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Case report

Holy water not so holy: Potential source of *Elizabethkingia* pneumonia and bacteremia in an immunocompromised host

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ABSTRACT

Elizabethkingia species are Gram-negative, glucose-non-fermenting bacilli predominantly found in soil and water, with *Elizabethkingia anophelis* increasingly recognized as a human pathogen. *E. anophelis* has also been reported in hospital outbreaks, suggesting the potential role of contaminated institutional water sources. Conventional microbiological methods often lead to misidentifying this pathogen for other members of the genus *Elizabethkingia*, suggesting a role for molecular methods for identification.

We report a 67-year-old female who developed multiorgan failure requiring intensive care unit admission and mechanical ventilation while being treated with chemotherapy for Burkitt lymphoma. She developed pneumonia with Gram-negative bacilli isolated from her endotracheal aspirate culture, later identified as *E. anophelis*. She later developed bacteremia due to the same pathogen, which was confirmed by MALDI-TOF and whole genome sequencing. Waterborne transmission via holy water administration was postulated to be potential source of infection.

Our case report highlights that *E. anophelis* may cause significant infection and should not be disregarded as contaminant, especially in immunosuppressed individuals. As a waterborne pathogen that may be brought into hospital environments, emphasis on educating family members, close nursing monitoring, and reporting of suspected, unsupervised manipulation of medical equipment should be undertaken to prevent contamination by this organism from outside sources.

Introduction

The genus *Elizabethkingia* comprises six species of Gram-negative, glucose-non-fermenting bacilli that are predominantly distributed in soil and water [1]. *Elizabethkingia anophelis* was first described as a new species in 2011 when isolated from the midgut of *Anopheles gambiae* [2]. Since then, several reports of human infections such as pneumonia, meningitis and catheter-related bacteremia have been published highlighting its potential as a pathogen with high mortality, especially in immunocompromised hosts [2–6]. Although its route of transmission and exact mechanism of infection remain unclear, the occurrence of hospital outbreaks has proffered the potential role of contaminated institutional water sources [5–10]. Proper identification of *E. anophelis* is not feasible with phenotypic characteristics, thus the need for molecular

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methods or matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) with the updated reference database [1,5]. Without an extended database, MALDI-TOF may misidentify *E. anophelis* as *E. meningoseptica* or other species as noted in previous reports [11]. Herein we present a case of *E. anophelis* ventilator-associated pneumonia (VAP) with bacteremia in an immunocompromised patient due to a suspected contaminated water source.

Case presentation

A 67-year-old woman with a past history of hip osteoarthritis, dyslipidemia, and recent cholecystectomy presented to the hospital with abdominal pain. Admission blood work showed leukocytosis with white blood cell count of 13.0×10^9/L, elevated creatinine of 180 μmol/L, normal lipase, and cholestatic pattern of abnormal liver enzymes with transiently elevated alanine transaminase and aspartate transaminase both $>$ 2–3x upper limit of normal, and alkaline phosphatase and gamma-glutamyl transferase both persistently $>$ 1000 U/L. Computerized tomography (CT) of the abdomen revealed peripancreatic, perinephric, and periureteric stranding without any stones or well-defined fluid collections. She was initially suspected of having pancreatitis with concomitant pyelonephritis and empiric ceftriaxone (2 g intravenous (IV) daily) was started.

Three days post-admission, her creatinine continued to rise precipitously with concurrent hyperkalemia and anuria necessitating transfer to the intensive care unit (ICU) and initiation of dialysis. Notably, she had persistent lymphocytosis in the preceding weeks and peripheral blood smear showed the presence of blasts concerning for underlying hematologic malignancy. Her acute renal injury was thought to be secondary to tumor lysis syndrome. Bone marrow biopsy histopathology confirmed Burkitt lymphoma. Magnetic resonance imaging (MRI) of abdomen evaluating the query pancreatitis and persistent cholestatic hepatitis, confirmed a bulky pancreas head with adjacent stranding suspicious of disease involvement. MRI brain showed diffuse bilateral nodular pachymeningeal thickening and enhancement with scattered areas of acute hemorrhage and a small left frontal subdural hematoma atypical of trauma, suspicious for leptomeningeal involvement with lymphoma. Cerebrospinal fluid sent for cytology was negative for malignancy.

She was subsequently started on modified rituximab, cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate (R-CODOX-M). She also received intra-thecal methotrexate, hydrocortisone, and cytarabine for possible leptomeningeal involvement of lymphoma. Granulocyte colony-stimulating factor (G-CSF) was initiated one week after chemotherapy to maintain her absolute neutrophil count above 1.0×10^9/L.

Her kidney function improved after 9 days of continuous renal replacement therapy. Due to a febrile neutropenic episode, meropenem (1 g IV every 8 h) was initiated and she required mechanical ventilation for increasing oxygen requirements. CT chest showed no pulmonary embolism, but bilateral ground-glass opacities and multifocal consolidations suggesting pneumonia. Bacterial cultures (blood, urine), serum cryptococcal antigen, and serum galactomannan were negative. Endotracheal aspirate (ETA) for bacterial culture was also negative. Lower respiratory samples by bronchoalveolar lavage could not be obtained due to clinical and respiratory instability. In addition to a 2-week course of meropenem IV, empiric mold coverage against *Aspergillus* spp. for possible invasive fungal infection (i.e., pneumonia) was also initiated. She was maintained on caspofungin (70 mg IV loading dose, then 50 mg IV daily) as alternative antifungal therapy, due to worsening liver enzymes with triazoles (alanine transaminase and aspartate transaminase both $>$ 5x upper limit of normal), and persistent and refractory hypokalemia (2.5–3.0 mmol/L) with liposomal amphotericin B IV despite electrolyte replacement therapy. She improved thereafter, was extubated after two weeks and transferred to the medical ward.

However, over subsequent days her level of consciousness worsened, with the need for increasing oxygen supplementation. She was transferred back to ICU and reintubated. Her caspofungin IV was continued and she was restarted on meropenem (1 g IV every 8 h).

Over the coming month, she made steady progress weaning from mechanical ventilation but required a tracheostomy. She remained in the ICU due to persistent low-level vasopressor support requirements. Six weeks after her ICU admission, she then developed mucopurulent secretions from tracheostomy and pressure support ventilation requirements increased, with positive end-expiratory pressure (PEEP) being increased from 8 to 12 cmH_2O while fraction of inspired oxygen was increased from 30 % to 35 %. She also developed an episode of fever of 38.0 °C while on meropenem IV and caspofungin IV. Repeat blood and urine cultures as part of septic workup were both negative.

Gram stain from ETA collected for bacterial culture showed 1 + pus cells and few Gram-negative bacilli. The species was found to be non-lactose-fermenting and oxidase-positive with typical coliform appearance of the colonies on sheep blood agar (SBA). Species was identified as *E. anophelis* by Bruker Biotyper® MALDI-TOF mass spectrometry. Antibiotic susceptibility testing (AST) performed with Kirby-Bauer disk diffusion method showed that the *E. anophelis* was susceptible to piperacillin-tazobactam, trimethoprim-sulfamethoxazole, intermediate to ciprofloxacin and resistant to meropenem, amikacin, ceftazidime, gentamicin, and tobramycin.

Based on the AST results, her meropenem IV was changed to piperacillin-tazobactam 4.5 g IV every 8 h. Chest radiography showed persistent bilateral opacities with slight worsening in the left lung. The source of her *E. anophelis* was initially unclear but on further assessment for environmental sources for this uncommon pathogen, it was noted that her family had been administering holy water through her nasogastric tube.

The patient completed a 1-week course of piperacillin-tazobactam IV for VAP due to *E. anophelis*. However, one day after completing her antibiotic course, she was noted to have a temperature of 37.9 °C and increased norepinephrine requirement.

Two sets of blood cultures were obtained, one from peripheral venipuncture another from peripherally inserted central catheter (PICC). The blood culture drawn from the PICC demonstrated Gram-negative bacilli with no capsule on the Gram stain (Fig. 1). Medium-sized tan-white colonies with mucoid appearance grew with aerobic incubation on SBA plate (Fig. 2). Bruker Biotyper® MALDI-TOF again identified the organism as *E. anophelis,* with further confirmation of species by 16 S ribosomal DNA (rDNA) (endpoint PCR test followed by Sangar sequencing).

Kirby-Bauer disk diffusion susceptibility testing showed the same susceptibility pattern as the previous isolate from ETA specimen.

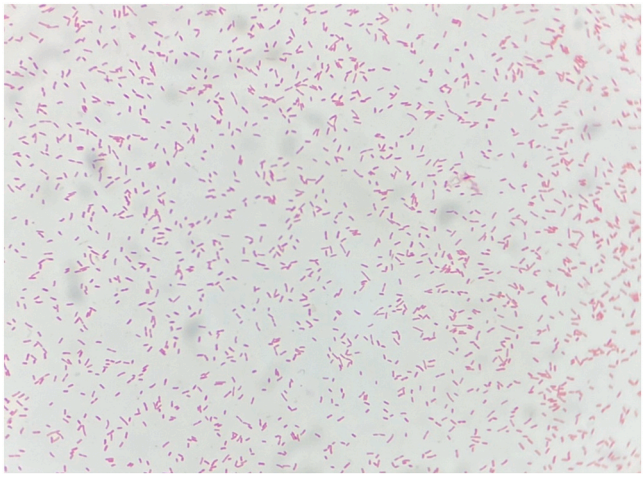

Fig. 1. Gram stain of blood culture. Gram-negative bacilli with no capsule visualized under light microscopy, with 100x magnification.

Fig. 2. Colony isolated from blood culture incubation on SBA plate. Medium-sized tan-white colonies with mucoid appearance visualized after 48 h aerobic incubation at 35°C on SBA.

Additional AST with E-strips were performed for minocycline to which the isolate showed susceptibility. ETA culture collected on the same day as the blood cultures grew *Stenotrophomonas maltophilia*.

Piperacillin-tazobactam 4.5 g IV every 8 h was restarted along with trimethoprim-sulfamethoxazole IV (15 mg/kg/day of trimethoprim component) for *S. maltophilia* coverage. The patient remained dependent on central venous access without the ability for line-free interval, and so antibiotics were continued with attempt of PICC line salvage. Repeat blood cultures from PICC and peripheral sites conducted 48 h later showed no growth.

She completed four weeks of piperacillin-tazobactam IV with a brief subsequent improvement in her hemodynamics. Unfortunately, she developed recurrent *S. maltophilia* pneumonia and bacteremia as well as metabolic derangements, liver failure and subsequent lower gastrointestinal bleeding. Due to ongoing bleeding, supportive palliative measures were instituted and the patient subsequently succumbed.

Discussion

Our case describes the occurrence of *E. anophelis* VAP in an immunosuppressed patient receiving chemotherapy for Burkitt lymphoma. Although the patient fulfilled the criteria for the diagnosis of VAP [12], the isolation of *E. anophelis* from tracheal aspirate was surprising. This organism was believed to be the cause of her pneumonia, with the same pathogen subsequently isolated from a blood culture sample further substantiating infection rather than environmental contamination or colonization. In light of this, the only possible source would have been the holy water being administered by the patient's family. However, this hypothesis could not be confirmed because, at the time the source investigation was initiated, the holy water sample was already discarded by the family and not available for analysis. At our institution, no other clinical isolates of *E. anophelis* were reported (including from the ICU and other medical and surgical units) within a year of our case, supporting the view that this was an isolated incident with holy water administration as the suspected source.

E. anophelis is usually considered a benign organism. It is ubiquitous in the environment, and rarely causes disease in humans [1]. In previous reports, most patients had risk factors, such as comorbidities and healthcare exposure before the onset of infection. Besides immunosuppression by chemotherapy and cancer, this patient had prolonged hospital and ICU stays, central venous catheters, and difficult-to-wean mechanical ventilation. This case highlights the potential of immunosuppression as a contributing factor for invasive disease after colonization, a hypothesis yet to be further investigated by clinical studies [4,5,13–15]. *E. anophelis* should not be disregarded as a contaminant, especially when isolated from sterile samples as studies have shown that invasive disease may be difficult to treat owing to emerging resistance and subsequently lead to morbidity and fatal outcomes [4,16].

E. anophelis is a relatively novel pathogen, first reported in 2011, but retrospective studies suggest that culture-based identification techniques could have misidentified this species as other members of the genus *Elizabethkingia* [9,11]. In our case, the use of Bruker Biotyper® MALDI-TOF was essential for proper identification and a 16 S rDNA assay provided further confirmation. We consider that proper identification with MALDI-TOF, coupled with 16 S rDNA sequencing is important for future studies aiming at the investigation of novel sources and transmission dynamics.

To date, there are no clearly established treatment strategies for managing *Elizabethkingia* infections, which may differ between species including *E. meningoseptica* and *E. anophelis* [17–19]. Targeted antibiotics (as monotherapy or in combination) based on susceptibilities are suggested based on reported cases of invasive infections due to *Elizabethkingia* [17–19]. Due to its potential biofilm forming capabilities, a prolonged course of targeted antibiotic treatment is suggested for infections due to *E. anophelis* [16]. Considering our patient's immunocompromised state with underlying disease, febrile neutropenia, and the potential high mortality and ability to form biofilm due to this organism [16,20], we opted for longer duration of antibiotics. However, more studies on treatment strategies evaluating optimal duration and role of combination therapy are required to better rationalize management in immunocompromised populations.

An important feature of *E. anophelis* is the potential to cause outbreaks. Transmission dynamics are not fully understood, but a few have been proposed: mosquito-borne, vertical transmission, institutional water sources (tap water used for cleansing patients or devices, such as nasogastric tubes), and cross-transmission through contaminated hands [1,6,8,21]. An outbreak of *E. anophelis* from 2015 to 2016 in Wisconsin, USA, was thoroughly investigated and evolutionary molecular biology suggested a common reservoir, but the source remained unidentified [5]. Prior to our patient's presentation, five cases of *E. anophelis* had been investigated in our locale, the Greater Toronto Area, by Public Health Ontario with whole genome sequencing, but no common source was identified [9]. At the time of our case, no other cases due to *E. anophelis* were reported at our institution. We hypothesized that the holy water being administered by the family might have been a potential source. According to the family, the product was imported and sourced from a sacred well. Whole genome sequencing could help further investigate this hypothesis if samples from the water were available. Our patient's infection was considered an isolated incident. However, in cases concerning nosocomial outbreaks, standard infection control measures including hygiene practices and outbreak investigations including contact investigations, environmental sampling including from suspected sources (e.g., sink aerators, medical equipment) may be necessary [22].

In conclusion, our case report highlights that *E. anophelis* may indeed cause significant infection and should not be considered a contaminant especially in immunosuppressed individuals. The organism originates from water sources and may be introduced into the hospital environment from an outside source. Emphasis on educating family members, close nursing monitoring, and reporting of suspected, unsupervised manipulation of medical equipment should be considered to prevent contamination by this organism from outside sources.

Abbreviation

AST: antibiotic susceptibility testing; CT: computerized tomography; ETA: endotracheal aspirate; G-CSF: granulocyte colony-stimulating

factor; ICU: intensive care unit; IV: intravenous; MALDI-TOF: matrix-assisted laser-desorption/ionization time-of-flight; MRI: magnetic resonance imaging; PEEP: positive end-expiratory pressure; PICC: peripherally inserted central catheter; rDNA: ribosomal DNA; SBA: sheep blood agar; VAP: ventilator-associated pneumonia.

CRediT authorship contribution statement

Rahel T. Zewude: Writing – review & editing, Writing – original draft, Visualization. **Matheus O. Bastos:** Writing – review & editing, Writing – original draft, Data curation. **May AlFalahi:** Writing – review & editing, Writing – original draft. **Coleman M.F. Rotstein:** Writing – review & editing, Supervision, Conceptualization. **Carson K.L. Lo:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Patient consent

Written informed consent was obtained from the patient for the publication of this case report and the accompanying images.

Ethical approval

Not applicable.

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Author statement

The authors declare that this manuscript is original, has not been published before and is currently not being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all authors. We understand that the Corresponding Author is the sole contact for the Editorial process. He/She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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