Outbreak of Gram-Negative Bacteremia at St. Cloud Hospital
Investigation Summary, Minnesota Department of Health, 2011

Sept 14, 2012

Background

On February 21, 2011, the Minnesota Department of Health (MDH) Acute Disease Investigation and Control Section was contacted by an infection preventionist (IP) at St. Cloud Hospital (SCH) requesting assistance with a cluster of four patients on the Surgical Unit/Surgical Progressive Care Unit (Surgical/SPCU) with blood cultures that were positive for the bacterium Ochrobactrum anthropi during February 2011; two additional blood cultures from Surgical Unit/SPCU patients were pending.

SCH staff were concerned about the possibility of product contamination since these six patients had received hydromorphone, a narcotic pain medication, administered by a patient controlled analgesia (PCA) pump. PCA pumps are medication-dispensing units attached to an intravenous line that is inserted into a vein. Patients self-administer short-acting doses of pain medication by means of a push-button mechanism. On February 20, 2011 SCH staff reportedly collected eight hydromorphone bags in use by patients throughout the hospital, removed hydromorphone with the same lot numbers from the SCH Pharmacy, and began using hydromorphone with another lot number from the same manufacturer. On February 22, the CentraCare Laboratory (SCH Laboratory) reported that the two pending blood cultures were positive for the bacterium, Klebsiella oxytoca. Additionally, the SCH Laboratory reported that K. oxytoca and Stenotrophomonas maltophilia bacteria were detected in two of the eight hydromorphone bags in use by patients (see SCH Laboratory Results below).

O. anthropi and S. maltophilia are bacteria commonly found in the environment that can grow in liquids; however, they are rarely human pathogens. K. oxytoca are human pathogens that are less commonly found in the environment. MDH staff contacted the U.S. Centers for Disease Control and Prevention (CDC) for technical assistance and consultation. MDH, CDC, and SCH staff discussed a range of potential sources of the infections including hydromorphone contamination, laboratory contamination, phlebotomy practices, environmental contamination, surgical and anesthetic practices and instrumentation, and pharmacy contamination. MDH asked about infection prevention and control practices in place at SCH and any identified breaches or changes in practice (e.g. hand hygiene, cleaning and disinfection, products); none were reported.

The SCH IP provided a line list to MDH with the 6 patients who had K. oxytoca or O. anthropi bacteremia. This list included surgical procedures, surgeons and anesthesiologists, products and medical equipment used pre-, peri-, and post-operatively, and patient location. Five of the patients had Duraprep pre-op skin prep; 6 had peri-operative 0.9% NaCl irrigant; an electric warmer was used on 6 patients; a hotline warmer was used on 3 patients (hotline warmer use unknown for 2 patients); 5 received IV Lactated Ringers peri-operatively; and 6 received hydromorphone by PCA. MDH requested that SCH staff conduct a retrospective review of microbiology records to look for additional O. anthropi isolates. Three additional O. anthropi blood isolates were identified, the earliest being from December 2010.

Investigation of Possible Contamination Sources

The following summarizes the investigation of possible sources of contamination.

- Laboratory contamination  The SCH Laboratory had implemented a new automated system (Vitek2) for microbiology testing in December 2010. Quality control (QC) organisms were used to validate
the new equipment prior to use and *O. anthropi* was one of the organisms used for this validation. Concern was raised that contamination in the laboratory may have occurred. The SCH Laboratory submitted this QC organism to MDH for pulsed-field gel electrophoresis (PFGE) testing to assess genetic relatedness to the patient blood isolates. The QC organism did not match the *O. anthropi* blood isolates collected from SCH patients (see Figure 7). There was no evidence to support laboratory contamination.

- **Contamination from phlebotomy practices**  SCH staff reviewed blood culture collection phlebotomy practices (e.g. skin antisepsis, venipuncture vs. line blood draws, etc.), supplies (e.g. blood culture bottles), and staffing records. Different phlebotomists obtained blood samples from patients with positive blood cultures. No contamination of supplies was identified. Therefore, there was no evidence to support contamination during blood culture collection.

- **Environmental contamination**  MDH requested that SCH staff collect samples of environmental liquids associated with the procedures identified on the line list. Environmental samples included: water from the hotline warmer; water from the ice machines and water dispenser; distilled water; and unopened bags of normal saline. These samples were submitted to CDC for testing; no contamination with *O. anthropi*, *K. oxytoca*, or *Stenotrophomonas maltophilia* was detected (Appendix 1).

- **Surgical and anesthetic practices and instrumentation**  There were no common surgical or anesthetic practices, or surgical procedures identified among the patients with positive blood cultures.

- **Pharmacy contamination**  Review of the line list revealed that all patients had received IV hydromorphone via PCA prior to positive blood culture collection. Hydromorphone bags were prepared in the SCH Pharmacy per physician order. SCH staff evaluated the preparation steps (adding hydromorphone to normal saline bags under a safety hood), pharmacy staffing records to identify pharmacists who prepared the bags, and individuals who transported the bags to the patient care areas. No contamination was identified in the bag preparation process and no information was provided to MDH regarding the pharmacists who prepared the bags. Unopened vials of hydromorphone and bags of normal saline were tested for contamination in the SCH Laboratory; all were negative. There were no known U.S. Food and Drug Administration (FDA) recalls associated with the hydromorphone or normal saline products in use at SCH.

- **Hydromorphone bag transport from the SCH Pharmacy to patient care areas**  SCH staff reported that prepared hydromorphone bags were transported to the patient care unit by pharmacy staff and stored in locked narcotics boxes. Nursing staff accessed the narcotics boxes by obtaining keys through the Omnicell, a secure, automated system for medication dispensing that requires a unique user code. No source of contamination was identified in the transport process.

- **SCH staff access to hydromorphone bags**  Hydromorphone bags were stored in one of three locked narcotics boxes on the Surgical Unit/SPCU. Nursing staff accessed the keys to the narcotics boxes by using their Omnicell user code. Hydromorphone bags were spiked and hung at the patient bedside. At the beginning of the investigation on February 21, 2011, SCH staff reported that they were not aware of any unusual patterns of Omnicell access. On March 14, 2011 SCH staff reported that a review of Omnicell access logs indicated that a specific healthcare worker (healthcare worker A) had an Omnicell access rate several times greater than any other staff from July 2010 – January 2011.
SCH Laboratory Results

The SCH Laboratory cultured and tested the contents of 8 hydromorphone bags collected on February 20, 2011 by SCH staff. One bag (Bag A), obtained from a Surgical Unit/SPCU patient #21 grew K. oxytoca, S. maltophilia, and Pseudomonas aeruginosa. This patient’s blood cultures were positive for K. oxytoca, S. maltophilia, and O. anthropi. A second bag (Bag B) was collected from a Surgical Unit/SPCU patient #26 and grew K. oxytoca and S. maltophilia. This patient’s blood culture test was negative. SCH reported that the remaining 6 bags were negative for bacterial growth.

The identification of the same bacteria in two patients’ blood and the two hydromorphone bags in use by these patients led MDH investigators to consider drug diversion as the source of the positive blood cultures. Additionally, intravenous administration of the hydromorphone could facilitate transfer of the bacteria from the bag to the patient’s blood. MDH suggested that SCH staff investigate the possibility of drug diversion.

MDH Epidemiologic Investigation

MDH staff initiated an epidemiologic investigation to determine if there were additional cases associated with this cluster and to identify the source. A data collection form was developed to review medical records of patients that met the following case definition:

- Admission to the Surgical Unit/SPCU at SCH since October 1, 2010; and
- ≥1 positive blood culture for O. anthropi, K. oxytoca, and/or S. maltophilia; and
- Received narcotics via PCA or epidural route 36 hours prior to collection of positive blood culture.

Twenty-five patients met the case definition (case-patients) from October 2010 – March 2011 (Figure 1). MDH staff began a review of case-patient medical records on March 7 and 8, 2011. All 25 case-patients were post-surgical; median age was 61 years (range 35 – 84); 44% were female. Three case-patients had evidence of a surgical site infection at the time of blood culture. All 25 case-patients had documented symptoms that prompted blood culture collection (Table 1); other symptoms documented in the case-patient records included fever, tachycardia, tachypnea, diaphoresis, and increased pain. Within 36 hours prior to blood culture collection, one case-patient received fentanyl via epidural, one case-patient received fentanyl IV, and 23 case-patients received narcotics via PCA (19: hydromorphone; 3: morphine; and 1 hydromorphone and morphine). Eight of the 23 also received narcotics via IV (5: hydromorphone; 3: hydromorphone and fentanyl). Within 48 hours of symptom onset, six case-patients were transferred to an intensive care unit, three were returned to the operating room due to the unexplained nature of their symptoms, and one died (Table 1).

Among the 25 case-patients, 38 isolates of O. anthropi, K. oxytoca, and S. maltophilia grew from 35 blood cultures from October 2010 through March 2011 (Table 2). Ten of 35 blood cultures were polymicrobial (contained multiple bacteria); eight of these grew two different bacteria and two grew three different bacteria. In addition to O. anthropi, K. oxytoca, and S. maltophilia, bacterial species were Enterobacter agglomerans, Pseudomonas aeruginosa, Tatumella spp., Corynebacterium spp., and Acinetobacter junii (Table 2).

MDH staff analyzed SCH Laboratory microbiology data from October 2009 – March 2011 to assess trends of bacteremia due to O. anthropi, K. oxytoca, and S. maltophilia (Figures 2-5) among patients on the Surgical Unit/SPCU compared to patients hospital-wide. From October 2009 to November 2010 the rates of bacteremia due to these bacteria were nearly zero. From October 2010 through February 2011 a substantial increase in bacteremia due to O. anthropi, K. oxytoca, and S. maltophilia was noted among...
Surgical Unit/SPCU patients, reaching a five-fold increase in February 2011. In March 2011, the rate of bacteremia due to these bacteria returned to nearly zero on the Surgical Unit/SPCU.

MDH staff reviewed SCH Lab microbiology data for bacteremia due to common skin contaminants, *Staphylococcus aureus*, and Gram-negative bacteria other than *O. anthropi*, *K. oxytoca* and/or *S. maltophilia* among patients on the Surgical Unit/SPCU from January 2009 – March 2011. No rate increase was identified for common skin contaminants or *Staphylococcus aureus*. There was an increase in one family of Gram-negative bacteria, Enterobacteriaceae, between October – November 2010.

MDH staff requested staffing records for the Surgical Unit/SPCU to look for staffing patterns associated with case-patients. While conducting the medical record reviews at SCH on March 8, 2011, and before MDH staff had an opportunity to review Surgical Unit/SPCU staffing records, MDH staff were informed by the SCH IP that earlier that day a registered nurse with supervisory responsibilities assigned to the Surgical Unit/SPCU (healthcare worker A) was removed from practice for suspicion of narcotic diversion.

After removal of healthcare worker A, there were no further reports in March 2011 of bacteremia due to *O. anthropi*, *S. maltophilia*, or *K. oxytoca* among patients on the Surgical Unit/SPCU. SCH has not reported any additional cases of bacteremia with these organisms to MDH to date (6/13/2012).

**Laboratory Results from CDC, FDA, MDH, and SCH**

MDH Laboratory and CDC Laboratory tested each submitted isolate to confirm the bacterial species and conducted pulsed-field gel electrophoresis (PFGE). PFGE provides a DNA pattern, or DNA “fingerprint”, to describe genetic elements of the bacteria. The patterns can be quantitatively compared to show genetic relatedness. The “Tenover criteria”, established guidelines for comparing differences between PFGE patterns, were used; patterns were classified as “indistinguishable”, “closely related” (1-3 bands different between patterns which could be achieved by a single mutation), “possibly related” (4-6 bands different between patterns which required a minimum of two mutations”, or “unrelated” (> 7 bands different between patterns which requires a minimum of three mutations). It is incumbent that PFGE data be analyzed in the context of epidemiological data to best determine the likelihood of isolates originating from a common source. For a description of MDH laboratory methods, see Appendix 2.

**Isolates obtained from hydromorphone bags**

Four isolates (2: *K. oxytoca*, 2: *S. maltophilia*) recovered from two hydromorphone bags (Bag A and Bag B) were submitted by the SCH Laboratory to the MDH Laboratory and CDC Laboratory for PFGE testing. The *S. maltophilia* isolates had indistinguishable PFGE patterns from one another and the *K. oxytoca* isolates had indistinguishable PFGE patterns from one another (Figures 6 and 8). CDC (Appendix 1) and MDH PFGE testing results were identical for these *S. maltophilia* isolates and *K. oxytoca* isolates.

SCH staff gave a FDA Criminal Investigator two hydromorphone bags (Bag C and Bag D) that were in use by Surgical Unit/SPCU patients on February 22, 2011. Bag C was collected from patient #21 and Bag D from patient #26. Of note, Bag C was collected from the same patient as Bag A, and Bag D was collected from the same patient as Bag B. Two *K. oxytoca* isolates were recovered from Bag C and one *K. oxytoca* isolate from Bag D. The FDA Laboratory submitted these isolates to the MDH Laboratory for

---

PFGE testing and MDH Laboratory subsequently submitted them to the CDC Laboratory for PFGE testing. One *K. oxytoca* isolate from Bag C and the *K. oxytoca* isolate from Bag D had indistinguishable PFGE patterns; the second *K. oxytoca* isolate from Bag C was not related to these isolates (Figure 6). CDC Laboratory results (Appendix 1) were identical to MDH Laboratory results.

Bags A and B each grew one *S. maltophilia* isolate. The PFGE patterns of these isolates were indistinguishable (Figure 8). CDC Laboratory results (Appendix 1) were identical to MDH Laboratory results.

**Patient blood isolates**

SCH Laboratory submitted 16 blood isolates (8: *O. anthropi*, 7: *K. oxytoca*, 1: *S. maltophilia*) obtained from the case-patients to MDH Laboratory for PFGE testing. Two of the *O. anthropi* isolates were obtained from the same case-patient on the same day. All of the *O. anthropi* blood isolates had indistinguishable PFGE patterns. Five of the *K. oxytoca* blood isolates had indistinguishable PFGE patterns (KOXY1); two *K. oxytoca* isolate PFGE patterns were related to each other (KOXY2 and KOXY3). Neither KOXY2 nor KOXY3 were related to KOXY1 (Figure 6). CDC Laboratory results (Appendix 1) were identical to MDH Laboratory results.

Patient #21 was administered hydromorphone Bag A and hydromorphone Bag C. The PFGE pattern of the *K. oxytoca* isolate from Bag A, one of the *K. oxytoca* isolates from Bag C, and the *K. oxytoca* blood isolate from this patient had indistinguishable PFGE patterns (Figure 6).

**Environmental samples**

Environmental samples (IV saline, distilled water, water and ice from the ice machine, and hotline warmer water) collected in February 2011 by SCH staff were submitted to the CDC Laboratory for testing. None of these environmental samples grew *O. anthropi*, *K. oxytoca*, or *S. maltophilia* (Appendix 1).

Additional environmental samples were collected in March 2011 by MDH staff and were tested by the MDH Laboratory. These included samples from two bathrooms (faucets, drain and sink), three general sinks (drain, faucet, sink), one clean utility sink, and two air conditioners near the narcotic boxes on the Surgical Unit/SPCU. Two samples (SPCU bathroom drain and Surgery 1A drain) grew *K. oxytoca* and one sample (Surgery 1A drain) grew *S. maltophilia*. The PFGE patterns of these isolates were unrelated to PFGE patterns of case-patient blood isolates or hydromorphone bag isolates.

The FDA Criminal Investigator collected a saline bottle from healthcare worker A’s desk and submitted it to the FDA Laboratory for testing. The FDA Laboratory identified two *O. anthropi* isolates in the bottle and submitted these isolates to the MDH Laboratory for PFGE testing. These two *O. anthropi* isolates had indistinguishable PFGE patterns (OANT9) from one another; this PFGE pattern was closely related to the PFGE pattern of six *O. anthropi* case-patient blood isolates (OANT4) (Figure 7).

**Additional Laboratory Testing**

The SCH Laboratory reported that drug concentration testing was performed on a 100 mL hydromorphone bag in use by a Surgical Unit/SPCU patient from which bacteria were recovered. The date of bag collection and the patient from whom the bag was removed were not reported to MDH. The SCH Laboratory reported that the hydromorphone concentration in the bag was 0.014 mg/mL and the normal concentration of a 100 mL hydromorphone bag is reportedly 0.2 mg/mL.

**Other Investigative Activities**
On March 8, 2011 MDH staff were informed by SCH staff that healthcare worker A, assigned to the Surgical Unit/SPCU, admitted to drug diversion and replacement with saline. MDH took the following actions:

- On March 9, MDH contacted FDA and the U.S. Department of Justice Drug Enforcement Administration (DEA) regarding the possibility of narcotic diversion at SCH. MDH was concerned that the public’s health may be at ongoing risk if healthcare worker A continued to have access to narcotics in this or another healthcare facility.
- MDH contacted the Minnesota Board of Nursing after being informed by SCH staff that healthcare worker A was a registered nurse due to concern for potential ongoing risk of patient harm in the event that healthcare worker A was employed or sought new employment in another healthcare facility.
- MDH expressed concern to SCH staff, FDA, and DEA about potential bloodborne pathogen transmission to patients affected by possible narcotic diversion. SCH personnel contacted healthcare worker A who consented to bloodborne pathogen testing on March 9.
  - MDH cross-matched the name of healthcare worker A with MDH viral hepatitis B and C and HIV disease databases; no matches were found.
  - SCH staff subsequently informed MDH staff that healthcare worker A tested negative for HIV, hepatitis B virus, and hepatitis C virus.
- MDH was asked by DEA, FDA, and SCH personnel to interview healthcare worker A regarding diversion methodology in order to evaluate ongoing risk to patients. This interview was conducted on March 9 at SCH.
  - During this interview, healthcare worker A admitted to obtaining narcotic bags from the locked narcotic boxes, peeling the foil covering on the narcotic bag port, withdrawing narcotic from the bag with a syringe, and replacing the displaced liquid with saline. The narcotic bag was returned to the locked narcotic box.
- Healthcare worker A stated that diverted narcotics were used by healthcare worker A and shared with one other person. This raised concern for potential bloodborne pathogen transmission to patients. In consultation with CDC, MDH recommended that this other person also undergo bloodborne pathogen testing. SCH contacted this other person who agreed to have this testing done at SCH on March 11, 2011. SCH staff subsequently informed MDH staff that the results of the other person’s HIV, hepatitis B virus, and hepatitis C virus tests were negative.
- MDH recommended that SCH staff report this situation to the MDH Office of Health Facility Complaints (OHFC).
- On March 11, SCH staff informed MDH that they had reviewed the Omnicell access log and noted that healthcare worker A accessed the Omnicell more frequently than other staff.
- On March 14, CDC and MDH recommended that SCH notify all patients potentially affected by this narcotic diversion, not just those with positive blood cultures. A timely, transparent, and proactive approach was recommended to SCH, based on CDC’s extensive experience with similar situations.

Conclusions

This is the first documented report of a cluster of bacteremias where genetically closely related bacteria were found in three epidemiologically-linked sources: 1) normally sterile hydromorphone bags; 2) patient blood cultures; and 3) normal saline which a healthcare worker reportedly used to replace diverted narcotic from the hydromorphone bags. It is plausible that bacteria were introduced into the narcotic bags by healthcare worker A at any of several points during the diversion of narcotic from the bags and replacement with saline and the contaminated contents of the bags resulted in bloodstream infection in at least seven cases. This contamination could have occurred in multiple ways such as use of a contaminated syringe for diversion/replacement or healthcare worker A not using sterile technique when accessing the narcotic bags.
The identification of bacteremias among case-patients described in this report likely underestimates the impact of drug diversion in this setting. Other potentially affected patients not identified as part of this outbreak investigation included those on the Surgical Unit/SPCU who received narcotics administered intravenously and did not have a blood culture obtained, or who had a bloodstream infection caused by a bacterial species other than those included in this outbreak, or whose pain management was inadequate.

Discussion

The SCH IP contacted the MDH after identifying a cluster of bacteremias thought to be associated with product contamination. While multiple contamination sources were considered, the presence of polymicrobial blood cultures and unusual Gram-negative organisms were suggestive of controlled substance diversion and replacement.

MDH was primarily concerned about on-going risk to the public’s health after learning that healthcare worker A had been removed from practice yet held an active nursing license, allowing healthcare worker A continued access to narcotics at any Minnesota healthcare facility. FDA, DEA and MDH worked collaboratively to facilitate a timely epidemiologic investigation. MDH staff interviewed healthcare worker A regarding the mode of diversion to assess on-going patient risk and the need for bloodborne pathogen testing/prophylaxis.

Importantly, within 48 hours of symptom onset, six case-patients were transferred to an intensive care unit, three required unanticipated surgical procedures due to the unexplained nature of their symptoms, and one died. It is unclear whether case-patient outcomes were a result of symptoms of bacteremia or symptoms of inadequate pain management since healthcare workers responding to the case-patient would have assumed that the patient was receiving the prescribed dosage of narcotic. While it is possible that coincidental events led to these outcomes, given the microbiologic and epidemiologic data, this is highly unlikely.

While MDH did not conduct a medico-legal review of medical records, it is possible that other case-patients, whose pain medications were likely diverted and contaminated, also suffered undue, unnecessary harm. Although the likelihood of additional patients having experienced negative outcomes became apparent during our investigation, this was beyond the scope of the MDH investigation.

This epidemiologic investigation was challenging. Several SCH staff closely tied to the investigation had long-standing personal relationships with healthcare worker A, which impeded their ability to be objective. Despite concerns for hydromorphone product contamination, only two hydromorphone bags from Surgical Unit/SPCU patients taken on February 20, 2011 were tested by SCH Lab. On March 8, 2011, the date that healthcare worker A was removed from practice, all hydromorphone bags were reportedly pulled from Surgical Unit/SPCU. The content of these bags leaked before being collected by the FDA for testing.

Past outbreaks of hepatitis C have been linked to healthcare worker drug diversion and have shown that healthcare workers involved in diversion may be employed by multiple healthcare facilities as long as their professional license remains active. While SCH staff submitted reports to authorities (e.g. MN Board of Nursing, DEA, FDA, OHFC), the reports were delayed and the content lacked critical details (e.g. bacteremia detected in 25 patients on the Surgical Unit/SPCU within a defined time period). Healthcare worker A’s registered nurse license remained active without stipulation for three weeks after receiving notice of healthcare worker A’s drug diversion and the resultant patient injury. This created the opportunity for healthcare worker A to continue to practice and place patients unnecessarily at risk for...
uncontrolled pain management and bloodstream infections at SCH and other healthcare facilities. To our knowledge, healthcare worker A was not hired at another healthcare facility prior to the MN Board of Nursing placing a stipulation on the license.

To promote a culture of patient safety, all healthcare facilities need to have policies and mechanisms in place to prevent, detect and address narcotic diversion and resulting adverse events. Detection of infection clusters or unusual organisms should trigger an investigation and healthcare worker drug diversion should be considered.

Healthcare facilities should be aware that state health departments and other public health entities can contribute epidemiologic and laboratory expertise when investigating suspected drug diversion, and facilitate additional consultation with CDC.

**Post-investigation Recommended Action Steps for SCH**

1. Ensure compliance with DEA requirements to notify DEA in writing of the theft or significant loss of any controlled substances within one business day of discovery, and complete and submit DEA Form 106.

2. Ensure employees are aware of the hospital policy for addressing pharmaceutical diversion.

3. Promote an institutional culture that supports and encourages employees to notify appropriate personnel of suspicious activity or behavior involving pharmaceuticals.

4. The involvement of senior leadership, including medical staff, is essential throughout an investigation involving suspected drug diversion, including notifying appropriate authorities.

5. Ensure that the facility infection surveillance and microbiology data are reviewed and analyzed regularly. Information technology staff should be engaged to ensure that the infection surveillance system maximizes available resources (i.e. MedMined, laboratory data).

6. The Infection Prevention and Control Department should be adequately staffed to detect increases in incidence of pathogens, clusters or outbreaks, and the detection of unusual pathogens should be communicated to appropriate personnel (e.g. infection prevention, infectious disease, laboratory, and senior leadership) in a timely manner.

7. Ensure that Omnicell access data are regularly monitored to detect unusual patterns of use by staff.

8. Relocate the narcotic box from behind a door near the staff restroom on Surgical Unit 2.

9. Follow best practices as defined by the Minnesota Controlled Substance Diversion Prevention Coalition.

10. Objectivity in investigations is critical; avoid involving staff with close personal and/or professional relationships with individuals targeted in an investigation.

Table 1. Bacteremia Case-patients, SCH Surgical Unit/SPCU, 10/1/2010 – 3/18/2011: Demographics and Signs/symptoms within 48 hours of Onset Date
<table>
<thead>
<tr>
<th>Description</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total case-patients</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>61 (35 to 84)</td>
<td></td>
</tr>
<tr>
<td>Median length of stay, days (range)</td>
<td>10 (4 to 32)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>44%</td>
</tr>
<tr>
<td>Antibiotics at time of blood culture</td>
<td>15</td>
<td>60%</td>
</tr>
<tr>
<td>Signs/symptoms within 48 hours of onset date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
<td>12%</td>
</tr>
<tr>
<td>Chills</td>
<td>12</td>
<td>48%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>3</td>
<td>12%</td>
</tr>
<tr>
<td>Headache</td>
<td>5</td>
<td>20%</td>
</tr>
<tr>
<td>Cough</td>
<td>4</td>
<td>16%</td>
</tr>
<tr>
<td>Nausea</td>
<td>11</td>
<td>44%</td>
</tr>
<tr>
<td>Hypotension</td>
<td>6</td>
<td>24%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7</td>
<td>28%</td>
</tr>
<tr>
<td>Agitation/Confusion/Disorientation</td>
<td>12</td>
<td>48%</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>15</td>
<td>60%</td>
</tr>
<tr>
<td>Tachypnea</td>
<td>4</td>
<td>16%</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>5</td>
<td>20%</td>
</tr>
<tr>
<td>Diaphoresis</td>
<td>5</td>
<td>20%</td>
</tr>
<tr>
<td>Elevated C reactive protein (CRP)</td>
<td>9</td>
<td>36%</td>
</tr>
<tr>
<td>Fever</td>
<td>24</td>
<td>96%</td>
</tr>
<tr>
<td>Elevated white blood cell (WBC) count</td>
<td>9</td>
<td>36%</td>
</tr>
<tr>
<td>Signs/Symptoms of a skin/soft tissue infection</td>
<td>3</td>
<td>12%</td>
</tr>
<tr>
<td>Increased pain</td>
<td>15</td>
<td>60%</td>
</tr>
<tr>
<td>Outcome within 48 hours of symptom onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms resolved</td>
<td>15</td>
<td>60%</td>
</tr>
<tr>
<td>Transfer to ICU/CCU/MPCU</td>
<td>6</td>
<td>24%</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Additional (unplanned surgery)</td>
<td>3</td>
<td>12%</td>
</tr>
</tbody>
</table>
Table 2. Bacteremia Case-patients, SCH Surgical Unit/SPCU, 10/1/2010 – 3/18/2011: Positive Blood Culture Results

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Blood Culture 1</th>
<th>Blood Culture 2</th>
<th>Blood Culture 3</th>
<th>Blood Culture 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture Date</td>
<td>Result</td>
<td>Culture Date</td>
<td>Result</td>
</tr>
<tr>
<td>13</td>
<td>10/28/10</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>11/2/10</td>
<td><em>K. oxytoca, Tatumella spp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>11/9/10</td>
<td><em>K. oxytoca, E. agglomerans, P. aeruginosa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>11/10/10</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>11/16/10</td>
<td><em>K. oxytoca</em></td>
<td>11/17/10</td>
<td><em>K. oxytoca</em></td>
</tr>
<tr>
<td>16</td>
<td>11/21/10</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>11/23/10</td>
<td><em>K. oxytoca, E. agglomerans</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>12/1/10</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12/2/10</td>
<td><em>K. oxytoca</em></td>
<td>12/3/10</td>
<td><em>K. oxytoca, Tatumella spp.</em></td>
</tr>
<tr>
<td>12</td>
<td>12/15/10</td>
<td><em>O. anthropli, P. aeruginosa, Corynebacterium spp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>12/24/10</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>12/29/10</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1/11/11</td>
<td><em>O. anthropli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1/11/11</td>
<td><em>K. oxytoca, E. agglomerans</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>1/15/11</td>
<td><em>K. oxytoca</em></td>
<td>1/17/11</td>
<td><em>K. oxytoca</em></td>
</tr>
<tr>
<td>15</td>
<td>1/21/11</td>
<td><em>K. oxytoca, S. maltophilia</em></td>
<td>1/23/11</td>
<td><em>K. oxytoca</em></td>
</tr>
<tr>
<td>20</td>
<td>2/4/11</td>
<td><em>O. anthropli, A. junii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2/5/11</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2/5/11</td>
<td><em>K. oxytoca</em></td>
<td>2/9/11</td>
<td><em>O. anthropli</em></td>
</tr>
<tr>
<td>18</td>
<td>2/9/11</td>
<td><em>K. oxytoca</em></td>
<td>2/11/11</td>
<td><em>O. anthropli</em></td>
</tr>
<tr>
<td>5</td>
<td>2/12/11</td>
<td><em>K. oxytoca, O. anthropli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2/17/11</td>
<td><em>O. anthropli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>2/17/11</td>
<td><em>K. oxytoca</em></td>
<td>2/19/11</td>
<td><em>S. maltophilia</em></td>
</tr>
<tr>
<td>2</td>
<td>2/19/11</td>
<td><em>K. oxytoca</em></td>
<td>2/20/11</td>
<td><em>O. anthropli</em></td>
</tr>
<tr>
<td>4</td>
<td>3/5/11</td>
<td><em>K. oxytoca</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Bacteremia case-patients with positive blood cultures among SCH Surgical Unit/SPCU, October 2010 – March 2011. Each box indicates a blood culture for a case-patient that was positive for *K. oxytoca* (K), *O. anthropi* (O), and/or *S. maltophilia* (S).
Figure 2. Rate of positive blood cultures for *Ochrobactrum anthropi* per 1,000 patient-days, October 2009-March 2011.
Figure 3. Rate of positive blood cultures for *Klebsiella oxytoca* per 1,000 patient-days, October 2009 – March 2011.
Figure 4. Rate of positive blood cultures for *Stenotrophomonas maltophilia* per 1,000 patient-days, October 2009 – March 2011.
Figure 5. Rate of all positive blood cultures per 1,000 patient-days, October 2009 – March 2011.
Figure 6. Dendrogram of K. oxytoca PFGE patterns (A) and band differences between K. oxytoca PFGE patterns (B). Dendrogram (A) represents genetic differences between tested isolates based on PFGE patterns. Grid below the dendrogram (B) represents the number of PFGE bands that are different between each of the PFGE patterns identified. Indistinguishable patterns were assigned the same PFGE pattern designation (i.e. all KOXY1 isolates were indistinguishable). PFGE patterns that were 1-3 bands different were labeled as “closely related”. PFGE patterns that were 4-6 bands different were labeled as “possibly related” and PFGE patterns that were ≥7 bands different were called “unrelated”.

<table>
<thead>
<tr>
<th>MDH #</th>
<th>PFGE Pattern</th>
<th>Source</th>
<th>Patient #</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2011008655-3</td>
<td>KOXY9</td>
<td>SPCU bathroom drain</td>
<td>N/A</td>
</tr>
<tr>
<td>M2011008658-4</td>
<td>KOXY8</td>
<td>Surgery 1A drain</td>
<td>N/A</td>
</tr>
<tr>
<td>C2011006962</td>
<td>KOXY1</td>
<td>Blood</td>
<td>18</td>
</tr>
<tr>
<td>C2011006967</td>
<td>KOXY1</td>
<td>Blood</td>
<td>21</td>
</tr>
<tr>
<td>C2011006968</td>
<td>KOXY1</td>
<td>Blood</td>
<td>2</td>
</tr>
<tr>
<td>C2011007851</td>
<td>KOXY1</td>
<td>Blood</td>
<td>6</td>
</tr>
<tr>
<td>C2011007852</td>
<td>KOXY1</td>
<td>Blood</td>
<td>23</td>
</tr>
<tr>
<td>C2011013116</td>
<td>KOXY1</td>
<td>SCH, hydromorphone bag A</td>
<td>21</td>
</tr>
<tr>
<td>C2011013117</td>
<td>KOXY1</td>
<td>SCH, hydromorphone bag B</td>
<td>26</td>
</tr>
<tr>
<td>C2011034004</td>
<td>KOXY1</td>
<td>FDA, hydromorphone bag C (#2BB)</td>
<td>21</td>
</tr>
<tr>
<td>C2011034005</td>
<td>KOXY2</td>
<td>FDA, hydromorphone bag C (#2BC)</td>
<td>21</td>
</tr>
<tr>
<td>C2011034006</td>
<td>KOXY2</td>
<td>FDA, hydromorphone bag D (#2CA)</td>
<td>26</td>
</tr>
<tr>
<td>C2011006960</td>
<td>KOXY2</td>
<td>Blood</td>
<td>7</td>
</tr>
<tr>
<td>C2011006964</td>
<td>KOXY3</td>
<td>Blood</td>
<td>5</td>
</tr>
<tr>
<td>KOXY1</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOXY2</td>
<td>&gt;10</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>KOXY3</td>
<td>&gt;10</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>KOXY8</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td></td>
</tr>
<tr>
<td>KOXY9</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td></td>
</tr>
</tbody>
</table>

Indicates unrelated

Indicates closely related

Table: PFGE Pattern Differences

<table>
<thead>
<tr>
<th>PFGE Pattern</th>
<th>KOXY1</th>
<th>KOXY2</th>
<th>KOXY3</th>
<th>KOXY8</th>
<th>KOXY9</th>
</tr>
</thead>
<tbody>
<tr>
<td>KOXY1</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOXY2</td>
<td>&gt;10</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOXY3</td>
<td>&gt;10</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOXY8</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>KOXY9</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>--</td>
</tr>
</tbody>
</table>
Figure 7. Dendrogram of *O. anthropi* PFGE patterns (A) and band differences between *O. anthropi* PFGE patterns (B). Dendrogram (A) represents genetic differences between tested isolates based on PFGE patterns. Grid below the dendrogram (B) represents the number of PFGE bands that are different between each of the PFGE patterns identified. Indistinguishable patterns were assigned the same PFGE pattern designation (i.e. all OANT4 isolates were indistinguishable). PFGE patterns that were 1-3 bands different were labeled as “closely related”. PFGE patterns that were 4-6 bands different were labeled as “possibly related” and PFGE patterns that were ≥7 bands different were called “unrelated”. 

<table>
<thead>
<tr>
<th>MDH #</th>
<th>PFGE Pattern</th>
<th>Source</th>
<th>Patient #</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2011020196</td>
<td>OANT9</td>
<td>FDA, saline bottle (#1A)</td>
<td>N/A</td>
</tr>
<tr>
<td>C2011020197</td>
<td>OANT9</td>
<td>FDA, saline bottle (#1B)</td>
<td>N/A</td>
</tr>
<tr>
<td>C2011006959</td>
<td>OANT4</td>
<td>Blood</td>
<td>7</td>
</tr>
<tr>
<td>C2011006961</td>
<td>OANT4</td>
<td>Blood</td>
<td>18</td>
</tr>
<tr>
<td>C2011006963</td>
<td>OANT4</td>
<td>Blood</td>
<td>5</td>
</tr>
<tr>
<td>C2011006965</td>
<td>OANT4</td>
<td>Blood</td>
<td>3</td>
</tr>
<tr>
<td>C2011006966</td>
<td>OANT4</td>
<td>Blood</td>
<td>21</td>
</tr>
<tr>
<td>C2011006970</td>
<td>OANT4</td>
<td>Blood</td>
<td>20</td>
</tr>
<tr>
<td>C2011006971</td>
<td>OANT6</td>
<td>Blood</td>
<td>14</td>
</tr>
<tr>
<td>C2011007855</td>
<td>OANT6</td>
<td>Blood</td>
<td>14</td>
</tr>
<tr>
<td>C2011006969</td>
<td>OANT5</td>
<td>Quality control organism</td>
<td>N/A</td>
</tr>
<tr>
<td>C2011006972</td>
<td>OANT7</td>
<td>Cornea</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### A.

**PFGE Pattern**

<table>
<thead>
<tr>
<th>PFGE Pattern</th>
<th>OANT4</th>
<th>OANT5</th>
<th>OANT6</th>
<th>OANT9</th>
</tr>
</thead>
<tbody>
<tr>
<td>OANT4</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OANT5</td>
<td>&gt;10</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OANT6</td>
<td>2</td>
<td>&gt;10</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>OANT9</td>
<td>3</td>
<td>&gt;10</td>
<td>5</td>
<td>--</td>
</tr>
</tbody>
</table>

- **Indicates unrelated**
- **Indicates closely related**
- **Indicates possibly related**
A.  

![Dendrogram of S. maltophilia PFGE patterns (A) and band differences between S. maltophilia PFGE patterns (B). Dendrogram (A) represents genetic differences between tested isolates based on PFGE patterns. Grid below the dendrogram (B) represents the number of PFGE bands that are different between each of the PFGE patterns identified. Indistinguishable patterns were assigned the same PFGE pattern designation (i.e. all SMALT1 isolates were indistinguishable). PFGE patterns that were 1-3 bands different were labeled as “closely related”. PFGE patterns that were 4-6 bands different were labeled as “possibly related” and PFGE patterns that were ≥7 bands different were called “unrelated”.]

<table>
<thead>
<tr>
<th>MDH #</th>
<th>PFGE Pattern</th>
<th>Source</th>
<th>Patient #</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2011007853</td>
<td>SMALT1</td>
<td>SCH, hydromorphone bag A</td>
<td>21</td>
</tr>
<tr>
<td>C2011007854</td>
<td>SMALT1</td>
<td>SCH, hydromorphone bag B</td>
<td>26</td>
</tr>
<tr>
<td>C2011007856</td>
<td>SMALT1</td>
<td>Blood</td>
<td>15</td>
</tr>
<tr>
<td>M2011008658-2</td>
<td>SMALT6</td>
<td>Surgery 1A drain</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Environmental samples were submitted to the Environmental Microbiology lab for isolation and identification of *Ochrobactrum anthropi*, *Klebsiella oxytoca*, and *Stenotrophomonas maltophilia*, and for genetic relatedness testing of recovered environmental and patient isolates. Pulsed-field gel electrophoresis (PFGE) was performed on isolates received at CDC from the Minnesota Department of Health.

**Test Methods**

**Microbial Recovery and Isolation:** 0.9% IV saline, distilled water, water and ice from the ice machine, and the IV warmer water samples were filtered and cultured onto MacConkey II (Becton, Dickinson and Company, Sparks, MD) and R2A plates. All plates were incubated for 24-48 hours at 30°C. The plates from the IV saline samples were held for 14 days to test for sterility based on a membrane filtration standard protocol (1) with culture modifications described above. Suspected isolates with phenotypic characteristics similar to *O. anthropi*, *K. oxytoca*, and *S. maltophilia* were isolated and identified as described below.

**Identification:** Species identification confirmation of the patient isolates and suspect colonies recovered from the environmental samples was performed by an automated biochemical identification system (Vitek 2; bioMérieux, Durham, NC).

**PFGE:** The CDC PFGE protocol used is based on a standard *Yersinia* PulseNet protocol available at [http://www.cdc.gov/pulsenet/protocols.htm](http://www.cdc.gov/pulsenet/protocols.htm), was used with the following modifications. Chromosomal DNA from the *O. anthropi* and *S. maltophilia* isolates was digested with the restriction endonuclease SpeI and XbaI, respectively. Restriction fragments were separated with CHEF Mapper® XA Pulsed Field Electrophoresis System (Bio-Rad Laboratories, CA). PFGE conditions for the isolates were switch times of 2 and 50 seconds and total run time of 22 hours for *O. anthropi*, switch times of 5 and 40 seconds and total run time of 17.6 hours for *S. maltophilia*. The following PFGE conditions were used for *K. oxytoca* isolates:

- Batch 1: Molecular chromosomal DNA was digested with XbaI. Running conditions were switch times of 2.2 and 63.8 seconds for a total run time of 21 hours.
- Batch 2: A second batch of *K. oxytoca* isolates including repeat isolates of those originally received and new isolates recovered from IV medications were sent for confirmation by CDC of PFGE testing done at the MNDOH. PFGE was performed using a comparable MNDOH protocol which is based on a standard Salmonella PulseNet protocol (2). SpeI was used to digest the DNA and the PFGE running conditions were 2.2 seconds for the initial switch time, 64.0 seconds for the final switch time, and a total run time of 18 hours.

*Salmonella* serotype *Braenderup* (H9812 strain) was used as a universal standard in all the PFGE runs for all the isolates. The genetic relatedness of the isolates was analyzed by BioNumerics software (Applied Maths, Austin, TX). Similarity of PFGE patterns was based upon Dice coefficients and a dendrogram was built using the unweighted-pairing group method. The Tenover criteria (3) were used to interpret the comparison of the PFGE patterns from the environmental, IV medication and patient isolates; patterns were classified as indistinguishable (100% similarity), closely related (1-3 band difference), possibly related (4-6 band difference) or unrelated (>7 band difference).
Results Summary

*Ochrobactrum anthropi, Klebsiella oxytoca,* or *Stenotrophomonas maltophilia* were not recovered from the environmental samples (Table 1) submitted to CDC. PFGE was performed on isolates received at CDC from the Minnesota Department of Health.

**PFGE:**

1. *O. anthropi:* The PFGE band patterns of eight of eight patient isolates were genetically indistinguishable from one another (Table 2, Figure 1).
2. *S. maltophilia:* All three of the isolates were genetically indistinguishable from one another (Table 3, Figure 2).
3. *K. oxytoca:*
   a. Batch 1: Five of the seven isolates from Batch 1 were visually indistinguishable from each other (Table 4a, Figure 3a); the other two isolates (MDH ID #s C2011006960 and C2011006964) were closely related to one another, but were not related to the larger *K. oxytoca* genetically indistinguishable cluster.
   b. Batch 2: Two unrelated indistinguishable clusters (A&B) were identified (Table 4b, Figure 3b). Cluster A consisted of 5 blood isolates (C2011006962, C2011006967, C2011006968, C2011007851 and C2011007852) and isolates from hydromorphone bags (C20110013116, C20110013117, C20110034004). Cluster B consisted of isolates: C2011006960 (from Blood; similar source CDC 2011-09-02 and 2011-09-46) and isolates C20110034005 and C20110034006 (hydromorphone); blood isolate C2011006964 is closely related to isolates in Cluster B.

References


Revision History

Revision 0  Original issued 04/28/2011
Revision 1  Issued 02/28/2012; incorporated changes below based on feedback from MNDOH:
   - Page 1, last paragraph: The PFGE band patterns of eight of the nine (this should read eight of eight since PFGE was not performed on the cornea isolate)
   - Table 3: State ID isolate SO20110341 is from a PCA bag - not a human (blood) isolate.
Revision 2  Issued 06/06/2012; incorporated changes based on feedback from MNDOH
   - Page 2, Results Summary cont’d: State ID #s SO20110236 and SO20110236 should be changed to State ID #s SO20110236 and SO20110240
   - Added PFGE analysis of additional *K. oxytoca* Batch 2 isolates received
Tested by: Sarah Gilbert, Bette Jensen, Heather O'Connell, Alicia Shams

DISCLAIMER: The identification methods used and the results reported are for investigational or research purposes. These test results may not be used for diagnosis, treatment, or for the assessment of a patient's health.
Table 1. Summary of results of tested environmental samples sent to the outbreak lab.

<table>
<thead>
<tr>
<th>CDC Lab#</th>
<th>State ID</th>
<th>Origin</th>
<th>Description</th>
<th>Results (species ID confirmed with Vitek 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-09-15</td>
<td>SO20110270</td>
<td>IV Medications</td>
<td>0.9% NaCl bags</td>
<td>NG</td>
</tr>
<tr>
<td>2011-09-16</td>
<td>SO20110272</td>
<td>IV Medications</td>
<td>0.9% NaCl bags</td>
<td>NG</td>
</tr>
<tr>
<td>2011-09-17</td>
<td>SO20110273</td>
<td>IV Medications</td>
<td>0.9% NaCl bags</td>
<td>NG</td>
</tr>
<tr>
<td>2011-09-18</td>
<td>SO20110274</td>
<td>IV Medications</td>
<td>0.9% NaCl bags</td>
<td>NG</td>
</tr>
<tr>
<td>2011-09-19</td>
<td>SO20110265</td>
<td>Water (environmental)</td>
<td>1883 ice dispenser</td>
<td><em>Bordetella bronchiseptica</em>; pigmented yellow unidentified gram negative rod (GNR)</td>
</tr>
<tr>
<td>2011-09-20</td>
<td>SO20110266</td>
<td>Water (environmental)</td>
<td>1883 water dispenser</td>
<td>yellow pigmented unidentified GNR</td>
</tr>
<tr>
<td>2011-09-21</td>
<td>SO20110267</td>
<td>Water (environmental)</td>
<td>1894 ice dispenser</td>
<td>yellow pigmented unidentified GNR; <em>Pseudomonas</em> sp.</td>
</tr>
<tr>
<td>2011-09-22</td>
<td>SO20110268</td>
<td>Water (environmental)</td>
<td>1894 water dispenser</td>
<td>yellow unidentified pigmented GNR</td>
</tr>
<tr>
<td>2011-09-23</td>
<td>SO20110269</td>
<td>Water (environmental)</td>
<td>distilled water for topping up</td>
<td>red and yellow unidentified pigmented GNRs</td>
</tr>
<tr>
<td>2011-09-24</td>
<td>SO201110249</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>yellow pigmented unidentified GNR, non-tuberculosis mycobacteria (NTM); small grey fungus</td>
</tr>
<tr>
<td>2011-09-25</td>
<td>SO201110250</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>yellow pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-26</td>
<td>SO201110251</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>yellow pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-27</td>
<td>SO201110252</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>yellow pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-28</td>
<td>SO201110253</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>pink pigmented unidentified GNR, NTM; small grey fungus</td>
</tr>
<tr>
<td>2011-09-29</td>
<td>SO201110254</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>pink pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-30</td>
<td>SO201110255</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>pink pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-31</td>
<td>SO201110256</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>pink pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-32</td>
<td>SO201110257</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>pink pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-33</td>
<td>SO201110258</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>pink pigmented unidentified GNR, non</td>
</tr>
<tr>
<td>2011-09-34</td>
<td>SO201110259</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>yellow pigmented unidentified GNR, NTM; small grey fungus; <em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>2011-09-35</td>
<td>SO201110260</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>NTM; small grey fungus</td>
</tr>
<tr>
<td>2011-09-36</td>
<td>SO201110261</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>black and grey pigmented yeast and mold</td>
</tr>
<tr>
<td>2011-09-37</td>
<td>SO201110262</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>black and grey pigmented yeast and mold</td>
</tr>
<tr>
<td>2011-09-38</td>
<td>SO201110263</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>black and grey pigmented yeast and mold</td>
</tr>
<tr>
<td>2011-09-39</td>
<td>SO201110264</td>
<td>Water (environmental)</td>
<td>HOTLINE</td>
<td>black and grey pigmented yeast and mold</td>
</tr>
</tbody>
</table>
### Table 2. Summary of *O. anthropi* PFGE and identification results

<table>
<thead>
<tr>
<th>CDC Lab#</th>
<th>MDH ID</th>
<th>Origin</th>
<th>Description</th>
<th>Results (species ID confirmed with Vitek 2)</th>
<th>PFGE results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-09-01</td>
<td>C2011006959</td>
<td>Human</td>
<td>Blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-03</td>
<td>C2011006961</td>
<td>Human</td>
<td>Blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-05</td>
<td>C2011006963</td>
<td>Human</td>
<td>Blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-07</td>
<td>C201106965</td>
<td>Human</td>
<td>Blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-08</td>
<td>C2011006966</td>
<td>Human</td>
<td>Blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-11</td>
<td>C1011006969</td>
<td>Culture</td>
<td>QC organism</td>
<td><em>O. anthropi</em></td>
<td>Not related to cluster A</td>
</tr>
<tr>
<td>2011-09-12</td>
<td>C2011006970</td>
<td>Human</td>
<td>Blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-13</td>
<td>C2011006971</td>
<td>Human</td>
<td>Blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-14</td>
<td>C2011006972</td>
<td>Human</td>
<td>cornea donor</td>
<td><em>Rhizobium radiobacter</em></td>
<td>no PFGE performed</td>
</tr>
<tr>
<td>2011-09-44</td>
<td>C2011007855</td>
<td>Human</td>
<td>blood</td>
<td><em>O. anthropi</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
</tbody>
</table>

### Table 3. Summary of *S. maltophilia* PFGE and identification results

<table>
<thead>
<tr>
<th>CDC Lab#</th>
<th>State ID</th>
<th>Origin</th>
<th>Description</th>
<th>Results (species ID confirmed with Vitek 2)</th>
<th>PFGE results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-09-42</td>
<td>C2011007853</td>
<td>IV Medications</td>
<td>PCA Bag</td>
<td><em>S. maltophilia</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-43</td>
<td>C2011007854</td>
<td>IV Medications</td>
<td>Isolate (IV Medications)</td>
<td><em>S. maltophilia</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-45</td>
<td>C2011007856</td>
<td>Human</td>
<td>blood</td>
<td><em>S. maltophilia</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
</tbody>
</table>

### Table 4a. Summary of *K. oxytoca* PFGE and identification results – Batch 1

<table>
<thead>
<tr>
<th>CDC Lab#</th>
<th>State ID</th>
<th>Origin</th>
<th>Description</th>
<th>Results (species ID confirmed with Vitek 2)</th>
<th>PFGE results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011-09-02</td>
<td>C2011006960</td>
<td>Human</td>
<td>Blood</td>
<td><em>K. oxytoca</em></td>
<td>Not related to outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-04</td>
<td>C2011006962</td>
<td>Human</td>
<td>Blood</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-06</td>
<td>C2011006964</td>
<td>Human</td>
<td>Blood</td>
<td><em>K. oxytoca</em></td>
<td>Not related to outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-09</td>
<td>C2011006967</td>
<td>Human</td>
<td>Blood</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-10</td>
<td>C2011006968</td>
<td>Human</td>
<td>Blood</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-40</td>
<td>C2011007851</td>
<td>Human</td>
<td>blood</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>2011-09-41</td>
<td>C2011007852</td>
<td>Human</td>
<td>blood</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A</td>
</tr>
<tr>
<td>CDC Lab#</td>
<td>MDH ID#</td>
<td>Origin</td>
<td>Description</td>
<td>Organism ID (species ID completed by MNDOH)</td>
<td>PFGE results</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>------------</td>
<td>------------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>2011-09-46 (Repeat of 2011-09-02)</td>
<td>c2011006960</td>
<td>Human</td>
<td>Blood isolate</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster B; not related to cluster A</td>
</tr>
<tr>
<td>2011-09-47 (Repeat of 2011-09-04)</td>
<td>c2011006962</td>
<td>Human</td>
<td>Blood isolate</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-48 (Repeat of 2011-09-06)</td>
<td>c2011006964</td>
<td>Human</td>
<td>Blood isolate</td>
<td><em>K. oxytoca</em></td>
<td>Closely related to cluster B; not related to cluster A</td>
</tr>
<tr>
<td>2011-09-49 (Repeat of 2011-09-09)</td>
<td>c2011006967</td>
<td>Human</td>
<td>Blood isolate</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-50 (Repeat of 2011-09-10)</td>
<td>c2011006968</td>
<td>Human</td>
<td>Blood isolate</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-51 (Repeat of 2011-09-40)</td>
<td>c2011007851</td>
<td>Human</td>
<td>Blood isolate</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-52 (Repeat of 2011-09-41)</td>
<td>c2011007852</td>
<td>Human</td>
<td>Blood isolate</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-53</td>
<td>c20110013116</td>
<td>IV Medications</td>
<td>Isolate from Hydromorphone bag A</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-54</td>
<td>c20110013117</td>
<td>IV Medications</td>
<td>Isolate from Hydromorphone bag B</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-55</td>
<td>c20110034004</td>
<td>IV Medications</td>
<td>Isolate from Hydromorphone – FDA lab sample# - 2BB</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster A; not related to cluster B</td>
</tr>
<tr>
<td>2011-09-56</td>
<td>c20110034005</td>
<td>IV Medications</td>
<td>Isolate from Hydromorphone – FDA lab sample# - 2BC</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster B not related to cluster A</td>
</tr>
<tr>
<td>2011-09-57</td>
<td>c20110034006</td>
<td>IV Medications</td>
<td>Isolate from Hydromorphone – FDA lab sample# - 2CA</td>
<td><em>K. oxytoca</em></td>
<td>Genetically indistinguishable outbreak cluster B; not related to cluster A</td>
</tr>
<tr>
<td>2011-09-58</td>
<td>M2011008655-3</td>
<td>Environmental</td>
<td>Isolate from SPCU Bathroom Drain</td>
<td><em>K. oxytoca</em></td>
<td>Not related to cluster A and B</td>
</tr>
<tr>
<td>2011-09-59</td>
<td>M2011008658-4</td>
<td>Environmental</td>
<td>Isolate from SUR 1A Drain</td>
<td><em>K. oxytoca</em></td>
<td>Not related to cluster A and B</td>
</tr>
</tbody>
</table>
Figure 1. PFGE dendrogram of patient isolates of *O. anthropi.*

<table>
<thead>
<tr>
<th>percent similarity</th>
<th>MDH ID #</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C2011006959</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006961</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006963</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006965</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006966</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006970</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011007855</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C1011006969</td>
<td>Blood</td>
</tr>
</tbody>
</table>

Figure 2. PFGE dendrogram of *S. maltophilia* isolates.

<table>
<thead>
<tr>
<th>percent similarity</th>
<th>MDH ID #</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C2011007853</td>
<td>Hydromorphone bag A</td>
</tr>
<tr>
<td></td>
<td>C2011007854</td>
<td>Hydromorphone bag B</td>
</tr>
<tr>
<td></td>
<td>C2011007856</td>
<td>Blood</td>
</tr>
</tbody>
</table>

Figure 3a. PFGE dendrogram of *K. oxytoca* isolates – Batch 1 of 2

<table>
<thead>
<tr>
<th>percent similarity</th>
<th>MDH ID #</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C2011006960</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006964</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006962</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006967</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011006968</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011007851</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>C2011007852</td>
<td>Blood</td>
</tr>
</tbody>
</table>
Figure 3b. PFGE dendrogram of *K. oxytoca* isolates – Batch 2 of 2

<table>
<thead>
<tr>
<th>MDH ID#</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2011006964</td>
<td>Blood</td>
</tr>
<tr>
<td>C2011006960</td>
<td>Blood</td>
</tr>
<tr>
<td>C20110034005</td>
<td>Hydromorphone-FDA 2BC</td>
</tr>
<tr>
<td>C20110034006</td>
<td>Hydromorphone-FDA 2CA</td>
</tr>
<tr>
<td>C2011006962</td>
<td>Blood</td>
</tr>
<tr>
<td>C2011006967</td>
<td>Blood</td>
</tr>
<tr>
<td>C2011006968</td>
<td>Blood</td>
</tr>
<tr>
<td>C2011007851</td>
<td>Blood</td>
</tr>
<tr>
<td>C2011007852</td>
<td>Blood</td>
</tr>
<tr>
<td>C20110013116</td>
<td>Hydromorphone bag A</td>
</tr>
<tr>
<td>C20110013117</td>
<td>Hydromorphone bag B</td>
</tr>
<tr>
<td>C20110034004</td>
<td>Hydromorphone-FDA 2BB</td>
</tr>
<tr>
<td>M2011008655-3</td>
<td>SPCU Bathroom Drain</td>
</tr>
<tr>
<td>M2011008655-4</td>
<td>SUR1A Drain</td>
</tr>
</tbody>
</table>

Cluster A

Cluster B
MDH Public Health Laboratory Pulsed-field Gel Electrophoresis Testing Methodology

Pulsed-field Gel Electrophoresis (PFGE) testing provides a pattern, or DNA fingerprint, to describe genetic elements of bacteria. The patterns can be quantitatively compared to show genetic relatedness. PFGE is a tool that is most informative when used with epidemiologic data.

Below is a description of the PFGE testing performed on isolates associated with this investigation at the MDH Public Health Laboratory.

PFGE was performed on *K. oxytoca* and *S. maltophilia* using the standardized Salmonella PulseNet protocol [1] with the following exceptions: the initial optical density using the Dade turbidometer was 0.35-0.40 for *K. oxytoca* and 0.40-0.45 for *S. maltophilia*, and for both *K. oxytoca* and *S. maltophilia*, no proteinase K was added prior to cell lysis. *O. anthropi* was subtyped using the standardized PulseNet *Listeria monocytogenes* protocol [2] with the following exceptions: *Spe*I was used to digest the DNA and the PFGE running conditions were 2.2 seconds for the initial switch time, 64.0 seconds for the final switch time, and the run time was 18 hours. The protocol exceptions were necessary for optimization of the organism characteristics.

PFGE pattern analysis was performed at MDH using Bionumerics software utilizing the Dice coefficient. It has been determined that there is a high amount of PFGE pattern diversity between epidemiologically unrelated isolates of *O. anthropi* [3], *K. oxytoca* [4], and *S. maltophilia* [5]. Closely related PFGE patterns from organisms that have a high level of PFGE pattern diversity are more likely to have originated from a common source [6]. The Tenover criteria were used as a pattern interpretation guideline; patterns were classified as indistinguishable, related (1-3 bands different between patterns which could be achieved by a single mutation), possibly related (4-6 bands different between patterns which could be achieved by as few as 2 mutations) or not related (7 or greater bands different between patterns which requires a minimum of 3 mutations) [7].

References