Nosocomial Infections with *Aeromonas hydrophila* from Leeches

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The manner in which leeches are maintained before they are used for therapy has not been studied as a factor contributing to nosocomial infections. A 5-year retrospective survey of *Aeromonas hydrophila* nosocomial infections at a hospital in Marseille, France, revealed infections in 5 (4.1%) of an estimated 122 patients treated with leeches in the Hand Surgery Unit and 2 (2.4%) of an estimated 85 patients treated with leeches in other hospital units. The retrospective survey showed that the Hand Surgery Unit was the only unit that had its own aquarium for maintaining leeches; this aquarium was filled with tap water contaminated with *Aeromonas* species and was not regularly disinfected or cleaned. Leeches used in other units were maintained in non-carbonated water in a transport device. Use of leeches kept in aquariums that are filled with tap water and not disinfected or cleaned regularly may be linked to *A. hydrophila* infections.

For centuries, leeches have been used in medicine to treat a variety of conditions. Their use peaked during the military campaigns of the early 19th century, among the military surgeons of Napoleon’s army [1]. Leech therapy, abandoned for several decades, began to be used again in the 1960s, when it was reported to be successful for the treatment of compromised skin flaps [2] and, later, in 1981, for the treatment of replanted fingers [3]. Today, leech therapy is indicated in plastic and reconstructive surgery, to relieve venous congestion and to improve the microcirculation of flaps, grafts, or replants [4]. With use of leech therapy, the success rates of flap, graft, and replantation procedures have been reported to increase by 60%–83% [3, 4]. The medicinal use of leeches, however, is complicated by blood loss, which may necessitate blood transfusions [4], and by infections, mainly due to *Aeromonas hydrophila* [5]. Such infections may be localized to soft tissues or progress to cause myonecrosis with sepsis or septicemia. In the presence of infection, the success rate for flap salvage may decrease to ≤30% [4].

*Aeromonas* species are ubiquitous and are readily recovered from water, including tap water in hospitals and carbonated mineral water [6]. They have been identified in the mucus trails of leeches [7], where they are the main [5, 8, 9] or even the only [10] bacteria present. It is impossible to obtain *Aeromonas*-free leeches [11], because these ectoparasites lack the proteolytic enzymes necessary to digest blood meals; for these enzymes, they rely on *Aeromonas* species, with which they live symbiotically [1]. Various strategies have been suggested to prevent nosocomial *Aeromonas* infection due to leech therapy, but, to our knowledge, the importance of the quality of the water in which the leeches are maintained has never been studied. We examined *A. hydrophila* nosocomial infections due to leech therapy that occurred in our hospitals from June 1996 through December 2000 and performed a survey to determine the efficacy of infection-control measures that were used during this period.
Patients and Methods

Setting. The Assistance Publique Hôpitaux de Marseille (APHM; Marseille, France) is a 3500-bed teaching facility that comprises 4 acute-care hospitals. The same infection-control procedures are implemented by the infection-control teams in the 4 hospitals, and the overall infection-control program is coordinated by a central infection control committee.

Nosocomial infections surveillance. Hospital-wide laboratory-based surveillance of nosocomial infections has been ongoing at the APHM since 1996. It has focused on urinary tract infections, surgical-site infections (SSIs), pneumonia, and bloodstream infections. The criteria used to identify a nosocomial infection are essentially those used by the US Centers for Disease Control and Prevention (CDC) [12]. Every weekday (i.e., Monday through Friday), an infection-control nurse collects a list of suspected nosocomial infections identified by the microbiology laboratory and completes a data form for each case with the assistance of a nurse designated by the clinical unit concerned. The diagnosis of a nosocomial infection is confirmed during a meeting between a physician representing the clinical unit concerned and the infection control epidemiologist. The results of surveillance are sent monthly to the chiefs of the departments and to the physicians representing the clinical units.

We studied all A. hydrophila infections identified by the surveillance system from June 1996 through December 2000 and all the A. hydrophila isolates recovered from samples by the microbiology laboratory during the same period. Additional information about the A. hydrophila infections was collected by reviewing the case charts for each patient: this information included data on the age and sex of patients, the date and the type of surgical operation performed, the date of onset of infection and its clinical signs, the number of leeches used for therapy, and the clinical unit involved.

Microbiological surveillance of tap water. Samples of hospital drinking water (250 mL) were collected in each of the 4 hospitals of our institution every 2 months during the study period. Water samples were filtered with a 0.45-μm filter, and the filtration membrane was inoculated onto brom cresol purple agar (bioMérieux) and incubated at 30°C for 24 h. The number of colonies was counted, and identification of Aeromonas species was performed with an API 20E identification strip (bioMérieux) according to standard recommendations [13].

Use and maintenance of leeches. The purchase, maintenance, and distribution of leeches among the hospitals in the APHM is centralized and recorded on a computer in the pharmacy of one of the hospitals. From this pharmacy, we obtained data on the numbers of leeches delivered to the clinical units in the APHM each month from June 1996 through December 2000. However, the names of the patients treated with leeches and the actual number of leeches used per patient were not tracked. We also performed a retrospective survey to assess the conditions of maintenance and delivery of the leeches in the pharmacy and in the clinical units that used the most leeches. Infection control nurses collected information about the types of containers used to house and transport the leeches, the types and temperatures of water used in the containers, and any cleaning and disinfection procedures that were used.

Statistical analysis. Data were analyzed with Epi Info, version 6.02 (CDC). The proportions of leeches associated with infection in patients were compared by use of the Mantel-Haenszel test. Relative risks and associated 95% CIs were calculated; P values <.05 were considered significant.

Results

A. hydrophila nosocomial infections. From June 1996 through December 2000, we identified 14 nosocomial A. hydrophila infections: 9 SSIs, 3 cases of bacteremia, and 2 cases of pneumonia (figure 1). Of these, 7 SSIs were associated with leech therapy and occurred in the Hand Surgery Unit, the Plastic and Reconstructive Surgery Unit, or the Burn Unit. Three of these SSIs were associated with the application of leeches after finger replantations, and 4 were associated with leech therapy administered after skin or myocutaneous flap surgery (table 1). The infections occurred 2–11 days after the leeches were first applied; a mean of 10.1 leeches (range, 5–17 leeches) were used for each patient. The mean duration of the leech therapy was 4.7 days (range, 3–7 days). Two patients were receiving antibiotic therapy with amoxicillin plus clavulanic acid at the time of leech application, but this was ineffective against A. hydrophila infection. Localized cellulitis or myonecrosis was diagnosed during hospitalization in 4 patients who had flap surgery and after discharge in the 3 patients who had fingers replanted. Two of the 3 patients with infected finger replantations required rehospitalization. Six strains of A. hydrophila that caused nosocomial infections associated with leech therapy were resistant to ticarcillin. Three patients did not receive antibiotics; infections in these patients were treated with trimethoprim-sulfamethoxazole; in infection in 1 other patient was treated with ofloxacin. The replanted fingers proved non-viable in the patients with nosocomial infections and were subsequently removed. The patients with necrosis of myocutaneous flaps also required additional reconstructive surgery; in total, 9 additional surgical procedures were performed.

Use of leeches. From June 1996 through December 2000, leeches were purchased from a single supplier (Ricarimpex); in total, 2090 leeches were delivered to 13 clinical units. The use of leeches decreased over the study period, from 655 leeches in 1996 to 363 leeches in 2000. The Hand Surgery Unit and Plastic and Reconstructive Surgery Unit together...
used 85% of the leeches (1229 [59%] of 2090 leeches and 551 [26%] of 2090 leeches, respectively). Units that used only small numbers of leeches included the Pediatric Surgery Unit (76 [4%] of 2090 leeches) and the Maxillofacial Surgery Unit (50 [2%] of 2090 leeches).

**Infections associated with leech application.** Five of the leech-related infections occurred in the Hand Surgery Unit, 1 infection occurred in the Plastic and Reconstructive Surgery Unit, and 1 infection occurred in the Burn Unit. On the basis of the mean number of leeches used per patient (10.1), we estimated that 122 patients (i.e., 1229 leeches ÷ 10.1 leeches per patient) were treated with leeches in the Hand Surgery Unit and 85 patients (i.e., 861 leeches ÷ 10.1 leeches per patient) were treated with leeches in the other units during the study period. Accordingly, the rate of *A. hydrophila* infection after leech application was estimated to be 4.1% (5 of 122 patients) in the Hand Surgery Unit and 2.4% (2 of 85 patients) in the other units (difference, NS).

**Microbiological surveillance of tap water.** Forty (33%) of 120 hospital water samples yielded *Aeromonas* microorganisms at concentrations of 10–10$^3$ cfu/mL. Of these 40 isolates, 19 were identified as *A. hydrophila*, and 21 were identified as *Aeromonas sobria*.

**Delivery and maintenance of leeches.** After delivery from the supplier, leeches were kept in the pharmacy in a glass aquarium filled with noncarbonated mineral water. When needed, they were transported to units in a nonsterile plastic tanks that also contained noncarbonated mineral water (table 2). In the

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**Table 1. Descriptions of *Aeromonas hydrophila* surgical site infections (SSIs) identified at the Assistance Publique Hôpitaux de Marseille (Marseille, France) from June 1996 through December 2000.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type of surgery</th>
<th>Year of surgery</th>
<th>No. of leeches used$^a$</th>
<th>Time to diagnosis of SSI, days$^b$</th>
<th>Clinical signs of infection</th>
<th>Outcome of SSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Free tissue flap</td>
<td>1996</td>
<td>17</td>
<td>2</td>
<td>Fever (temperature, 38°C); inflammation of the flap; purid drainage</td>
<td>Loss of the flap; second free tissue flap surgery performed</td>
</tr>
<tr>
<td>2</td>
<td>Finger replantation</td>
<td>1997</td>
<td>13</td>
<td>No data</td>
<td></td>
<td>Finger amputation</td>
</tr>
<tr>
<td>3</td>
<td>Finger replantation</td>
<td>1998</td>
<td>6</td>
<td>11</td>
<td>Fever (temperature, 38.2°C); opening of the scar; purid stomp; no drainage</td>
<td>Finger amputation</td>
</tr>
<tr>
<td>4</td>
<td>Pedicled skin flap</td>
<td>1998</td>
<td>11</td>
<td>7</td>
<td>Fever (temperature, 38.2°C); no drainage</td>
<td>Loss of the flap and full-thickness skin graft</td>
</tr>
<tr>
<td>5</td>
<td>Finger replantation</td>
<td>1998</td>
<td>6</td>
<td>9</td>
<td>Fever (temperature, 39°C); purid purulent drainage</td>
<td>Finger amputation</td>
</tr>
<tr>
<td>6</td>
<td>Free tissue flap</td>
<td>1999</td>
<td>5</td>
<td>6</td>
<td>Fever (temperature, 40°C); opening of the scar</td>
<td>Partial necrosis of the flap</td>
</tr>
<tr>
<td>7</td>
<td>Free tissue flap</td>
<td>2000</td>
<td>13</td>
<td>6</td>
<td>No fever; inflammation of the flap; purid drainage</td>
<td>Partial necrosis of the flap</td>
</tr>
</tbody>
</table>

$^a$ No. of leech applications.

$^b$ Time from the first application of leeches to diagnosis of the SSI.
units, the leeches were kept in this tank for up to 5 days. Only the Hand Surgery Unit had its own aquarium, in which the transported leeches were placed. This tank was filled with tap water and was used to maintain leeches at room temperature for months. The aquarium was cleaned, but disinfectants were not used.

**DISCUSSION**

In the APHM, we found that leeches were most commonly used by the Hand Surgery Unit and the Plastic and Reconstructive Surgery Unit. This was expected, because leech therapy is recommended in the salvage of compromised pedicled flaps and in microvascular free tissue transfers [4]. The risk of nosocomial infections may have led to the decrease in the number of leeches used from 1996 through 2000. Instead, the leeches may have been reserved for use in more precise indications—namely, in cases of venous insufficiency in previously devascularized tissue or failing free tissue flaps [14] with adequate arterial inflow and in situations in which reintervention was not possible [15].

The mean duration of the leech application (4.7 days) and the mean number of leeches per infected patient (10.1 leeches per patient) were similar to those described in the literature [3, 4]. Foucher et al. [3] first described the application of 2 leeches per day for 5 days after finger replantation. A meta-analysis showed a mean duration of leech use of 4.2 days for series of both infected and noninfected patients [4]. Therefore, the use of leeches in the units of our institution seemed to be in accordance with international practice.

From June 1996 through December 2000, 7 nosocomial *A. hydrophila* infections associated with leech application were identified in the Hand Surgery Unit, the Plastic and Reconstructive Surgery Unit, and the Burn Unit. The *A. hydrophila* infection rate after leech therapy was impossible to calculate precisely, because the identities of the patients who received the therapy were not recorded by the pharmacy or by the unit. We therefore estimated the infection rate on the basis of the mean number of leeches used on patients and the numbers of leeches delivered by the pharmacy. This rate was probably an underestimation, because we could not take into account the leeches that died in the aquarium in the unit before they could be used and the leeches that did not attach to patients. However, our estimate is in agreement with the incidence rates previously reported in the literature, which range from 7% to 20% [16, 17].

Because the guts of leeches may contain pathogenic bacteria, viruses [13], or parasites [18], various infection-control strategies have been proposed to prevent nosocomial infections. First, the use of leeches is recommended only for salvageable tissue that has venous obstruction but intact arterial perfusion [3]. The leeches must be used only on a single patient and then discarded, to prevent the spread of infections between patients. Investigators have attempted to disinfect the guts of leeches before they are placed on patients by placing the ectoparasites in 0.02% chlorhexidine for 15 s [11, 15] or in antibiotic solutions (tetracycline or cefoperazone solutions) for 12 h [9], but these attempts were unsuccessful. Administration of a second- or third-generation cephalosporin or a fluoroquinolone to patients before the application of leeches has appeared to prevent the transmission of bacterial infection from the leeches [5, 10, 19, 20]. More recently, mechanical leech therapy has been developed, which simulates the mechanical action of the sucking of leeches and injects anticoagulant in situ [21].

Our retrospective survey of the conditions under which the leeches were distributed and maintained showed that only the Hand Surgery Unit, in which *A. hydrophila* infection was twice as common as in other units, had its own aquarium for leech storage. This aquarium was filled with tap water that was shown to be contaminated by *A. hydrophila*. Whereas contamination of tap water has been reported in various hospitals [22], *A. hydrophila* microorganisms were undetectable in 713 samples of bottled noncarbonated mineral water [6]. Therefore, our data suggest that filling the aquariums with tap water aided the colonization of leeches with *A. hydrophila* and, therefore, contributed to the development of SSIs. The risk of infection was increased by the lack of cleaning and disinfection of the aquarium. We therefore recommend that leeches be maintained strictly in the central maintenance site and they be maintained

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**Table 2. Conditions under which leeches used for medicinal purposes were maintained in the hospital units that regularly used them.**

<table>
<thead>
<tr>
<th>Unit or department</th>
<th>Container</th>
<th>Water</th>
<th>Temperature</th>
<th>Disinfection procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacy</td>
<td>Aquarium</td>
<td>Mineral</td>
<td>Room</td>
<td>None</td>
</tr>
<tr>
<td>Hand Surgery</td>
<td>Aquarium</td>
<td>Tap</td>
<td>Room</td>
<td>None</td>
</tr>
<tr>
<td>Plastic and Reconstructive</td>
<td>Sterile tank</td>
<td>Tap and mineral</td>
<td>Room</td>
<td>None</td>
</tr>
<tr>
<td>Pediatric Surgery</td>
<td>Sterile tank</td>
<td>None</td>
<td>Room</td>
<td>None</td>
</tr>
</tbody>
</table>

**NOTE.** The Maxillofacial Surgery Unit, which used a small number of leeches for therapy during the period under study, did not maintain its own supply of leeches.
in the same medium in the transport aquariums that are used to deliver them to the units of the hospital that requested them.

Acknowledgments

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References