Investigation of a Candida guilliermondii Pseudo-Outbreak Reveals a Novel Source of Laboratory Contamination

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Investigation of a *Candida guilliermondii* pseudo-outbreak reveals a novel source of laboratory contamination

Running title: *Candida guilliermondii* pseudo-outbreak

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ABSTRACT

*Candida guilliermondii* was isolated from sterile specimens with increasing frequency over a several-month period despite a paucity of clinical evidence suggesting true *Candida* infections. However, a healthcare-associated outbreak was strongly considered due to growth patterns in the microbiology laboratory more consistent with true infection than environmental contamination. Therefore, an extensive investigation was performed to identify its cause. With the exception of one case, patient clinical courses were not consistent with true invasive fungal infections. Furthermore, no epidemiologic link between patients could be identified. Rather, extensive environmental sampling revealed *C. guilliermondii* in an anaerobic holding jar, where anaerobic plates were pre-reduced and held prior to specimen inoculation. *C. guilliermondii* grows poorly under anaerobic conditions. Thus, we postulate that anaerobic plates became intermittently contaminated. Passage from intermittently contaminated anaerobic plates to primary quadrants of aerobic media during specimen planting, yielded a colonial growth pattern typical for true specimen infection, thus obscuring laboratory contamination. Molecular evaluation of the *C. guilliermondii* isolates confirmed a common source for pseudo-outbreak cases but not for the one true infection. In line with Reason’s model of organizational accidents, both active and latent errors coincided to contribute to the pseudo-outbreak. These included organism factors (lack of growth in anaerobic conditions obscuring plate contamination); human factors (lack of strict adherence to plating order, leading to only intermittent observation of aerobic plate positivity) and laboratory factors (novel equipment). All of these variables should be considered when evaluating possible laboratory-based pseudo-outbreaks.
INTRODUCTION

Repeated isolation of rare organisms may indicate either a true cluster of healthcare-associated infections (HAIs) or a pseudo-outbreak. Distinguishing between these two scenarios is both difficult and critical for determining appropriate follow up.

Recent true outbreaks of Exserohilum meningitis cases were traced to compounded pharmacy products from one supplier. Delayed identification of the underlying problem resulted in many HAIs (1). Similar true outbreaks have been associated with a wide variety of contaminated products and common exposures.

Pseudo-outbreaks result from specimen contamination during or after collection. Maki (2) and Weinstein (3) suggest that pseudo-outbreaks should be considered when clusters of positive sterile cultures with an unusual organism and without compatible associated clinical symptoms and/or without an obvious endogenous source are identified. Contamination can lead to inappropriate treatment and adverse outcomes related to unnecessary antimicrobial use (2).

Many organisms are ubiquitous in the environment and can directly contaminate microbiological media. Practically, contamination is usually readily identified based on growth pattern. Because standard plating practices leads to successive organism dilution, in true infections, the largest number of colonies appear in the first streaked quadrant, followed by successively less growth in the second, third, and fourth quadrants. In contrast, contaminated specimens typically have no dilution effect, and growth is often seen on plate edges with no relationship to specimen inoculation site or streak lines. If these typical patterns are not found, contamination can be masked, leading to confusion with true infection.

On 9/2/12, the Clinical Microbiology Laboratory notified the Division of Infection Control/ Hospital Epidemiology (IC/HE) of five patients in whom Candida guillermondii was
isolated from sterile specimens during a 24-hour period. Although clinical infections caused by non-albicans *Candida* are increasing, *C. guilliermondii* infections account for only 1% of all *Candida* infections, raising the question of whether these isolates represented a true healthcare-associated outbreak or a pseudo-outbreak (4). Microbiology laboratory review of culture plate growth patterns was strongly suggestive of true infection. Thus, an extensive epidemiologic, microbiologic and molecular investigation into potential sources of this cluster was launched. Here, we describe the investigation and characterize the identification of a novel source of laboratory contamination.

**MATERIALS AND METHODS**

**Setting.** Beth Israel Deaconess Medical Center is a 672 bed academic tertiary care medical center in Boston, Massachusetts. The medical center includes East and West campuses situated approximately 0.25 miles apart. Patients are housed on both campuses and procedures are performed at both sites.

**Epidemiologic cased definition and investigation.** An epidemiologic case was defined as any patient with a microbiologic culture from a sterile site (blood, body fluid, or tissue) that grew *C. guilliermondii* during the six-month period before and 18 months after 9/2/2012. The institution’s microbiology database was queried for the 14-year period prior to the outbreak to identify baseline hospital incidence of *C. guilliermondii*. An epidemic curve and a line list were then used to identify common exposures and guide further investigation during the timeframe of the epidemiologic investigation. Additional investigations included: manual review of all cases to determine the presence of true infection, observation of common procedures by infection control staff, and environmental culturing of key procedural areas and equipment in locations...
where *C. guilliermondii* positive cultures had been collected. In the clinical microbiology laboratory, specimen processing was observed from point of intake through the work up of positive cultures. Standard methods of cleaning and quality assurance steps were reviewed in depth.

**Microbiology procedures.** Standard culture practices for sterile source specimens (referred to hereafter as “planting”) included plating on sheep blood, chocolate, and lactose MacConkey agar for aerobic cultures; CDC anaerobic agar and laked-blood kanamycin agar (LKV) plates for anaerobic cultures; and, for fungal cultures, when ordered, Sabouraud dextrose agar (SAB) and inhibitory mold agar (IMA). Per standard laboratory practice, planting involved lining up media plates in a HEPA-filtered biosafety cabinet (Fig. 3D); transferring a small volume of sample to each using a sterile, disposable transfer pipette; and streaking the primary quadrant of each plate consecutively with a single cotton-tipped swab, moving from least selective to most selective medium. Secondary and tertiary quadrants were then streaked out for each plate individually with a flame-sterilized metal loop.

*C. guilliermondii* was identified using the VITEK 2 Yeast ID card (bioMérieux, Durham, NC). A subset of isolates was sent to the Mayo Clinic Reference Laboratories (Rochester, MN) for confirmation of identification using MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight). Environmental cultures were plated on Sabouraud dextrose agar (SAB) media (Remel, Lenexa, KS) and incubated at 30°C for 14 days.

**Genome mapping.** Pulsed-field gel electrophoresis using standard techniques was performed at the University of Iowa Reference Laboratory (5). Whole Genome Mapping was performed at OpGen (Gaithersburg, MD) as previously described (6). Specifically, yeast genomic DNA was adsorbed to a solid surface, digested with a six-base cutter, stained, and
imaged. Restriction fragments were aligned with the eight large supercontigs of the *C. guilliermondii* ATCC6260 reference genome sequence (7) using MapSolver™ (OpGen, Gaithersburg, MD). Each supercontig corresponded to a single chromosome numbered in decreasing order of size. Notably, Whole Genome Mapping detected a 400KB repetitive region in supercontig 5 not previously resolved in the reference, next generation sequenced-based, genome sequence. Therefore, to preserve the convention of numbering chromosomes according to their actual size, we reference the previously designated supercontig 5 sequence as chromosome 4, and supercontig 4 as chromosome 5, respectively. Dendrograms were generated using Un-weighted Pair Group Method with Arithmetic Mean (UPGMA)(8) such that the length of each horizontal dendrogram line at each branch point corresponds to the fractional difference between whole-genome maps. The fraction difference was then calculated by adding together the length of the restriction fragments that differ between the two maps and dividing the sum by the combined length of the two genomes.

**Ethical Considerations.** All studies were performed as part of normal quality assurance activities in our clinical microbiology and hospital infection control departments. Thus, the study was exempt from Institutional Board Review.

**RESULTS**

**Epidemiological Investigation.** Twenty-five *C. guilliermondii* case patients (36 specimens) were identified; the first identified case occurred on 4/21/12. No additional cases were identified until June. An epidemic curve for the outbreak is shown in Fig. 1A. Fig. 1B shows distribution of *C. guilliermondii* isolated at our institution from 1998-2012; no cases were
detected during the two-year period immediately preceding this outbreak. Prior to 2010, identification of *C. guilliermondii* occurred, on average, one to two times per year. The majority of pre-outbreak isolates were from blood cultures, followed by nail isolates (Fig. 2). Isolates during the outbreak period were primarily from procedural specimens, with the exception of blood culture specimens from a single patient.

Manual review of case patient records suggested one true *C. guilliermondii* infection in September in the middle of the peak of the pseudo-outbreak (the single blood culture isolate); the clinical courses of other affected patients were not consistent with a true *Candida* infection (Table 1). Specifically, the only patient who appeared to have a true infection was severely immunocompromised due to multiple myeloma and Crohn’s disease. During the course of her hospitalization, she developed severe sepsis with five blood culture sets positive for *C. guilliermondii*. The first two sets were drawn through a central venous catheter and also grew *Candida glabrata*. The last three sets were obtained by venipuncture and grew *C. guilliermondii* in aerobic bottles only. The patient subsequently died. Autopsy cultures from liver and lung were positive for both *C. guilliermondii* and *C. neoformans*.

All other case patients were either clinically stable or demonstrated improvement despite limited or no directed antifungal therapy and thus were deemed unlikely to have a true clinical *Candida* infection. Eight patients received antifungal therapy. Four of seven patients with *C. guilliermondii* isolated from peritoneal fluid were not treated; an additional patient was treated after a second positive culture was reported, despite a benign course during the two-month period between positive cultures. Another patient had positive pericardial and pleural fluid cultures after a pericardial window, without significant associated morbidity. One patient with obvious clinical morbidity had an infected hip with 18 joint fluid and tissue cultures taken over
the course of four days. However, although four of 18 were positive for *C. guilliermondii*, almost all were positive for *Proteus mirabilis*, presumably the true cause of the joint infection.

In addition, no associations were found between a sterile site culture positive for *C. guilliermondii* with patient demographics, comorbidities, procedure type or clinical staff members caring for the patient (Table 1). Nine (36%) case patients were male and the mean age was 59 years (range, 24-88). Case patients were admitted to a variety of services including hospital medicine, oncology, cardiology, orthopedics, and general surgery. Twenty-four (96%) had *C. guilliermondii* isolated after unrelated procedures performed in diverse locations throughout the medical center (East campus, 28%, West campus, 56%, outpatients, 16%). Affected procedures included bedside procedures (8), operating room surgical procedures (5), interventional radiology procedures (5), bronchoscopy (4), dermatology biopsy (1), and interventional cardiology procedures (1).

The only common exposure identified was receipt of lidocaine injections during procedures (24 patients, 96%). However, the vials of lidocaine were ruled out as a source based upon several factors. First, the lidocaine came from 11 different types of kits, including temporary central venous catheter insertion, peripherally inserted central venous catheter insertion, paracentesis, thoracentesis, arthrocentesis, and lumbar puncture kits. Second, multiple lots of lidocaine from different suppliers were used. Third, sampling from lidocaine vials used for sterile procedures and from single and multi-dose vials used throughout the institution was undertaken, and all cultures were sterile. Institutional findings were supported by communications with the Massachusetts Department of Public Health and the Centers for Disease Control and Prevention, who reported no other isolation of *C. guilliermondii* from lidocaine vials.
Finally, procedures during which sterile fluid was obtained were observed by IC/HE staff. No breaks in sterile technique were noted. Environmental cultures were obtained during and immediately following procedures, including from the automated pharmacy dispensing system that contained additional lidocaine vials for use and all were negative for *C. guilliermondii*. Thus, the clinical epidemiologic investigation did not support a true outbreak.

**Clinical Microbiology Laboratory Investigation.** Growth patterns on culture plates were examined and initially found to be consistent with true infection rather than environmental contamination (Fig. 3A). Growth was always observed in the first inoculation quadrant, occasionally also in the secondary quadrant, and was never observed outside of streak lines. Positive specimens were transported by either courier or pneumatic tubes from two different campuses and received in different types of transport tubes including sterile cups, syringes, and swabs. They were processed in two biosafety cabinets on different shifts by a large variety of technologists.

Multiple environmental cultures (n=225) were obtained from the clinical microbiology laboratory to evaluate any area potentially in contact with specimens and/or technologists planting specimens. These included transfer pipettes, cotton swabs, plastic loops, flammable loop handles, incubators, biosafety cabinets, cold rooms, floors, countertops, front specimen receipt desk, work cards, air vents, phones, gloves, media, door handles, plate racks, and surfaces where plates were stored. *C. guilliermondii* was isolated from four swabs obtained from the interior side, bottom and rim of the anaerobic media holding jar (Fig. 3B).

The anaerobic holding jar used in our laboratory is part of the Advanced Anoxomat (Advanced Instruments, Inc., Norwood, MA). Plates were held in the anaerobic holding jar prior to use in setting up anaerobic cultures. The Anoxomat system is a microprocessor-controlled
instrument for rapidly establishing anaerobic environments inside airtight, acrylic chambers. It achieves anaerobiosis by rapidly evacuating and injecting a mixture of 5% H2, 10% CO2, 85% N2 gas in three separate cycles. A pouch is present in the chamber lid to hold palladium catalyst to aid in consumption of oxygen present in the chamber. Cultures of baked catalyst in line for future use were all negative. Culture of gas line ports that connect the anaerobic gas cylinder to the Anoxomat and the Anoxomat to the anaerobic holding jar and additional Anoxomat anaerobic chambers used to incubate anaerobic plate cultures after specimen inoculation were all negative.

Based on these findings, procedures for anaerobic plate processing were further investigated. Standard laboratory procedure is to streak out plates in a defined hierarchical order: blood, chocolate, CDC anaerobe sheep blood, Sabouraud dextrose lactose MacConkey, laked blood kanamycin vancomycin (LKV), and inhibitory mold agar (Fig. 3D). Interviews with the microbiology technologists revealed that the recommended hierarchy was not rigorously followed during the cluster period. In addition, the same anaerobic holding jar was used continuously during the outbreak period without a regular, defined cleaning schedule.

Moisture condensate on the inside walls of the holding jar was noted on inspection and was attributed to excessive moisture present in anaerobic agar plates (Fig. 3C). During the Anoxomat evacuation and injection process, water droplets were observed moving on the inside of the lids of these excessively moist anaerobic agar plates. Technologists noted excessive moisture in unopened anaerobic CDC anaerobe sheep blood and LKV agar media (Remel, Lenexa, KS) as early as April 2012, roughly coincident with the first isolation of C. guilliermondii during the pseudo-outbreak. Un-inoculated anaerobic media, that had not been
pre-reduced in the holding jar, was sampled extensively both through aerobic incubation and swabbing the surface of media to plates, and *C. guilliermondii* was never isolated.

**Molecular investigation.** Pulsed-field gel electrophoresis was performed on representative environmental and patient samples. In *Bss*HI, *Not*I, and *Sfi*I restriction digests, isolates from procedural specimens and the anaerobic holding jar had identical banding patterns. The single blood culture isolate from the patient with a clinical course consistent with true infection had a different banding pattern, showing at least one band difference on *Bss*HI-, *Not*I-, and *Sfi*I-digested samples in comparison with the other pseudo-outbreak isolates (Fig. 4).

Whole Genome Mapping was then performed because of increased resolution when compared to traditional pulsed-field gel electrophoresis. Importantly, optical mapping patterns from holding jar isolates and procedural specimens were essentially identical. However, the blood culture isolate appeared to be distinct (Fig. 5). Specific and prominent differences between the blood culture isolate and a representative procedural isolate include an unaligned telomeric region in chromosome 1, a 7 KB deletion in chromosome 2, a 15 KB insertion in chromosome 3, and a 25 KB insertion in chromosome 8 (Fig. 5B).

**Intervention and follow up.** After the source of *C. guilliermondii* source was identified, several steps were taken. First, the holding jar was thoroughly cleaned and a daily disinfection protocol was implemented. Second, the importance of plating order was reinforced with technologists, and a pictorial representation of plating order was placed above each specimen processing area as a reminder (Fig. 3D). Third, to prevent condensation from occurring inside the jar, suppliers of anaerobic media were changed to secure drier plates, and anaerobic plates were placed into an ambient air drying rack for several hours prior to pre-reduction. In 18
months since these interventions, C. guilliermondii was not isolated from any additional patient specimens or environmental samples.

DISCUSSION

Pseudo-outbreaks caused by bacterial, fungal, and viral pathogens are well documented and have been traced to a variety of sources, including contaminated medical or laboratory equipment, tap water rinsing, use of non-sterile solutions in the microbiology laboratory and specimen cross-contamination (9-16). They have also been traced to highly contaminated environments, such as drinking water and ice machines (17, 18), which may introduce microorganisms into patients or clinical specimens during collection.

Candida pseudo-outbreaks are rare. Two pseudo-fungemias with C. guilliermondii were traced to contaminated hands and nails of healthcare workers collecting blood cultures and to contaminated heparin used to flush butterfly needles prior to blood draws (19, 20). Three other pseudo-outbreaks were attributed to different Candida species: Candida versatilitis associated with supplementation of blood culture bottles with olive oil (21), Candida parapsilosis associated with grinding tissue in contaminated salt solution prior to plating (22), and C. parapsilosis associated with manual aeration of an older blood culture system by a colonized technologist (23). There are no prior reports of pseudo-outbreaks definitively traced to anaerobic culture handling, although one report did suggest that a Clostridium sordellii pseudo-outbreak might be associated with storage of pre-reduced plates in an anaerobic chamber (24).

Several simultaneous factors coincided to yield this pseudo-outbreak, and to obscure the distinction between true infection and media contamination. In this sense, we invoke Reason’s model of organizational accidents (25), with both active errors (streaking plates out of order) and
latent errors (handling of anaerobic plates, cleaning techniques), aligned for an adverse event to take place (26).

Specifically, we postulate that the forcible evacuation of the Anoxomat overcame the swan neck barrier of petri dishes, and forced aerosolized organisms to enter the interior of the anaerobic plates. Normally, primary quadrants of different culture plate media are successively streaked with the same swab in a defined order during specimen planting (Fig 3D). However, this order was not always followed during the outbreak period. Organisms were therefore inadvertently transferred during specimen planting from randomly contaminated anaerobic plates sequentially to the primary quadrants of other media. Because plates were only sometimes streaked out of order, contamination of aerobic plates occurred only intermittently. *C. guilliermondii* does not grow anaerobically, obscuring the random localization of organisms on anaerobic media that otherwise would have facilitated recognition of environmental contamination. The subsequent intermittent growth on aerobic medium in primary and also sometimes secondary quadrants simulated the pattern seen during a true outbreak.

Additional contributing factors included initial contamination of the surface of the holding jar coupled with excessively moist anaerobic media used during the outbreak period, leading to condensation in the holding jar. This moisture presumably enhanced growth of *C. guilliermondii* and potential for aerosol dissemination during forcible evacuation and filling of the chamber by the Anoxomat. The potential for contamination was also enhanced by the lack of a scheduled disinfection protocol for the device. Of note, the manufacturer’s operation manual specifies which types of disinfectant should be used, but does not provide guidance for the frequency of its application (27).
The unlikely occurrence during the height of the pseudo-outbreak of a true *C. guilliermondii* case with multiple positive blood and post-mortem cultures, also reinforced initial concern that additional cases might be due to a large outbreak of HAIs and led to a thorough epidemiologic investigation. However, molecular diagnostics demonstrated that this one rare true infection was distinct from the clonal organisms isolated from the anaerobic holding jar and pseudo-outbreak specimens.

This pseudo-outbreak highlights the need for vigilance during the repeated isolation of an unusual organism. The late recognition of this unusual and unsuspected type of laboratory contamination led to unnecessary clinical workup, treatment, and follow up; and a large epidemiological investigation, including input from public health agencies. While several simultaneous events contributed to the pseudo-outbreak, other factors prompted its recognition, In particular, one astute microbiology technologist noted the first cluster of cases and reported to IC/HE suspicion of laboratory contamination. Almost simultaneously, an Infectious Diseases consultant relayed observation of serial cases lacking the clinical characteristics suggestive of deep *Candida* infection.

Our investigation also emphasizes several quality assurance considerations for clinical microbiology laboratories. First, pre-emptive meticulous, regular, targeted, and documented disinfection of equipment and surfaces are prudent to minimize contamination risk related to any technology that alters airflow, such as the Anoxomat—specifically, because such technologies may inadvertently lead to unrecognized contamination through compromise of swan neck barriers that normally keep media sterile. Second, stringent procedures for media acceptability must be followed (28). Third, rigorous adherence to standard operating procedures, even for seemingly less compelling practices like plating order, should be emphasized.
Taken together, these observations indicate how several deficiencies in practice and unrecognized technical vulnerabilities, perhaps minor by themselves, can align in a classic Reason’s Model of organizational accidents, also known as the “Swiss cheese” model to lead to adverse events.

Acknowledgements:

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References


27. Operation Manual Anoxomat Mark II CTS, p. 60. In B.V. MM (ed.).

# Table 1. Brief Clinical Course of Patients with Positive Cultures with *C. guilliermondii* during the Pseudo-Outbreak Period.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Clinical History</th>
<th>Specimen</th>
<th>Procedure</th>
<th>Pathology</th>
<th>Procedures in 90 Day Window prior to Positive Culture</th>
<th>Antifungal Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4/21/2012</td>
<td>Ascites</td>
<td>Peritoneal fluid</td>
<td>Paracentesis</td>
<td>N/A</td>
<td>Paracentesis (Outside facility, 4/16/2016)</td>
<td>Micafungin</td>
</tr>
<tr>
<td>2</td>
<td>6/8/2012</td>
<td>Ascites</td>
<td>Peritoneal fluid</td>
<td>Paracentesis</td>
<td>N/A</td>
<td>Paracentesis (5/9/2012)</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>6/8/2012, 8/22/2012</td>
<td>Ascites</td>
<td>Peritoneal fluid</td>
<td>Paracentesis (Performed by interventional radiology)</td>
<td>N/A</td>
<td>None</td>
<td>None after first positive culture; voriconazole after second</td>
</tr>
<tr>
<td>4</td>
<td>6/22/2012</td>
<td>Abscess</td>
<td>Abscess fluid</td>
<td>Aspiration of abdominal abscess (Utrasound-guided)</td>
<td>N/A</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>7/10/2012</td>
<td>Knee pain, total joint replacement</td>
<td>Joint fluid</td>
<td>Right revision total knee arthroplasty</td>
<td>Special stains negative for fungi</td>
<td>Floroscopy-guided right knee aspiration (4/24/2012)</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>7/10/2012</td>
<td>Shoulder pain</td>
<td>Bone</td>
<td>Bone biopsy (CT-guided)</td>
<td>N/A</td>
<td>Right shoulder cortisone injection at outside facility, date unknown</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>8/15/2012</td>
<td>Pulmonary nodules</td>
<td>Lung tissue</td>
<td>Transbronchial biopsy (Bronchoscopy)</td>
<td>Special stains negative for fungi</td>
<td>Biopsy of hypopharyngeal mass and femur fracture repair (7/30/2012)</td>
<td>Micafungin</td>
</tr>
<tr>
<td>8</td>
<td>8/27/2012</td>
<td>Pericardial effusion</td>
<td>Pericardial tissue</td>
<td>Pericardial window</td>
<td>GMS stain negative</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>8/27/2012</td>
<td>Ascites</td>
<td>Peritoneal fluid</td>
<td>Paracentesis</td>
<td>N/A</td>
<td>Paracentesis (Outside facility, 8/20/2012)</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>8/28/2012</td>
<td>Pericardial effusion</td>
<td>Pericardial fluid</td>
<td>Pericardial window</td>
<td>N/A</td>
<td>Pericardiocentesis (8/21/2012)</td>
<td>Voriconazole</td>
</tr>
<tr>
<td>11</td>
<td>8/28/2012</td>
<td>Wrist pain</td>
<td>Synovial tissue</td>
<td>Left wrist synovectomy</td>
<td>GMS stain negative</td>
<td>None</td>
<td>Fluconazole</td>
</tr>
<tr>
<td>12</td>
<td>8/29/2012</td>
<td>Hip pain and total joint replacement</td>
<td>Bone</td>
<td>Resection arthroplasty</td>
<td>Not performed</td>
<td>Drainage of right hip hematoma 8/22/2012 and right hip total arthroplasty 8/1/2012</td>
<td>Fluconazole</td>
</tr>
<tr>
<td>13</td>
<td>9/17/2012</td>
<td>Pleural adhesions</td>
<td>Pleural tissue</td>
<td>Video-assisted thorascopy with exploration and washout</td>
<td>Special stains for fungi negative</td>
<td>Biopsy of mediastinal mass (6/21/2012) and radical thymectomy 8/6/2012</td>
<td>None</td>
</tr>
<tr>
<td>14</td>
<td>9/18/2012</td>
<td>Sepsis</td>
<td>Blood</td>
<td>None</td>
<td>N/A</td>
<td>Arterial line insertion 8/25/2012, Peripherally-inserted central catheter 7/16/2012</td>
<td>Micafungin</td>
</tr>
<tr>
<td>15</td>
<td>9/23/2012</td>
<td>Ascites</td>
<td>Peritoneal fluid</td>
<td>Paracentesis (Utrasound-guided)</td>
<td>N/A</td>
<td>Multiple paracenteses</td>
<td>None</td>
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<tr>
<td>16</td>
<td>9/23/2012</td>
<td>Sepsis, rash</td>
<td>Skin joint fluid aspirate</td>
<td>Punch biopsy</td>
<td>Left hip revision arthroplasty</td>
<td>N/A</td>
<td>None</td>
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<tr>
<td>17</td>
<td>9/24/2012</td>
<td>Hip pain and total joint replacement</td>
<td>Joint fluid aspirate</td>
<td>Left hip revision arthroplasty</td>
<td>N/A</td>
<td>Left hip aspiration (Interventional radiology, 8/24/2012)</td>
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<tr>
<td>18</td>
<td>9/26/2012</td>
<td>Pleural effusion</td>
<td>Pleural fluid</td>
<td>Thoracentesis</td>
<td>N/A</td>
<td>Tunneled peripherally-inserted central catheter insertion (7/16/2012)</td>
<td>Voriconazole</td>
</tr>
<tr>
<td>19</td>
<td>9/28/2012</td>
<td>Ascites</td>
<td>Peritoneal fluid</td>
<td>Aspiration of abdominal fluid</td>
<td>N/A</td>
<td>Pancreatic debridement (8/16/2012) Exploratory</td>
<td>Micafungin</td>
</tr>
<tr>
<td>Date</td>
<td>Description</td>
<td>Location</td>
<td>Procedure</td>
<td>Additional Details</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>9/28/2012</td>
<td>Mediastinal adenopathy</td>
<td>Bronchial tissue</td>
<td>Bronchoscopy</td>
<td>Special stains for fungi negative</td>
<td></td>
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<td>Bone biopsy (CT-guided, 7/31/2012)</td>
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<td>Bronchial tissue</td>
<td>Bronchoscopy</td>
<td>Special stains for fungi negative</td>
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<td>Punch biopsy</td>
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<td>Bronchial tissue</td>
<td>Bronchoscopy</td>
<td>Special stains for fungi negative</td>
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<td>10/14/2012</td>
<td>Ascites</td>
<td>Peritoneal fluid</td>
<td>Paracentesis</td>
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<td>Multiple prior paracenteses (ultrasound-guided), Embolization procedure</td>
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<td>Pleural effusion</td>
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<td>Pleurex catheter placement (Ultrasound-guided, 10/5/2012), Video-assisted thoracic surgery (8/10/2012)</td>
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446
Figure Legends.

Figure 1. *C. guilliermondii* isolation over time at our institution.
(A) Epidemic curve of *C. guilliermondii* cases by week during pseudo-outbreak period.
(B) Historical isolation of *C. guilliermondii* by year.

Figure 2. Distribution of sample types from which *C. guilliermondii* was isolated before and during outbreak.
(A) Years 1998-2011.
(B) Year 2012. Multiple isolates from the same specimen source from any given patient are only counted once with the exception of two peritoneal fluids from the same patient isolated one month apart in Panel B, which were considered independent events and counted separately. In addition, one patient in panel B had isolates from both pleural and pericardial fluid, and another patient had isolates from blood, post-mortem liver and post-mortem spleen all counted separately.

Figure 3. Laboratory investigation of *C. guilliermondii* outbreak.
(A) Examples of *C. guilliermondii* growing in primary streak areas from two patient samples.
(B) Anaerobic holding jar.
(C) Example of excess moisture in unopened anaerobic media packages.
(D) Proper plating order, beginning on the left with the most enriched medium and progressing to the most selective on the right (CHOC, chocolate agar; BAP, sheep blood agar; CDC, CDC anaerobic agar; SAB, Sabouraud dextrose agar; MAC, lactose MacConkey agar; LKV, laked-blood kanamycin vancomycin agar; IMA, inhibitory mold agar).
Figure 4. Pulsed-field gel electrophoresis of pseudo-outbreak strains.

(A) *BssH*I digest.

(B) *Not*I digest. Patient pseudo-outbreak isolates designated as “*” with the one blood culture outbreak isolate examined labeled as “B”; anaerobic holding jar isolate, “H1”; molecular weight marker, “mw”; and environmental isolates from the University of Iowa Reference Laboratory unrelated to outbreak isolates, “C1, C2”. Arrows highlight a single band difference between blood and patient/holding jar isolates. *Sfi*I restriction is not shown.

Figure 5. Whole Genome Mapping.

(A) Dendogram shows that pseudo-outbreak and holding jar isolates are closely related and fall within the same (*, H1, H3) or very closely related map types (H2). The single exception was the blood culture isolate examined (‘B’), which appeared more closely related to an environmental isolate (C1) from the University of Iowa than to pseudo-outbreak and holding jar isolates. The X axis denotes fractional difference between whole-genome maps as described in the supplementary materials.

(B) *Afl*III restriction maps of *C. guilliermondii* chromosomes from a representative pseudo-outbreak strain designated as “*” in panel (A). Because of the high density of restriction sites and condensed nature of the chromosome maps, lines denoting restriction sites have merged into dark and light banding patterns corresponding to areas with greater or lesser restriction site density. Arrow and arrow heads point to the location of and adjoining text describes differences found in the whole-genome map of the blood culture isolate (‘B’) compared to the whole-genome map pattern shared by pseudo-outbreak and anaerobic holding jar strains shown.