Emerging Outbreak of *Elizabethkingia Anopheles*: A Systematic Review

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Abstract

The recent outbreak of Elizabethkingia anopheles in Midwest countries has caused a number of deaths. Notably Elizabethkingia anopheles causes neonatal meningitis, bacteraemia, sepsis, blood stream infections and respiratory infections. This infection may pose serious threats to public health because of lack of sufficient research and its endemic potential unknown.

This systematic review was meant to develop a deeper insight into the current status of *E. anophelis* related evidence and to highlight areas that need further research. Reviewing existing literature will help other researchers in identifying and addressing the knowledge gaps.

Various free access databases such as Google Scholar, Scopus, PubMed, and Science Direct were employed for literature survey. All articles that have been published since 2011, when the outbreak was reported for the first time have been included in this systematic review. The research related to this subject is in earlier stages and little information is currently available. Future studies must focus on the molecular basis, control, prevention, and therapeutics of *E. anophelis* infection to mitigate its increasing risk. This review is meant to provide baseline data

for future research. Scientific community must carry out research on infections caused by *E. anophelis* mosquito else it will result in a disastrous outbreak.

Keywords: *Elizabethkingia anophelis,* meningitis, phylogenetics, strain diversity,

1. Introduction

Genus *Elizabethkingia* is a part of family *Flavobacteriaceae* and phylum *Bacteroidetes*. It is a non-motile, ubiquitous, and aerobic bacterium mostly found in the gut of *Anopheles* mosquito and colonizes the human respiratory tract. Four different species that belong to genus *Elizabethkingia* are *E. miricola*, *E. meningoseptica* , *E. endophytica,* and *E. anophelis*.

E. meningoseptica is a nosocomial pathogen that affects patients on hemodialysis [1, 2], and are responsible for bacteremia [3], septicaemia [4], endophthalmitis [5], and meningitis [2, 6-9]. *E. miricola* has the potential to cause ventilator-associated pneumonia, sepsis, and bacteremia [10, 11]. *E. endophytica* was isolated from *Zea mays* [12]. Phylogenetic analysis revealed that E. *anophelis* is different from closely related species *E. miricola* and related group *E. meningoseptica* **(**Fig 1**)**.

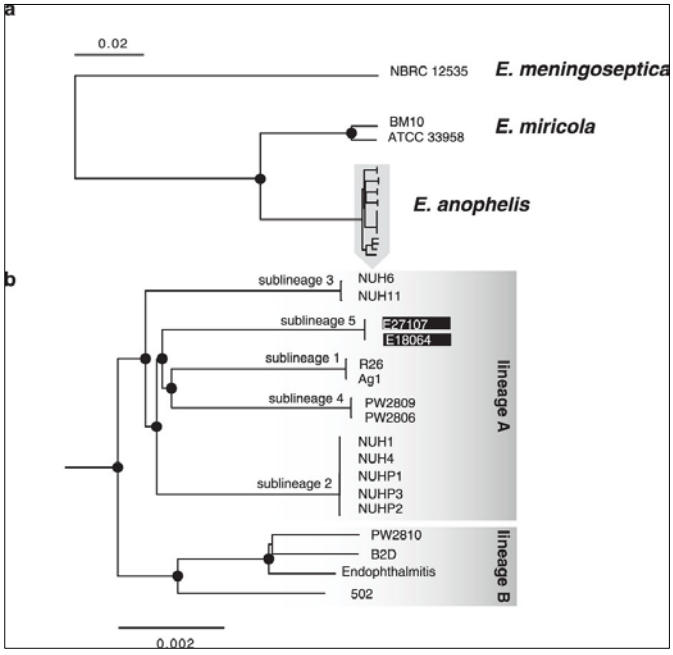

Fig 1: Phylogenetic tree of Elizabethkingia species [13].

E. anophelis is a gram-negative bacteria isolated from the midgut of anopheline mosquitos.

Studies involving a three years long outbreak spanning a time period from 2015 to **2018 in** Taiwan resulted in the identification of a specific *E. Anophelis* strain. Transmission mechanism patterns in 26 patients were studied using Pulsed-field gel electrophoresis (PFGE) and complete-genome sequencing [14].

A study was carried out in Saudi Arabia including 27 patients who had been **hospitalized from** June 2013 to May 2019 suspected of having Chryseobacterium/Elizabethkingia spp infections. Blood culture studies showed that *Elizabethkingia spp,* indeed, was the most prevalent amongst the pathogens isolated [15]

In another study undertaken in Singapore involving 79 blood culture isolates from 2009 to 217 were probed. PCR assisted results showed 78/79 of these isolates were of *E. Anophelis* showing an overwhelming dominance of the strain under review [16].

Recently, *E. anophelis* infection was responsible for a public health crisis in Michigan, Illinois, and Wisconsin with 65 confirmed cases and 20 deaths as of June 2016 [17]. The high mortality rate associated with this infection has caused trouble in the past few years (Fig 2).

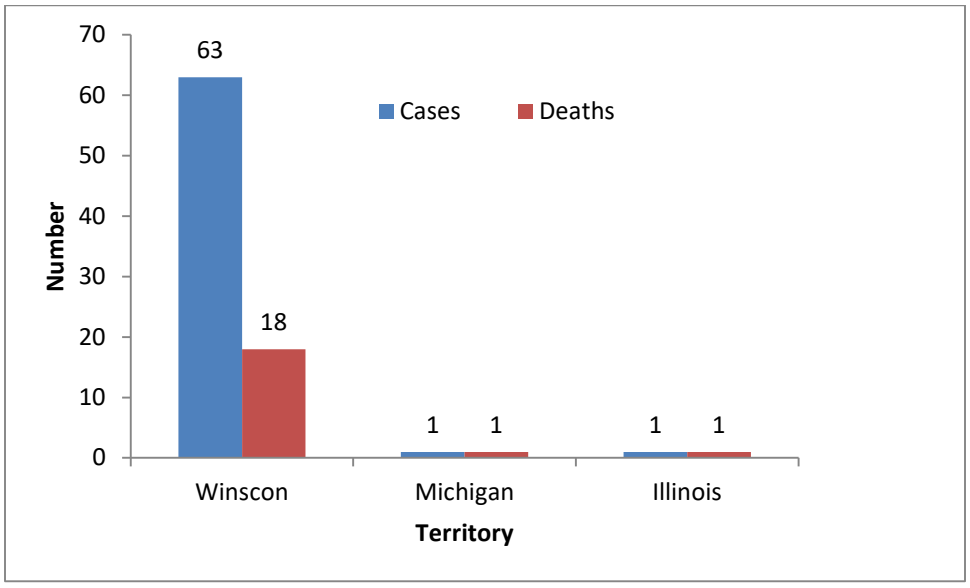

Fig 2: Confirmed cases and deaths of during recent deadly outbreak of *E. anopheles* in MidWest countries.

Strains of Elizabethkingia are usually found in fresh and marine environments. Mostly, immunocompromised individuals acquire *E. anophelis* infection during hospital stay [18, 19]. Most of people had bloodstream infections but respiratory infections were also reported in some cases during 2015-16 *E. anophelis* outbreak. *Elizabethkingia* is resistant to many antibiotics [16]. According to some clinicians *E. anophelis* bacteria are susceptible to antibiotics such as fluoroquinolones and rifampin therefore, treating patients with a combination of antibiotics may improve the outcome [20]. Common symptoms include shortness of breath, cough, chills, fever, cellulitis, headache, and joint pain. An evidence of vertical transmission has been reported recently but the proper transmission path and different modes of transmission are still vague [21-24].

This systematic review was meant to develop a deeper insight into the current status of *E. anophelis* related research and to highlight areas that need further research. Reviewing existing literature will help other research scientists in identifying and addressing the knowledge gaps.

2. Methodology

2.1. Literature survey and data screening

Various free access databases such as Google Scholar, Scopus, PubMed, and Science Direct were employed for the literature survey. Little research has been done on *E. anophelis*. We used different keywords such as: *E. anophelis* infections, *E. anophelis* transmission, *E. anophelis* symptoms, *E. anophelis* strain diversity, *E. anophelis* future prospects, *E. anophelis* treatment etc. Our comprehensive research yielded 17 records.

2.2. Quality assessment

Eligible publications included all research articles or original studies related to *E. anophelis* since it was initially reported in 2011 to 2016. Authors analyzed available literature independently and removed the duplicates.

2.3. Data Synthesis

A total of 13 articles were included and Microsoft Excel spreadsheet was employed to record information such as authors, methods, key findings, and conclusion.

3. Results

A global primary literature was compiled after a literature search on *Elizabethkingia anophelis* that was published since it was first reported. All the records were peer reviewed articles available in English language. Table 1 depicts the key findings, methods, study areas, and study design of all the documents that met the inclusion criteria.

Table 1: Currently available studies related to *Elizabethkingia anophelis*

Ref.	Methodology	Key Findings	Conclusion
1	Pathogenesis associated features and phylogenetic relationships of two African neonatal meningitis *E. anophelis* isolates and compared with *Elizabethkingia* isolated from other sources and regions [13].	Distinct sublineages observed in African *E. anophelis* genetically related. Specific resistance genes acquired as a result of horizontal transfer were also observed in African isolates.	The emerging pathogen *Elizabethkingia* is dynamically evolving over time.
2	Complete circularized genome sequences of 4 strains collected during E. *anophelis* outbreak were studied [25].	Mapping of outbreak strains showed similarity with the genome of strain CSID_3015183678. Ordered arrangement was observed at three segments A, B, and C belonging to the position 3929927.	Complete gene sequences have been deposited at GenBank under BioProject no. PRJNA315668.
3	The molecular and clinical epidemiology of Elizabethkingia-like species isolated from bacteraemia patients admitted in 5 regional hospitals of Hong Kong was analyzed [26].	16S rRNA based gene sequencing revealed that out of total 45 episodes of bacteremia associated with Elizabethkingia-like species, *E. anophelis*, *E. meningoseptica*, *E. miricola,* and other diverse genera/species were responsible for 17, 1, 3, and 24 episodes, respectively.	*E. anophelis* is the predominant cause of Elizabethkingia bacteremia and the morbidity and mortality associated with this life-threatening condition.
4	All four species of*Elizabethkingia* were sequenced [27].	Results revealed that *Elizabethkingia endophytica* and *Elizabethkingia anophelis* belongs to genomospecies 1, whereas, *Elizabethkingia miricola* is similar to to genomospecies 2.	The complete genome sequences have been deposited at GenBank under BioProject no. PRJNA301708.

5	Researchers sequenced *Elizabethkingia anophelis* from asian malaria vector *Anopheles stephensi* [28].	This whole-genome shotgun project has been deposited at GenBank under the accession no. LFKT00000000.	This genome sequence provides baseline data to analyze host-microbe interactions in mosquitoes.
6	Researchers examined the number of *E. anophelis* in gut and its physiological requirements using selectable markers, reporter systems (green fluorescent protein [GFP] and NanoLuc), and transposons that function in *E. anophelis* for genetic manipulation and P*ompA* based flavobacterial expression system integrated into the *E. anophelis* to enhance promoter activity and that leads to increased production of NanoLuc and GFP [29].	A 71%, 82% and 3% infection rate was determined with *A. gambiae, A. stephensi*, and *Aedes triseriatus* respectively, when fed with NanoLuc-tagged cells at larval stage. Arginine was found to be an important amino acid for *E. anophelis* whose growth was promoted by animal erythrocytes *in vivo* and *in vitro* suggesting that erythrocyte lysis in the mosquito midgut provides nutrients.	The study revealed the molecular manipulation and interaction of *E. anophelis* with mosquito hosts and shows that *E. anophelis* adapts to various mosquito midgut environments differently.
7	Sequencing of *E. anophelis* NUHP1 was done and its response to oxidative stress was also assessed [30].	Results indicated the presence of 4,369,828 base pairs long circular genome containing 4,141 predicted coding sequences. Sequence analysis also revealed that *E. anophelis* possess an organized system stress response and iron scavenging. We further showed that hemoglobin facilitates the growth, hydrogen peroxide tolerance, cell attachment, and biofilm formation of *E. anophelis* NUHP1.	Heme uptake and production of siderphore act as key players of stress response and virulence of *E. anophelis*.
8	JM-87(T) bacterial strain was isolated from corn plant and studied for taxonomic classification [12].	The bacteria appeared rod-shaped and gram-negative. Based on 16S rRNA gene sequences, isolate exhibited 99.1, 97.8, and 97.4% similarity to *Elizabethkingia anophelis*, *Elizabethkingia meningoseptica* and *Elizabethkingia miricola*, respectively.	JM-87(T) proved to be novel species named as *Elizabethkingia endophytica*.

9	$R26^T$ and Ag1, two different strains of E. *anophelis*, isolated from different strains of *A. gambiae* were sequenced [31].	Both strains of bacteria were identical. Different TonB dependent transporters with different substrate specificities were observed in E. *anophelis* genome. E. *anophelis* genome also contain several different genes with broad antibiotic resistance, genes that encode efflux pumps and β-lactamases, and genes important for mosquito carbohydrate metabolism. E. *anophelis* encodes various hemolysins that increase hemolytic activity leading to erythrocytes digestion in the mosquito gut. Antioxidant genes and OxyR regulon provide defense against the oxidative stress that is associated with blood digestion.	The study elucidated functional characteristics symbiotic relationship of bacterium with the mosquito host.
10	Researchers used rapid genome sequencing to examine 3 isolates of *E. anophelis* obtained from 1 mother and 2 neonates who had chorioamnionitis and meningitis, respectively [24].	Genomics revealed that bacteria transmitted from mother to her neonate. Genome of 2 strains HKU37 and HKU38 were identical to each other but different from third strain HKU36, thus excluding a clonal outbreak.	The study suggested the vertical transmission of *E. anophelis* associated infections.
11	Whole-genome sequencing of seven isolates of E. *anophelis* collected from different hospitals and compared with five *Elizabethkingia* spp. Genomes available over NCBI. Researchers applied pan-genomic approach for identification of core- and pan-genome for the *Elizabethkingia* genus [32].	The genome of *E. anophelis* strains were identical to *E. anophelis* Ag1 and R26 strains that were isolated from malaria mosquito vector *Anopheles gambiae.*	The results of the study highlighted the nosocomial transmission of *E. anophelis* infection.
12	Researchers isolated R26(T) from midgut of the mosquito Anopheles gambiae and studied its growth properties, antibiotic	Isolates appeared as rod-shaped gram-negative cells. Optimum growth of bacteria was observed at 30-31 °C and 37	The study proposed that strain R26 (T) represented novel species that was named

	resistance characteristics, and taxonomic allocation [23].	°C. Bacteria showed resistance against streptomycin, chloramphenicol, kanamycin, tetracycline, and ampicillin. R26 (T) was 98.2% similar to *Elizabethkingia miricola* GTC 862(T) and 98.6% similar *Elizabethkingia meningoseptica* ATCC 13253(T) based on 16S rRNA gene sequence analysis.	*Elizabethkingia anophelis*.
13	Authors presented case-study of 8-year old girl brought to Complexe Pédiatrique in Bangui, Central African Republic, in March, 2011 [33].	Strain was identified as *E. meningoseptica* using API 20NE system strip. Phylogenetic analysis based on 16SrRNA gene exhibited that isolate belongs to *E. anopheles*.	*E. anopheles* associated meningitis was first time reported in Arfica in 2011.
14	We present the draft genome sequences of two strains of *E. anophelis*, $R26^T$ and Ag1, which were isolated from the midgut of the malaria mosquito *Anopheles gambiae* [34].	CLC Genomics Workbench v.4.9 based de novo assembly generated 51 contigs, totaling 4.05 Mbp and DNASTAR NGen v 10.0 based *de novo* assembly of $R26^T$ genomic reads (652 Mbp) yielded 66 contigs, totaling 4.03 Mbp with an average GC content of 35.4%. NCBI Prokaryotic Genome Automatic Annotation Pipeline revealed 3,648 protein coding sequences CDS and 38 RNA genes in Ag1 and 3,687 protein coding sequences (CDS).	The draft genome sequences of strains Ag1 and $R26^T$ are available in DDBJ/EMBL/GenBank under the GenBank accession numbers AHHG00000000 and ANIW00000000, respectively.

4. Discussion

In this review, 14 records were identified that included 11 original research articles and 2 case reports published between 2011 and 2016. Studies highlighted the vector potential of mosquitoes for transmission of *E. anophelis* to humans.

There is strong evidence that *E. anophelis* transmits from mother to fetus and this infection is currently circulating in Michigan, Illionois and Wisconsin. The prevalence of *E. anopheles* is

much higher than *E. meningoseptica* and *E. miricola* [25]. Previous studies show that *E. anophelis* associated bacteremia carries high morbidity and mortality. [26]Accumulating evidence suggests that *E. anophelis* is misidentified as *E. meningoseptica* but MALDI-TOF MS is the most appropriate choice for accurate and rapid diagnosis of *E. anophelis* infections. The complete genomic sequences of four different strains collected during a recent outbreak of 2015-16 have been deposited to GenBank under the BioProject no. PRJNA315668 [26, 28]. Previously, complete genomic sequences of two strains $R26^T$ and Ag1 isolated from midgut of the malaria mosquito *Anopheles gambiae* are available under the GenBank accession numbers ANIW00000000 and AHHG00000000, respectively. Likewise, the genomic sequence of *E. anopheles* strain EaAs1 isolated from the Asian malaria mosquito *Anopheles stephensi* has been deposited at GenBank under the accession no. LFKT00000000 [27].

One of the study demonstrates the molecular basis of *Elizabethkingia* infections and host mosquito interactions and introduced the development of techniques for integration of foreign DNA into the chromosome and expression of gene of interest in commensal *Elizabethkingia* [29, 35]. This study provided future avenues for the development of novel biocontrol agent diseases caused by the mosquitoes. The reporter strain specifically GFP-based or NanoLuc-based allowed the understanding of bacterial infection, *in vivo* cell localization, and gene regulation [35].

Immunocompromised patients are known to be mostly infected by genus Elizabethkingia and a number of new species of this genus have been reported in the last decade. Elizabethkingia anopheles is the most prevalent species of this genus. This genus of pathogen is sensitive to minocycline, however, it is resistant to β-lactam inhibitors, aminoglycosides, β-lactams, and carbapenems.

5. Conclusion

In conclusion, *E. anopheles* related research is in initial stages. This review identified knowledge gaps with respect to therapeutics, pathogenesis, transmission, phylogenetics, and molecular biology of infection.

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