Pseudo-Outbreak of *Rhizobium radiobacter* Infection Resulting from Laboratory Contamination of Saline Solution

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We report a pseudo-outbreak of *Rhizobium radiobacter* infections resulting from contamination by a saline dispenser in the microbiology laboratory. Isolates from clinical specimens had identical antimicrobial susceptibilities and electrophoretic fingerprints. The episode resolved with autoclaving of the dispenser. This demonstrates the importance of timely, thorough investigation of unusual organisms, particularly when they appear as a cluster.

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A pseudoepidemic is defined as a real clustering of false infections or an artifactual clustering of real infections (10). Nosocomial pseudo-outbreaks result in unnecessary expenditure of resources. An investigation conducted by the Center for Disease Control of 181 nosocomial epidemics occurring over 20 years concluded that 11% were pseudoepidemics (10), leading to unnecessary use of resources.

We report a pseudo-outbreak of sterile-site infections with an unusual human pathogen, *Rhizobium radiobacter*, resulting from a contaminated saline dispenser in the laboratory. The source of the outbreak was determined using standard epidemiological methods. There has been one previous report of an outbreak of *R. radiobacter* pseudobacteremia (9). This is the first documented report of a nonbacteremic pseudo-outbreak of *R. radiobacter*.

Species in the genus *Rhizobium* are aerobic, non-spore-forming, oxidase-positive, gram-negative bacilli, found in the environment and associated with tumorigenic diseases in plants (11). *R. radiobacter* is the species most commonly associated with human disease, with bacteremia being the most common manifestation, usually catheter associated (7, 11).

The National University Hospital is a 900-bed tertiary care center in Singapore, caring for pediatric, obstetric, gynecological, medical, and surgical patients. The pseudo-outbreak spanned from 14 to 24 January 2008. The infection control department commenced investigation on the 8th day of the pseudo-outbreak, after being notified by the microbiology department of the unusual number of isolate cultures growing *R. radiobacter*.

Case patients were those who had *R. radiobacter* isolated from a sterile-site specimen during the outbreak period. Cases were identified from microbiology records and plotted onto an epidemic curve. Patient details, including any evidence of clinical infection extracted from the clinical notes and environmental exposures investigated, are shown in Table 1.

Clinical specimens were collected aseptically and placed into sterile specimen containers and then diluted with saline in the laboratory and inoculated onto blood, MacConkey, and chocolate agar plates for culture. Plates were incubated aerobically at 35°C for 48 h. Colonies of *R. radiobacter* were identified and drug sensitivities were determined using the Vitek 2 system (bioMérieux Inc., Durham, NC).

Once a pseudoepidemic was suspected, environmental samples were taken from the mouth of the saline dispenser in the laboratory, from saline before and after it was put through the saline dispenser, and from skin-cleaning preparations used in operating rooms. Environmental swabs and one drop of cleaning solution or saline dispensed via the laboratory dispenser were inoculated onto a blood agar plate and incubated for 48 h at 35°C. Colonies were identified and tested for susceptibilities by using the Vitek 2 system (bioMérieux Inc., Durham, NC).

Repetitive bacterial sequence-based PCR (rep-PCR) was performed on isolates by using the BOX1AR primer (5’-CTAACGGGCAAGGCGACGCTGACG-3’) (8). rep-PCR was performed on crude extracts from bacterial suspensions boiled for 10 min in sterile water. PCRs were performed in a thermal cycler (GeneAmp 9700; Applied Biosystems, Foster City, CA) with a HotStar PCR Taq master mix kit (Qiagen, Hilden, Germany). Amplification conditions were 95°C initially for 15 min followed by 95°C for 30 s, 50°C for 30 s, and 72°C for 4 min for over 30 cycles, followed by a final 10-min extension period at 72°C. PCR products were analyzed on ethidium bromide-stained agarose gels. Clinical and environmental isolates were compared.

Cultures from nine patients over an 11-day period yielded 11 isolates of *R. radiobacter* (Fig. 1). Nine specimens from the seven adult patients were tissue or bone obtained intraoperatively, and the specimens from the two pediatric patients were tips from peripherally inserted central catheters. Patient characteristics are shown in Table 1. None of the patients were immunosuppressed. Three patients had other documented infections, with clinical syndromes not characteristic of *R. radiobacter* infection. No patient was specifically treated for *R.
### TABLE 1. Patient characteristics and clinical data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Admission (day/mo/yr)</th>
<th>Reason for admission/procedure</th>
<th>Date of procedure (day/mo/yr)</th>
<th>Material(s) used</th>
<th>Surgeon(s)</th>
<th>Anesthetist(s)</th>
<th>Ward</th>
<th>Lines</th>
<th>Fever</th>
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<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>Male</td>
<td>14/1/08</td>
<td>Excisional lymph node biopsy</td>
<td>14/1/08</td>
<td>DS05</td>
<td>Ethilon 5/0, Surgicel A, B</td>
<td>P</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>Male</td>
<td>31/12/07</td>
<td>Pelvic debridement for osteomyelitis complicating open reduction and internal fixation of pelvic fracture</td>
<td>14/1/08</td>
<td>OR15</td>
<td>Povidone iodine plus ethanol, Versafoam, gentamicin beads, VAC</td>
<td></td>
<td>No</td>
<td>Nil</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>Male</td>
<td>16/1/08</td>
<td>Total knee replacement for osteoarthritis of right knee</td>
<td>17/1/08</td>
<td>OR02</td>
<td>Povidone iodine plus ethanol, Monocryl, Stryker Scorpio system D, E, F, G</td>
<td></td>
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<td>No</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>Male</td>
<td>16/1/08</td>
<td>Wound debridement and external fixation for traumatic left distal tibial fracture</td>
<td>18/1/08</td>
<td>OR14</td>
<td>Povidone iodine plus ethanol, Pulsavac lavage, VAC</td>
<td></td>
<td>No</td>
<td>No</td>
<td>51</td>
</tr>
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<td>5</td>
<td>44</td>
<td>Female</td>
<td>15/1/08</td>
<td>Elective revision of Girdlestone's procedure (bone graft and total hip replacement)</td>
<td>18/1/08</td>
<td>OR01</td>
<td>Drains and implants E, F, G, H</td>
<td></td>
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<td>6</td>
<td>69</td>
<td>Male</td>
<td>11/1/08</td>
<td>Debridement and synovectomy of right wrist for acute gout</td>
<td>18/1/08</td>
<td>DS04</td>
<td>Povidone iodine, Prolene, saline via pulsator lavage</td>
<td></td>
<td>No</td>
<td>Nil</td>
<td>53</td>
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<tr>
<td>7</td>
<td>8</td>
<td>Male</td>
<td>4/1/08</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>8</td>
<td>43</td>
<td>Male</td>
<td>15/1/08</td>
<td>Right knee arthroscopy for gonococcal septic arthritis</td>
<td>17/1/08</td>
<td>OR07</td>
<td>Povidone iodine plus ethanol, Prolene, drain CW, X</td>
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<tr>
<td>9</td>
<td>0.16</td>
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<td>4/1/08</td>
<td>Small bowel obstruction</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>48</td>
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</table>

**Notes:**
- NA, not applicable (patient did not undergo any surgical procedures).
- Individual surgeons and anesthetists are each represented by a different alphabetical letter.
- Presence of a peripherally inserted central catheter or central venous catheter.
- VAC, vacuum-assisted closure.
radiobacter infection. One patient had a nonurgent surgical procedure delayed by 48 h.

Review of surgical procedures, operating theaters, materials used, and surgeons and anesthetists involved did not reveal any common links between cases (Table 1). Povidone iodine was used for skin preparation in all surgical procedures. Investigation revealed that all specimens were diluted with saline from a common dispenser; in addition, the laboratory had recently started sourcing sterile saline externally and had stopped boiling saline prior to dispensing. The dispenser had inadvertently gone for a prolonged time without autoclaving. Identification of these common links between cases prompted sampling of povidone iodine skin preparations, the saline dispenser, and the commercial saline for microbiological investigation.

Cultures of samples taken from the mouth of the saline dispenser revealed heavy growth of *R. radiobacter*. Cultures of saline from the dispenser revealed a light growth of *R. radiobacter*. There was no growth of organisms from other environmental specimens. rep-PCR yielded an identical pattern, and antibiograms were identical for all isolates from clinical and environmental samples.

Following the investigation, regular autoclaving of the saline dispenser and boiling of all saline prior to use were instituted. Subsequently, there were no further isolates of *R. radiobacter* from clinical specimens.

*Rhizobium* species are ubiquitous in soil and water; however, they are rarely opportunistic human pathogens (3, 5, 6). The increased number of *R. radiobacter* isolates from clinical specimens in our patients was not compatible with an outbreak of *R. radiobacter* infection, prompting further investigation for a source of potential contamination.

Other studies have demonstrated laboratory contamination resulting in pseudo-outbreaks. Sources have included culture media and medical equipment (1, 2, 4). This pseudo-outbreak highlights the importance of ensuring the sterility of saline used in the laboratory as well as regular autoclaving of equipment.

It was fortunate, in this pseudo-outbreak, that the organism involved was an uncommon human pathogen, resulting in early suspicion of specimen contamination. This suspicion was confirmed by isolation of the organism from a common source and supported by demonstrating identical antibiograms and rep-PCR electrophoretic patterns among environmental and clinical isolates. As a result, appropriate interventions were instituted, quickly aborting the pseudo-outbreak before a significant adverse impact on patient care occurred. The pseudo-outbreak may not have been detected as rapidly had the organism been a more common human pathogen.

The potential unnecessary antibiotic treatment and adverse effects on patients from a pseudo-outbreak illustrate the importance of adherence to strict sterilizing protocols in the laboratory, especially in processing specimens.

**REFERENCES**