessory gene regulator) in *S. epidermidis* seems required for survival in tissues, since *agr* mutants are less capable of surviving in rabbit peri-implant tissue than are wild type *S. epidermidis* \textsuperscript{15}.

The presence of high numbers of bacteria in subcutaneous tissue at catheter insertion sites may partially explain the relatively high frequency of infection when catheters are inserted at or in the vicinity of former catheter tunnels, for instance by exchange over a guidewire \textsuperscript{44-46}, and underline that repeated catheter insertion at the same site should be avoided.

**ACKNOWLEDGMENTS**

We thank the physicians of the intensive care unit of the Academic Medical Center in Amsterdam, The Netherlands, for their cooperation, René Sijmons, Rob Poeser, and the other autopsy assistants for their help and guidance, and Jos Mulder and Jan Stap for immunohistochemistry and advice on immunofluorescence microscopy.

**REFERENCES**


