Characterization of a Novel Pathogen in Immunocompromised Patients: *Elizabethkingia anophelis*

Mohamad Yasmin[1,2], Steven H. Marshall[2], Emma Marshall[2], Anna Cmolik,[2], Helen W. Boucher[3], Alejandro Vila[4], Robert A. Bonomo*[1,2,5,6-8]

[1]DEPARTMENT OF MEDICINE, CASE WESTERN RESERVE UNIVERSITY (CWRU); [2] LOUIS STOKES CLEVELAND DEPARTMENT OF VETERANS AFFAIRS MEDICAL CENTER; [3]TUFTS CENTER FOR INTEGRATED MANAGEMENT OF ANTIMICROBIAL RESISTANCE, TUFTS MEDICAL CENTER; [4] INSTITUTO DE BIOLOGÍA MOLECULAR Y CELULAR DE ROSARIO (IBR); [5]DEPARTMENT OF MOLECULAR BIOLOGY AND MICROBIOLOGY, CWRU; [6]DEPARTMENTS OF PHARMACOLOGY, [7]BIOCHEMISTRY, AND [8]PROTEOMICS AND BIOINFORMATICS, CWRU; #CURRENT ADDRESS: THE JACKSON LABORATORY FOR GENOMIC MEDICINE

REVISED ABSTRACT

A 60-year-old woman with acute myelogenous leukemia , AML, developed fever and cough post induction chemotherapy. Meropenem and vancomycin were administered as empiric therapy. A bloodstream infection due to resistant *E. meningoseptica* was identified. Ceftazidime-avibactam (CZA) and aztreonam (ATM) were administered, but lab testing revealed resistance to the combination. Cefiderocol MIC was 2 mg/L; treatment was initiated. Minocycline and trimethoprim/sulfamethoxazole (TMP/SMX) were also added as the patient deteriorated and died ten days later.

Methods

MIC testing /disc diffusion assays were done according to CLSI guidelines. Whole genome sequencing (WGS) was performed, assembled and annotated using PATRIC.org and compared to Institute Pasteur core genome multilocus sequence typing, cgMLST, and Bacterial Isolate Genome Sequence Database (BIGSdb). Resistant genes and plasmids were identified by the Center for Genomic epidemiology.

Results

CgMLST and average nucleotide identity of 99.46% revealed the organism as *E. anophelis,* not *E. meningoseptica*. The WGS assembly resulted in 173 contigs with a chromosome of 4,090,739 bp. Plasmids were not identified. Resistance genes $bla_{B\text{-}11}$, $bla_{GOB\text{-}13}$ (both metallo-β-lactamases) and $bla_{CME\text{-}1}$ (a class D b-lactamase). *E. anophelis* was resistant to multiple drug classes including aminoglycosides, vancomycin, cephalosporins, and carbapenems. Further mechanism-based susceptibility testing (MBST) using double-disc diffusion assays demonstrated susceptibility to linezolid, cefepime/zidebactam, piperacillin/avibactam, with modestly wider zones of inhibition with the addition of ATM.

Conclusions

Using WGS, we correctly identified a highly drug resistant E. anopheles in an immunocompromised patient. Rapid analysis of the genetic background is required to inform better selection antimicrobial therapy.

INTRODUCTION

- *Elizabethkingia* is a genus of Gram-negative bacilli ubiquitous in nature and the healthcare setting. The role of this pathogen in infection in immunocompetent and immunocompromised hosts is not well defined.
- *E. meningoseptica* is the most identified species within this genus, however, *E. anophelis* and other species are misidentified due to phenotypic similarity and/or imprecise MALDI-TOF databases.
- According to recent CDC surveillance data, *E. anophelis* outbreaks have occurred in Illinois, Michigan and Wisconsin in 2016. The genesis of these outbreaks is unknown.
- *Elizabethkingia* infection is particularly challenging to treat due to its intrinsic production of Metallo-β-Lactamases (MBLs) which confer resistance to carbapenems as well as more contemporary β-Lactam/β-Lactam inhibitor (BL/BLI) combinations such as ceftazidime/avibactam (CZA).
- Our goal was to understand the antimicrobial susceptibility pattern of infection by *E. anophelis* in an immunocompromised host with AML with the intent to offer more targeted therapy.

METHODS

- Antimicrobial susceptibility testing (AST): MIC testing / broth microdilution, E-testing, and disc diffusion assays were done according to CLSI guidelines.
- Whole genome sequencing (WGS) was performed, assembled and annotated using PATRIC.org and compared to Institute Pasteur cgMLST instance of the BIGSdb database tool.
- Genes conferring antimicrobial resistance were identified by the Center for Genomic epidemiology.
- Phylogenetic tree was generated using the PATRIC.org codon phylogenetic tree building service based on 1000 genes.

RESULTS

Table 1. Antimicrobial susceptibility testing using various BLIs and combinations with Aztreonam

Single Disk-Diffusion assays (in mm)

	MIN	TIG	MOX	CIP	RIF	LZD	VAN	TMP-SMX
E. anophelis	32	20	27	20	27	30	6	33

Beta lactam/BLI combinations (in mm)

	CAZ-AVI	PIP-AVI	IMI-AVI	MEM-VAB	MEM-NAC	FEP-ZID	IMI-REL	PIP-NAC
E. anophelis	11	22	6	6	6	22	6	24

Combinations with Aztreonam (in mm)

	CAZ-AVI+ATM	PIP-AVI + ATM	MEM-VAB + ATM	FEP-ZIDE+ATM	IMI-REL + ATM
E. anophelis	15	24	8	24	6

Abbreviations: PIP: piperacillin; CAZ: ceftazidime; IMI: Imipenem; TMP/SMX: trimethoprim/sulfamethoxazole; AVI: avibactam; MIN: minocycline
MEM: meropenem; VAB: vaborbactam; REL: relebactam; NAC: nacubactam; FEP: cefepime; CIP: ciprofloxacin; MOX: moxifloxacin; RIF: rifampin
VAN: vancomycin; LZD: linezolid; TIG: tigecycline; ATM: aztreonam

Table 1. Antimicrobial susceptibility tests were performed and interpreted according to CLSI criteria. Additional combination susceptibility testing was conducted using newer β-lactamase inhibitors based on mechanisms of resistance. Abbreviations are presented in the table above.

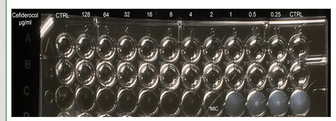

Figure 1. Broth microdilution MIC method used to measure the in vitro activity of Cefiderocol against *Elizabethkingia anophelis*

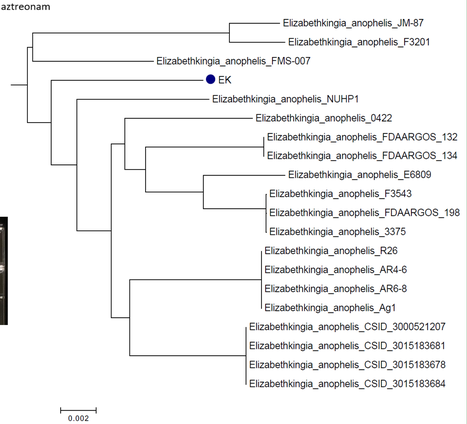

Figure 2. *E. anophelis* phylogenetic tree generated by PATRIC.org SNP analysis comparing the genome of our isolate (EA) with other *E. anophelis* isolates of different origins.

Sample	Length (bp)	Number of Contigs	Mean Contig Length (bp)	Longest Contig (bp)	Shortest Contig(bp)	N50 (bp)
EA	4090739	173	23645.9	90896	610	47449

Table 2. Assembly statistics pertaining to isolate EA

AMR Gene	%Identity	AMR gene length (bp)	%Coverage	Class
$bla_{B\text{-}11}$	99.2	750	100.0	β-lactamase
$bla_{GOB\text{-}13}$	98.635	879	100.0	β-lactamase
$bla_{CME\text{-}1}$	99.004	888	90.2	β-lactamase

Table 3. Isolate EA antimicrobial resistance genes. WGS and PCR detected genes encoding two Metallo-β-Lactamases (MBL): B and GOB. The genus *Elizabethkingia* is known to harbor chromosome-mediated intrinsic MBL genes responsible for widespread β-Lactam hydrolysis. No plasmids were detected.

- **Figure 3.** Ceftazidime-avibactam (CZA) and aztreonam (AT) *E*-test strips were utilized to screen for potential synergy against MBL producing *E. anophelis. The E*-test strips were applied to the inoculated culture plates in a side-by-side formation. Alternatively, *E*-test strips may be placed in a cross formation. Above image illustrates that the addition of AT to CZA did not decrease the MIC of CZA alone.

CONCLUSIONS

- Herein, we report our clinical experience treating a woman who suffered from AML and succumbed to a highly drug resistant infection. *E. anophelis* was first described in 2011 as a new species. Host factors likely contributed to the higher mortality associated with this infection, as most cases are reported from stem cell recipients and lymphoma patients (1)
- Prompt identification and thorough testing is needed to distinguish between species in the *Elizabethkingia* genus as delays in effective antimicrobial therapy can lead to higher mortality in immunocompromised patients. WGS was necessary and confirmed the presence of genes encoding MBLs: $bla_{B\text{-}11}$ and $bla_{GOB\text{-}13}$. No known plasmids were detected. Based on phylogenetic tree/SNP analysis, our isolate (EA) appears to be most closely related to NCBI GenBank submitted strain NUHP1.
- Phenotypically, the isolate was resistant to all tested b-lactams including carbapenems, piperacillin/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam, meropenem/nacubactam, and imipenem/relebactam. The only BL/BLI combinations that resulted in wider zones of inhibition were cefepime/zidebactam, piperacillin/avibactam (neither commercially available), and piperacillin/nacubactam (not commercially available).
- The isolate was susceptible to cefiderocol which the patient received (MIC 2 µg/ml). Cefiderocol is a poor substrate for hydrolysis by MBLs owing to the presence of a cyclic quaternary ammonium moiety on it's C-3 side chain (2). We plan to further investigate cefiderocol against other isolates within this genus to understand whether the stability of this antibiotic to hydrolysis or affinity for Iron transport contributes to this observation.
- **Why did the combination of TAZ AVI and ATM fail**? The answer to this question, we must consider the unanticipated complex genetic background of this isolate.
- We did not predict the presence of **two MBLs** (GOB-13 and B-11) nor did we expect the concomitant carriage of CME-1, a **class D** enzyme. B-11 likely contributed the higher resistance to carbapenems than GOB-13. Mechanistically, we employed AVI to inhibit class A&C β-lactamases and ATM to bypass MBLs. We conclude that AVI was ineffective in inhibiting CME-1, and the overproduction of MBL was sufficient to diminish the impact of ATM.
- In contrast, zidebactam may be more effective at inhibiting class D β-lactamases and may have an additional effect on PBPs [β-lactam enhancer, (3)]. We also hypothesize that cefepime may be a poor substrate for these MBLs and CME-1. Surprisingly, piperacillin was highly effective when combined with a variety of DBOs. Piperacillin may selectively target multiple PBPs and have a unique potency as it does against other non-fermenters.
- Therapy should be based on susceptibility testing. In cases where access to newer agents (e.g. cefiderocol) is not possible, potential regimens include rifampin in combination with trimethoprim-sulfamethoxazole, a fluoroquinolone, linezolid, or minocycline as determined by susceptibility testing.
- Although this is an uncommon infection in an immunocompromised host, much more needs to be determined to fully understand the best therapeutic approaches to these complex infections.

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