Spread of Coagulase-Negative Staphylococci During Cardiac Operations in a Modern Operating Room

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Background. Coagulase-negative staphylococci cause 33% to 62.5% of wound infections after cardiac operations. The aim of this study was to investigate the sources of coagulase-negative staphylococci in the sternal wound.

Methods. Twenty operations performed in zonal ventilated operating rooms were investigated prospectively. Cultures were taken from all persons present in the room, the sternal wound, and the air. Isolates macroscopically judged to be coagulase-negative staphylococci were metabolically classified, and similar isolates were investigated by pulsed-field gel electrophoresis.

Results. Bacterial counts in the operating room air were very low. Wound contamination was found in 13 of 20 operations. Six wound isolates could be traced, three to the patients' sternal skin, one to the patient's groin, one to the surgeon's nose, and one to the surgeon's arm and forehead and the assistant's nose. Three operating field air cultures could be traced to the scrubbed theatre staff. The single case of superficial sternal wound infection was caused by Staphylococcus aureus, which was not isolated from the wound at operation.

Conclusions. In an ultraclean environment, bacteria in the sternal wound originated from the patients' own skin and from the surgical team.
ment or plasty, and 3 patients had combined coronary artery bypass grafting and valve replacement. The median operating time was 165 minutes (range; 73 to 294 minutes).

Preoperative hair shortening was performed with electric cutters, and the patient was washed three times with chlorhexidine soap 4% (Zeneca, Göteborg, Sweden). Prophylactic antibiotic treatment was given with dicloxacillin 1.5 g intravenously at the start of the operation. An additional dose was given 4 hours later. All three operating theaters were fitted with high-efficiency particulate air-filtered vertical laminar airflow operating room ventilation [16]. Airflow in the central zone was 0.4 m/second, and in the peripheral zone 0.2 m/second. The staff wore special scrub suits with short sleeves and elastic cuffs at the neck, sleeves and trouser legs, made of tightly woven, fluorocarbon-treated cotton and polyester [17].

Cultures From the Patient
Before washing and draping in the operating room, cultures were taken with a cotton swab from the patient from the nose, presternal skin, and groin. The operating field was then washed with 0.5% chlorhexidine in 70% ethanol and draped with disposable drapes. Wound cultures were taken with absorbent polyvinyl alcohol foam pads from the open sternal wound edges, in the subcutaneous fat. For the first 11 patients the cultures were taken with five 10-mm diameter circular pads, and for patients 12 to 20, with two pads, 79 × 29 mm (Mölnlycke Clinical Products AB, Mölnlycke, Sweden). These pads have recovered high microbial counts in skin samplings [18].

Cultures From Operating Room Personnel
Cultures were taken from all persons present in the operating room. The number of scrubbed staff ranged from three to five persons, and the nonscrubbed staff ranged from seven to 14. From the scrubbed staff, cultures were taken with cotton swabs from the forehead, the forearm, the nose, and the groin, before scrubbing. Cultures were obtained from the nose and groin of all nonscrubbed staff, with cotton swabs. All staff in the operating room wore caps and facemasks. The scrubbed staff wore helmets, facemasks, double sterile gloves, and disposable operating gowns.

Cultures From Air
Air samples were taken with the Sartorius MD8 filter sampler (Sartorius GmbH, Göttingen, Germany) with an airflow of 6 m³/hour, for a duration of 10 minutes at six different times during the operation [19]. The samples were taken from the air immediately above the open sternal wound. Gelatin Disposable Filters with a 3-μm retention rate were used (Sartorius AG, Göttingen, Germany). Four 14-cm diameter sedimentation plates were placed in the peripheral zone in the operating theater at the beginning of the operation and remained in place until the skin of the sternal wound was closed.

Further Handling of Cultures
After the end of the operation, cultures were carried to the adjacent hospital laboratory and handled by accredited methods. Swabs were placed in Stuart’s transport medium and plated on horse blood agar on arrival in the laboratory, where they were incubated aerobically for 48 hours at 36°C, as were the sedimentation plates. All six Sartorius air filters were placed on horse blood agar plates during the operation and cultured for 48 hours at 36°C, three aerobically and three anaerobically. The absorbent polyvinyl alcohol foam pads were placed in a sterile container and transported to the laboratory within 20 minutes; the smaller pads were immersed in 10 mL of peptone water in test tubes and the larger pads in 50 mL in polyethylene bags. The tubes were vibrated for 10 minutes at 25 MHz in an ultrasonic cleaning bath (Langford Ultrasonics Ltd, Birmingham, United Kingdom). The bags were blended for 1 minute in a Stomacher Lab-Blender (Seaward Laboratories, London, United Kingdom).

From the tubes, three 10-μL samples were taken with a sterile loop and three 100-μL samples were taken with a calibrated pipette. From the bags, three 100-μL samples were taken with a calibrated pipette and three × 10 mL was filtered through cellulose nitrate filters with a pore size of 0.45 μm (Sartorius AG, Göttingen, Germany). The fluid samples and filters were cultured on horse blood agar, hematin agar, and anaerobic horse blood agar plates. The plates were incubated for 48 hours at 36°C in air, carbon dioxide, and an anaerobic jar, respectively.

Experienced laboratory staff read all cultures. Up to ten colonies per plate of suspected CoNS with different morphologic characteristics were subcultivated on individual blood agar plates at 36°C for 24 hours. The isolates were then inoculated in freeze broth and stored at −70°C. Isolates that, on the basis of macroscopic examination of primary or secondary cultures, obviously belonged to other species were discarded.

Metabolic Fingerprinting
The strains were subtyped by metabolic fingerprinting [14]. From each isolate, one colony was cultured in 8 mL of bromthymol blue stock solution 0.11%, and 150 μL were inoculated into wells containing 24 different biochemical reagents by using the PhenePlate system (Bio-Sys inova, Stockholm, Sweden). The color change was measured at 620 nm by a spectrophotometer at 16, 40, and 64 hours. Reference strains were included in each analysis. The reaction patterns were compared using PhPWIN software (BioSys inova, Stockholm, Sweden). Isolates that showed reactions with 96% or more similarity (judged by intensity and speed of reaction) were further analyzed with pulsed-field gel electrophoresis (PFGE).

DNA Fingerprinting-PFGE
Bacterial DNA preparation was performed according to a modification of Goering and Winters [20]. The photographed gels were scanned into a personal computer and
analyzed using the Molecular Analyst/PC Fingerprinting Software (BioRad Laboratories, Hercules, California). Similar strains were also compared by visual evaluation of the gel photographs.

**Protocol for Pulsed-Field Gel Electrophoresis**

One colony from each isolate was suspended in 5 mL of Brain Heart Infusion and was incubated shaking at 36°C overnight. One milliliter was harvested, washed with 0.5 mL Ten buffer (Karolinska Laboratories, Stockholm, Sweden), resuspended in EC buffer, and incubated at 55°C for 1 hour. Half a milliliter of 2% EC SeaPlaque GTG agarose (FMC BioProducts, Rockland, Maine), and 30 μL of Lysostaphin 1 mg/mL (Sigma Chemical Co, St Louis, MO) was added and blocks were cast. The blocks were incubated at 36°C for 1 hour in EC buffer (Karolinska Laboratories, Stockholm, Sweden), then at 55°C in TE buffer (Karolinska Laboratories, Stockholm, Sweden) after which the buffer was changed and the blocks were stored at 4°C until analyzed. In preparation for the electrophoresis, half a block was incubated at room temperature with 3 μL of restriction endonuclease SmaI (Boehringer Mannheim, Mannheim, Germany), 225 μL of distilled water and 25 μL of buffer (Boehringer Mannheim) overnight at room temperature. The blocks were then inserted into 1% SeaKem gold agarose and run in a contour clamped homogenous electric field system CHEF-DR II (BioRad Laboratories, Hercules, California). The gel was run in 0.5 TBE buffer (Karolinska Laboratories, Stockholm, Sweden) for 24 hours at 14°C at 6 V/cm with pulses ranging from 1 to 30 seconds. The gels were then colored using ethidium bromide and photographed.

For statistical analysis, Spearman rank order correlation analysis with the Statistica software was used (Statsoft Inc, Tulsa, OK).

**Results**

**Patient Population**

One patient had superficial wound infection with *Staphylococcus aureus*. At the time of operation *Neisseria mucosa* was found in the sternal wound (operation 5). Three patients had urinary tract infection, with cultures taken at the fourth and fifth postoperative days, (operations 4, 8, and 12). One patient with urinary tract infection also had superficial wound infection at the vein-harvesting site with CoNS and *S aureus* (operation 12). One patient had suspected pneumonia and CoNS growth on temporary pacing wires (operation 7), and 2 patients had fever of unknown origin (operations 10 and 16). There were no signs of wound infection, but all 3 had positive wound cultures at the time of operation. In addition, 2 patients had positive cultures with CoNS of temporary pacing wires. Culture from the surgical incision, taken at the time of operation, grew CoNS in both (operations 13 and 18). One of them had a slight fever but neither developed a wound infection. All 20 patients were discharged, 8 patients to other hospitals, 2 to the cardiology department, 8 to convalescent homes, and 2 to their homes.

**Cultures**

From the 934 cultures, 1,621 isolates with macroscopic properties similar to CoNS were harvested. The bacteria and amounts for each operation are shown in Table 1. The wound culture was positive in 13 of 20 operations. The numbers of bacteria on the pads were median four colony-forming units (cfu/cm²) (range, 0 to 2,904 cfu/cm²). Eight cultures grew only CoNS, four cultures grew CoNS and an additional bacterium, and one culture grew *N mucosa*. Sartorius air cultures were positive in 17 of 20 operations. The median value of CoNS was 0.33 cfu/m³ air (range, 0 to 450 cfu/m³). In two operations *Propionibacterium* species 0.83 cfu/m³ was found in addition to CoNS. The *Propionibacterium* grew on one of six filters in one of the operations, and on two in the second. The sedimentation plates were positive in 19 of 20 operations, median 1.30 cfu/m³ (range; 0–7.24 cfu/m³). The bacteria found were CoNS only in 16 operations, and CoNS and *Bacillus* in three operations. The *Bacillus* was present with one colony on one of four sedimentation plates.

By Spearman rank-order correlation, no correlation was found between bacterial counts in wound and Sartorius air cultures (r = −0.171), nor was there any correlation between Sartorius and sedimentation counts (r = −0.178) or between wound and sedimentation counts (r = 0.151).

**Fingerprinting**

All isolates were metabolically fingerprinted. Sedimentation plate cultures were excluded from further analysis. Two hundred seventy-six isolates that showed reactions with 96% or more similarity were included in the PFGE analysis. Details from operations where CoNS could be traced by PFGE are shown in Table 2. Six wound cultures were traced. Four isolates were traced to the patient’s skin. Two isolates were traced to scrubbed staff, one to a surgeon’s nose and one to a surgeon’s forehead and arm or the assistant’s nose (only minor bands differed) (Fig 1). The cultures from the air above the operating field were traced to scrubbed staff in three operations.

**Comment**

No deep postoperative infections with CoNS occurred during the nine months’ observation period. Because the mediastinitis rate at our clinic is approximately 1%, many operations would have to have been included to find one case of mediastinitis among the study population. This would have significantly strengthened this work, but unfortunately is not feasible. One superficial sternal wound infection with *S aureus* occurred. In the sternal wound culture from this patient’s operation *N mucosa* was found. The culture may have missed *S aureus*, as the efficiency of recovery is about 80% in clean wounds [24]. The wound might also have become infected at a later date, which seems probable because it presented 18 days postoperatively and infection with *S aureus* usually has a shorter incubation period [21].

Twelve of 13 contaminated wounds healed unevent-
fully, including the wound colonized with *S. aureus*, although some of them were heavily contaminated. It is not known what distinguishes the contaminated wound that will become infected from the one that will not.

In our study, four of the traceable sternal wound isolates originated from the patient’s skin, mostly from the sternum. In clean general surgery it has been shown that the more bacteria that colonized the skin, the more likely the contamination of the wound [4]. The most likely route for contamination would be continuous spread. The sterile adhesive plastic drape may become loose during the operation or the skin could have been prepared inadequately, which create a potential for spread of bacteria from the patient’s skin.

Bacteria were transferred from the scrubbed staff to the patients’ wound in two operations although the standard

### Table 1. Type and Amount of Bacteria Found in Wound, Sartorius Air Cultures, and on Sedimentation Plates

<table>
<thead>
<tr>
<th>Operation</th>
<th>Wound culture, bacteria (cfu/cm²)</th>
<th>Sartorius air culture, Median cfu/m³ (range)</th>
<th>Sedimentation plate, Median cfu/m³ (range)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>CoNS 1.29 (0–4)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>CoNS + Micrococi 0.50 (0–2)</td>
<td>CoNS 0.18 (0–1)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>CoNS 0.67 (0–3)</td>
<td>CoNS 1.49 (1–6)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>CoNS 0.17 (0–1)</td>
<td>CoNS 0.87 (1–4)</td>
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<tr>
<td>5</td>
<td><em>Neisseria mucosa</em>, 51</td>
<td>CoNS 0.17 (0–1)</td>
<td>Bacillus 0.12 (1)</td>
</tr>
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<td>6</td>
<td>CoNS, 255</td>
<td>CoNS 0.17 (0–1)</td>
<td>CoNS 1.01 (0–5)</td>
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<tr>
<td>7</td>
<td>CoNS, 306</td>
<td>CoNS 0.67 (0–2)</td>
<td>CoNS 0.76 (0–4)</td>
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<tr>
<td>8</td>
<td>CoNS, 2,803</td>
<td>0</td>
<td>CoNS 4.59 (1–6)</td>
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<td>9</td>
<td>CoNS + <em>Staphylococcus aureus</em>, 76</td>
<td>0</td>
<td>Bacillus 0.21 (1)</td>
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<tr>
<td>10</td>
<td>CoNS, 2,904</td>
<td>CoNS + Micrococi 0.17 (0–1)</td>
<td>CoNS 6.09 (0–18)</td>
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<tr>
<td>11</td>
<td>0</td>
<td>CoNS 0.67 (1)</td>
<td>CoNS 1.19 (0–3)</td>
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<td>12</td>
<td>CoNS + <em>Propionibacterium</em>, 46</td>
<td>CoNS 2.33 (0–13)</td>
<td>Bacillus 0.17 (1)</td>
</tr>
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<td>13</td>
<td>CoNS, 109</td>
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<td>CoNS 0.18 (0–1)</td>
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<tr>
<td>14</td>
<td>0</td>
<td>CoNS 75.00 (0–450)</td>
<td>CoNS 5.16 (0–28)</td>
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<td>CoNS, 5</td>
<td>CoNS 0.17 (0–1)</td>
<td>CoNS 7.24 (0–34)</td>
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<td>16</td>
<td>CoNS, &lt; 1</td>
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<td>CoNS 1.31 (0–3)</td>
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<td>17</td>
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<td>0</td>
</tr>
<tr>
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<td>19</td>
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<td>20</td>
<td>CoNS + Micrococi, 3</td>
<td>CoNS 0.17 (0–1)</td>
<td>CoNS 0.86 (0–3)</td>
</tr>
</tbody>
</table>

**CoNS** = coagulase-negative *staphylococci*;  
**cfu** = colony forming unit.

**Table 2. Sources of Coagulase-Negative Staphylococci in Wound and Air**

<table>
<thead>
<tr>
<th>Operation</th>
<th>Wound*</th>
<th>Patient Sternum</th>
<th>Patient Groin</th>
<th>Surgeon I Nose</th>
<th>Surgeon II Forehead, Forearm</th>
<th>Assistant Nose</th>
<th>Assistant Arm</th>
<th>Scrub Nurse Nose, Groin</th>
<th>Operating Field Air</th>
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<td>5</td>
<td></td>
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<td>Lane 2</td>
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<tr>
<td>10</td>
<td></td>
<td>Lane 8</td>
<td>lane 9</td>
<td>lanes 9 and 10</td>
<td>lane 6</td>
<td>lane 7</td>
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<tr>
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<td></td>
<td>Lanes 13 and 14</td>
<td>lane 12</td>
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<td>Lane 16</td>
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<td>15</td>
<td></td>
<td>Lane 18</td>
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</table>

* The lanes indicated are those in Figure 1.

x = indicates findings not represented in Figure 1.
for ultraclean air (< 10 cfu/m³) was fulfilled in our operating theaters. Air-borne contamination is further reduced by use of occlusive clothing, and nonairborne spread may dominate [12,22]. It is interesting to note that only the patient or a person standing very close to the wound contributed to the bacterial flora in the wound. That finding and the lack of correlation between air and wound counts leads us to conclude that nonairborne spread predominated. The surgeon’s nose was the source of CoNS in one operation, which indicates that the face mask was inadequate. In the second operation the isolate resembled the CoNS of the surgeon’s forehead and arm and of the assistant’s nose. Although the isolate from the assistant differed by minor bands from the isolates of the surgeon, they may represent an epidemic strain [23]. This PFGE pattern was not found in any other isolates. The headlight might rub bacteria-carrying skin particles off the forehead. An improved fitting of the headlight might eliminate this source of skin rubbing. The amount of bacteria dispersed increases in scaly skin disease and infected skin [24]. Attention should be given to such seemingly minor issues, both in patients and in staff. Dispersal tests before and during employment at an operation ward have been described [24]. This practice may eliminate most of the heaviest dispersers, although an administrative problem of reassignment of affected staff could occur. One wonders what effect this practice would have in an already ultraclean environment.

Seven isolates found in the wound could not be traced. This can perhaps be explained by the fact that not all isolates present on the skin are picked up on a swab or rinse culture [25]. Exclusive use of the absorbent polyvinyl alcohol foam pads with a high recovery rate might have increased the number of traceable isolates.

At Karolinska Hospital two changes have been implemented since 1997. We moved into newly built operating rooms ventilated with zone vertical laminar airflow and we started to use double gloving. The mediastinitis rate decreased from 2% to 1%. It is impossible to determine which change had the decisive effect; perhaps both contributed to the reduction in mediastinitis incidence. It is interesting to note that the proportion of CoNS-caused mediastinitis only decreased somewhat despite use of occlusive scrub suits since 1994 (from 46% in 1992 and 1993 to 37% in 1998), and that transfer from the skin of the scrubbed team still occurs.

The number of persons who were present in the operating room at some point during the operation ranged from 10 to 19 persons including two researchers. This high number of individuals participating results from changes of nonscrubbed staff and scrub nurses during lengthy operations. This practice is perhaps not desirable from a bacteriologic point of view. The air counts were low despite this practice.

To prevent postoperative infections the following steps should be taken: (1) Proper prophylactic antibiotics should be administered. (2) Efforts should be directed at preserving the integrity of the tissues by frugal use of electrocautery and careful wound closure. (3) The patient’s skin should be meticulously prepared and draped. (4) Airborne contamination should be minimized by effective ventilation in the operating room, by use of occlusive scrub suits for the staff, and occlusive drappings for the patient. (5) Attention should be paid to skin conditions of staff and patients.

This study could not have been done without the skilled and diligent work of Anna-Lisa Belin, microbiological technician. In addition we thank all the staff and colleagues in the Department of Thoracic Surgery and Thoracic Anesthesia for so cheerfully participating in the collection of samples. This study was done with the support of the Swedish Heart Lung Foundation.

References

9. Bitkover CY, Gårdlund B. Mediastinitis after cardiovascular