Epidemic Septic Arthritis Caused by *Serratia marcescens* and Associated with a Benzalkonium Chloride Antiseptic

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During a 6-week period, 10 patients were admitted to a hospital for treatment of knee or shoulder joint infections due to *Serratia* species. Isolates from eight patients were identified as *Serratia marcescens* with identical biochemical characteristics and antibiotic susceptibility patterns. Before the onset of infections, all patients had been treated by two orthopedic surgeons who shared an office. Studies revealed that infections were associated with previous joint injections (P = 4.44 × 10−5) of methylprednisolone and lidocaine. Environmental cultures revealed that a canister of cotton balls soaked in aqueous benzalkonium chloride and two multiple-dose vials of methylprednisolone previously used by office personnel were contaminated with the epidemic strain of *S. marcescens*. The canister may have served as a potential reservoir for contamination of sterile solutions and equipment used for joint injections, of skin at the injection site, and of hands of personnel. No further cases occurred after the use of aqueous benzalkonium chloride was discontinued.

Septic arthritis due to *Serratia* species is rare, with fewer than 50 cases having been reported in the literature (3, 5, 19); no epidemics of these infections have been reported. However, nosocomial epidemics of infections at other sites as a result of *Serratia* species have been traced to multiple sources including contaminated solutions and disinfectants, intravenous fluids, arterial-pressure monitors, mechanical respirators, intermittent positive-pressure breathing machines, intravenous catheters, ultrasonic nebulizers, fiberoptic bronchoscopes, and hand-to-hand transmission by hospital personnel (9, 25).

When septic arthritis due to *Serratia marcescens* was diagnosed in 10 patients admitted to a community hospital, intrinsic contamination of a product used for joint injections in the office of three orthopedic physicians was suspected. However, because the product was widely distributed and the reports of these infections were localized to only one geographic location, we believed that this was an unlikely source of the outbreak. We report here the results of the outbreak investigation, which identified contaminated aqueous benzalkonium chloride as a possible source.

MATERIALS AND METHODS

Epidemiologic investigation. A case was initially defined as the occurrence of joint fluid that was culture positive for *Serratia* species in a patient. To ascertain cases, we reviewed the microbiology laboratory records of the hospital from June 1981 to January 1982 for all cultures positive for *Serratia* species, for the sources of these cultures, and for the dates the specimens were taken. We reviewed the medical records of the patients with joint fluid cultures positive for *Serratia* species and abstracted information on age, sex, underlying illnesses, primary joint diagnoses, joint surgery, indications and dates for joint cultures, clinical parameters of infection, and medications. We also reviewed physicians office records to obtain information on the indications for and dates of joint injections.

To assess potential risk factors for developing joint infections, we performed two case-control studies. For the first study, 35 control patients were selected at random from an appointment log of all 265 patients who visited the physicians’ office from 6 to 14 January 1982. Office medical records of cases and controls were reviewed for the following information: appointment time, whether joint injection(s) was given at the visit, and medications used for injection. For the second study, all 42 patients who had received joint injections or aspirations during the period 6 to 14 January, but who did not become infected, were selected as control patients. In this study, the following predisposing risk factors were examined: diagnosis, previous surgery on the involved joint, and the number of previous injections to the involved joint, excluding the injection received during the period 6 to 14 January. We interviewed the two orthopedic surgeons who had injected the patients who later developed septic arthritis and the two office nurses for information on techniques used for joint injections and general office practices.

Laboratory studies. The hospital microbiology laboratory identified patient isolates by using standard biochemical procedures and the API system (Analytab Products, Plainview, N.Y.) (14, 23). Antimicrobial susceptibility tests were done by the Bauer-Kirby disk diffusion method (2). Four subcultures of *S. marcescens* obtained from patient specimens and one from a vial of methylprednisolone sampled in the office by one of the nurses (nurse B) and found culture positive for *S. marcescens* were submitted to the Centers for Disease Control (CDC) for confirmation and serotyping. The following multiple environmental samples were obtained on sterile cotton-tipped swabs and transported to CDC in Cary-Blair media and in tryptic soy broth (Bactec; Difco Laboratories) for culture: Phisohex soap (Winthrop Laboratories, Div. Sterling Drug Inc.), Lehn and Fink Instrument Germicide (National Laboratories), Betadine (Purdue Frederick Co., Norwalk; Conn.), hydrogen peroxide, liquid dishwashing soap, and hand cleansers. Other samples also sent to CDC for culture included new cotton balls, aqueous benzalkonium chloride stock solution (Zephiran chloride [aqueous solution, 1:750]; Winthrop Lab-

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oratories) from a partially used container, benzalkonium chloride-soaked cotton balls obtained from two canisters in the office, and a 50-ml vial of lidocaine (Xylocaine 1%; Astra Pharmaceutical Products, Inc.) which had been partially used.

Since intrinsic contamination of multiple-dose vials of methylprednisolone (Depo-Medrol; The Upjohn Co.) was initially suspected by one of the physicians, three 10-ml vials (lot numbers 014JH and 609JP) had been sampled in the office by nurse B on January 14, three (lot number 014JH) had been sampled on January 15, and three (lot number 014JH) had been sampled on January 18, and the samples had been sent to the hospital microbiology laboratory for culture. The exact technique used to acquire the samples could not be determined. All nine vials with remaining contents were also sent to CDC for culture. Twenty unopened vials of Depo-Medrol (lot number 014JH) were obtained from The Upjohn Co. and were also cultured at CDC. At CDC, all isolates were identified by standard biochemical tests (7). Bauer-Kirby antimicrobial susceptibility testing (2) and serotyping (7) were also completed.

Twenty-six unopened vials of Depo-Medrol (lot number 014JH) were returned to The Upjohn Co. from the doctors' office and were cultured at The Upjohn Co. laboratory. The Food and Drug Administration cultured three unopened Depo-Medrol vials (lot number 014JH) obtained from the doctors' office.

**RESULTS**

**Clinical and epidemiologic findings.** During the period June 1981 to January 1982, 47 patients had cultures positive for *Serratia* species (Fig. 1). From June to September 1981, 19 patients had cultures positive for *Serratia* species, but none of the cultures were from joint fluids. In contrast, from October 1981 to January 1982, 28 patients had cultures positive for *Serratia* species, and 11 (39%) of the cultures were from joint fluids.

Joints involved for the 11 patients with positive joint fluid cultures included one knee in 9 patients, both knees in 1 patient, and one shoulder in 1 patient. Ten patients developed severe pain several hours (up to 24 h) after their last joint injection. All had diagnostic arthrocentesis 3 to 24 days after the injection. Cultures of joint fluid were positive for *S. marcescens* in nine patients and for *Serratia* species in two patients. Ten patients were hospitalized for therapy. One patient with cultures positive for *S. marcescens* did not develop clinical evidence of infection and was not hospitalized. He had one joint fluid specimen culture positive for *S. marcescens* on 25 November 1981, following an intra-articular injection on November 16. He received no antibiotics, and subsequent cultures of joint fluid on December 7 and 13 were negative.

Six patients were men, and five were women. Their mean age was 57.8 years (range, 34 to 76 years). Of the 10 hospitalized patients, 8 had fever above 37.8°C. Four had peripheral leukocyte counts greater than 10,000/mm³. Joint fluid cell counts were available for five patients, and these ranged from 33,524 to 158,572/mm³. All hospitalized patients required prolonged therapy with intravenous antibiotics; six patients received surgical drainage of infected joints, five patients received intra-articular antibiotics, and surgical intervention was being considered for several other patients. At the time the investigation was concluded, most hospitalized patients were still undergoing therapy in the hospital.

Figure 2 shows the cases of *Serratia* joint infections by date of the last joint injection. Isolates from two patients whose last joint injection was in December 1981 were characterized as a *Serratia* sp. with the same antibiotic susceptibility pattern: resistant to ampicillin, cephalothin, colistin, and tetracycline; susceptible to carbenicillin, chloramphenicol, gentamicin, kanamycin, tobramycin, and cefamandole. This pattern differed slightly from that of isolates from patients whose last joint injection was in January 1982. Isolates from these patients were characterized as *S. marcescens* with the following antibiotic susceptibility pattern: resistant to cephalothin, colistin, and tetracycline; susceptible to ampicillin, carbenicillin, chloramphenicol, gentamicin, kanamycin, tobramycin, and cefamandole. On the basis of antibiotic susceptibility testing and lack of definitive speciation, the two patients whose last injection was in December 1981 were thought to possibly represent a separate cluster and were excluded from further studies. Additionally, the one patient who had a single joint culture positive for *S. marcescens* in November but whose symptoms had resolved within 24 h without specific therapy was also excluded from subsequent studies, since it was unclear whether he represented a definite case. Thus, for the purpose of case-control studies, a case was redefined as the occurrence of joint fluid culture positive for *S. marcescens* in a patient who had received his last joint injection in January 1982.
In the first case-control study, all 8 case patients but only 7 of 35 control patients had received joint injections during their appointments from 6 to 14 January ($P = 4.44 \times 10^{-2}$; Fisher's exact test, one-tailed). All eight case patients and five of seven control patients who received joint injections were injected with Depo-Medrol and Xylocaine.

In the second case-control study, the characteristics of case patients that may have predisposed them to develop *Serratia* arthritis were compared with those of 42 control patients who had also received joint injections during the period 6 to 14 January (Table 1). A total of 7 of 8 case-patients and 19 of 42 control patients had had at least one additional injection before 6 to 14 January ($P = 0.032$; Fisher’s exact test, one-tailed). Previous joint surgery was also significantly associated with case patients: 4 of 8 case patients but only 4 of 42 control patients had had previous joint surgery ($P = 0.016$; Fisher’s exact test, one-tailed).

**Review of office procedures.** Since infections appeared to have been acquired in the doctors’ office, practices in the office were reviewed. Three orthopedic surgeons (surgeons A, B, and C) shared the office. Surgeon C saw only 20 to 30 patients per week and performed few joint injections. No cases were associated with surgeon C. Surgeon A, on the other hand, saw 90 to 150 patients per week, and surgeon saw B 60 to 80 patients per week. Six of eight case patients were associated with surgeon B, and two were associated with surgeon A. Two office nurses (nurses A and B) assisted the surgeons. Nurse A, who usually assisted surgeon A, was on vacation during the period from 25 December 1981 through the epidemic period. During this time, nurse B assumed the duties of nurse A. Surgeons A and B and nurses A and B were interviewed about the technique used for joint injections. All intra-articular medications were drawn from multiple-dose vials by the nurse on duty. Usually, Depo-Medrol and Xylocaine were drawn into 6 to 10 3-ml disposable syringes at the beginning of each office day. The needle was removed from each syringe after it had been filled and was replaced with a sterile cap. According to nurse B, all prefilled syringes were usually used the same day; however, unused syringes were not discarded but were saved at room temperature to be used the next day. Multiple-dose vials of Depo-Medrol and Xylocaine were stored at room temperature as recommended by the manufacturer.

Office personnel reported that for the past 17 years, the main antiseptic used in the office had been an aqueous benzalkonium chloride product, Zephiran. Two glass canisters containing cotton balls soaked in Zephiran were present in the office: one in the utility room, where syringes for joint injections were prepared, and one in the cast room. The canisters were cleaned with a commercial dishwashing detergent at approximately 1-month intervals and rinsed with tap water. The time of the most recent cleaning could not be ascertained. After being cleaned, the canisters were filled with cotton balls (100% cotton fiber), and Zephiran solution from a stock bottle stored under the sink was poured over the cotton. Between cleanings, additional cotton and Zephiran were added to the canisters as needed. The canisters and the cotton balls were never sterilized. Until at least mid-December, Zephiran-soaked cotton was routinely used to wipe off the tops of multiple-dose vials of Depo-Medrol and Xylocaine before syringes were filled and to prepare the skin at the injection site. Zephiran was the antiseptic found in greatest quantity in the office during the investigation in late January.

**TABLE 1.** Risk factors for development of joint infection by *S. marcescens* after joint injection in the second case-control study

<table>
<thead>
<tr>
<th>Population studied (no.)</th>
<th>Risk factor</th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age (yr)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. male&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No. given joint injections before 6 to 14 January&lt;sup&gt;c&lt;/sup&gt;</td>
<td>No. undergoing previous joint surgery&lt;sup&gt;d&lt;/sup&gt;</td>
<td>No. with prosthetic joint injected&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Case patients (8)</td>
<td>51.5</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Controls (42)</td>
<td>58.0</td>
<td>20</td>
<td>19</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Not significant by t test.
<sup>b</sup> Not significant by one-tailed Fisher exact test.
<sup>c</sup> $P = 0.032$ by one-tailed Fisher exact test.
<sup>d</sup> $P = 0.016$ by onetailed Fisher exact test.
The typical joint injection by surgeon A required the use of one needle and two syringes, one containing Xylocaine and the other containing Depo-Medrol. The skin was prepped, and the Xylocaine syringe was used to enter the joint space and to inject the local anesthetic. While the needle remained in the joint space, the empty Xylocaine syringe was removed and replaced with the Depo-Medrol syringe. Approximately 1 ml of Depo-Medrol was then injected into the joint space. No gloves were worn, and no additional hand preparation was done.

On 30 January, to test the hypothesis that an environmental office contaminant could have been introduced into a sterile multiple-dose vial, we asked nurse B to withdraw a small sample of broth from a vial of commercial culture medium (trypsin soy broth) by the same technique she would have used when filling syringes before mid-December. This vial was similar to the multiple-dose Depo-Medrol and Xylocaine vials in that it had to be entered by puncturing the rubber diaphragm on the top with a needle. Nurse B used a Zephiran-soaked cotton ball from the canister in the utility room to disinfect the vial diaphragm before withdrawing the sample. The vial with remaining contents was sent to CDC for culture.

**Microbiology results.** Of the samples sent to CDC, the following were positive for *S. marcescens*: subcultures of joint fluid isolates from four patients who had had their last injection in January 1982; two vials of Depo-Medrol, from two different lot numbers (014JH and 609JP), which had been sampled by nurse B in the office on 14 January; and samples from the canister of Zephiran-soaked cotton balls in the utility room. Quantitative counts of *S. marcescens* in the Depo-Medrol vials were $2.1 \times 10^2$ and $1.7 \times 10^2$ CFU/ml. Fluid expressed from the Zephiran-soaked cotton balls contained $2.3 \times 10^4$ CFU/ml. All patient and environmental isolates had identical biochemical characteristics, antibiotic susceptibility patterns, and serotype (O5:H-undetermined). The vial of culture media sampled by nurse B was also positive for the same strain of *S. marcescens*.

Reports confirmed by telephone communication indicated that the 26 unopened vials of Depo-Medrol (lot number 014JH) cultured by The Upjohn Co. and the 3 vials cultured by the Food and Drug Administration were sterile. The 20 unopened vials of Depo-Medrol (lot number 014JH) received by CDC from The Upjohn Co. were cultured and found to be sterile.

**DISCUSSION**

The results of our investigation revealed that 10 patients developed joint infections with *Serratia* spp. in two clusters: one cluster of 2 patients with *Serratia* sp. infections in December 1981, and one cluster of 8 patients with *S. marcescens* infections in January 1982. Of the several possible mechanisms for the pathogenesis of septic arthritis, the most important in the present study appeared to be the introduction of organisms into the joint space at the time of intra-articular injection. This could have occurred if one or more of the following were contaminated: medication(s) used to inject the joint, instruments used to inject the joint, skin overlying the site of injection, or the hands of personnel performing the procedure.

Intrinsic contamination of Depo-Medrol, i.e., contamination introduced at the time of manufacture, appeared unlikely in this outbreak for two reasons. First, cultures of unopened medication vials were reportedly sterile; the two vials that were culture positive for *S. marcescens* had been previously sampled by nurse B in the office. Second, the "implicated" lots of Depo-Medrol had been distributed widely across the nation, yet neither CDC nor the Food and Drug Administration had received other reports suggesting epidemics of septic arthritis associated with intrinsic contamination of the product.

The available evidence suggested that for the eight patients who developed *S. marcescens* septic arthritis following joint injections in January 1982, the canister of Zephiran-soaked cotton balls served as a reservoir for the epidemic strain of *S. marcescens*. Use of the contaminated cotton balls in the described fashion could have led to the contamination of sterile multiple-dose vials of medications used for injections, of the needle used to puncture the diaphragm of the medication vial, of hands of personnel administering injections, and of the skin of the patient at the time of the injection. Information that Zephiran use was discontinued in mid-December appears inconsistent with this hypothesis. However, one might speculate that the ready availability of canisters of Zephiran-soaked cotton balls would make inadvertent use of such materials a strong possibility.

The reasons why the two clusters occurred when they did and why many patients who received joint injections similar to those received by case patients during the period 6 to 14 January 1982 did not develop clinical infection remain unexplained. An intermittently contaminated reservoir, or the intermittent use of a contaminated reservoir, might explain the two clusters. Although intermittent or varying levels of contamination of medications and equipment might also explain why some patients receiving injections during the epidemic period escaped infection, host factors might also have played an important role. Indeed, our second case-control study revealed that patients who had undergone previous joint surgery or who had a history of prior joint injections appeared to be at the greatest risk of infection. Although the presence of underlying illnesses other than prior joint surgery and multiple injections was not determined for our controls, previous reports document the association of multiple underlying problems with septic arthritis due to *Serratia* spp. (1, 3, 5, 6, 11, 15, 16, 18, 20-22, 24). Additionally, instances of septic arthritis associated with intra-articular steroid injections have been reported (10, 12, 20).

Intrinsic and extrinsic contamination of aqueous quaternary ammonium compounds such as Zephiran has been well documented previously and has been the subject of concern for many years (4). Contamination is usually due to gram-negative organisms and has resulted in a number of nosocomial outbreaks, including outbreaks due to *Serratia* spp. (4, 8, 9). Cotton and other materials have been shown to inactivate aqueous quaternary ammonium compounds after prolonged contact with them (13). We found it interesting that the preservative in the Depo-Medrol product used during this outbreak, γ-myristyl picolinium chloride, is also an aqueous quaternary ammonium compound. Laboratory studies determined that the strain of *S. marcescens* recovered in this outbreak was relatively resistant to Zephiran as well as to the preservative in Depo-Medrol (17).

Since the removal of Zephiran as an antiseptic and disinfectant from the physicians' office, no further cases of arthritis due to *S. marcescens* have been reported.

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LITERATURE CITED