Genomic features, phylogenetic relationships, and comparative genomics of *Elizabethkingia anophelis* strain EM361-97 isolated in Taiwan

Jiun-Nong Lin\(^1,2,3\), Chung-Hsu Lai\(^2\), Chih-Hui Yang\(^4\), Yi-Han Huang\(^1\) & Hsi-Hsun Lin\(^2\)

*Elizabethkingia anophelis* has become an emerging infection in humans. Recent research has shown that previous reports of *E. meningoseptica* infections might in fact be caused by *E. anophelis*. We aimed to investigate the genomic features, phylogenetic relationships, and comparative genomics of this emerging pathogen. *Elizabethkingia anophelis* strain EM361-97 was isolated from the blood of a cancer patient in Taiwan. The total length of the draft genome was 4,084,052 bp. The whole-genome analysis identified the presence of a number of antibiotic resistance genes, which corresponded with the antibiotic susceptibility phenotype of this strain. Based on the average nucleotide identity, the phylogenetic analysis revealed that *E. anophelis* EM361-97 was a sister group to *E. anophelis* FMS-007, which was isolated from a patient with T-cell non-Hodgkin’s lymphoma in China. Knowledge of the genomic characteristics and comparative genomics of *E. anophelis* will provide researchers and clinicians with important information to understand this emerging microorganism.

*Elizabethkingia* is a genus of aerobic, nonfermenting, nonmotile, catalase-positive, oxidase-positive, indole-positive, and gram-negative bacilli that are usually distributed in soil and water environments\(^1\)–\(^3\). Genus *Elizabethkingia* has been reported to cause human infection since Elizabeth O. King’s original work in 1959\(^4\). However, this genus had rarely been responsible for infections in humans before. These microorganisms have been recently reported to cause life-threatening infections in immunocompromised patients, such as pneumonia, bacteremia, meningitis, and neutropenic fever\(^1\)–\(^7\).

Among genus *Elizabethkingia*, *E. meningoseptica*, previously known as *Chryseobacterium meningosepticum*, is the most well-known species that causes opportunistic infection in humans\(^2,3\). In contrast, little is known about *E. anophelis*. *Elizabethkingia anophelis* was first isolated from the midgut of a mosquito, *Anopheles gambiae*, in 2011\(^8\) and has caused several outbreaks of infections in Africa\(^7,9\), Singapore\(^10\), Hong Kong\(^11\), and the USA\(^12\). The Centers for Disease Control and Prevention of the USA reported two outbreaks of infections caused by *E. anophelis* in the Midwest. A total of 63 patients in Wisconsin were confirmed to have *E. anophelis* infection between November 1, 2015 and April 12, 2017, and this outbreak caused 19 deaths\(^13\). Another cluster of 10 patients with *E. anophelis* infection was reported in Illinois, and six of the patients died of this infection\(^5\). Pulsed-field gel electrophoresis and whole-genome sequencing revealed that the strains of *E. anophelis* in these two outbreaks were genetically different\(^6\). However, recent research has shown that *E. anophelis* was frequently misidentified as *E. meningoseptica*, and previous reports of *E. meningoseptica* infections might in fact be caused by *E. anophelis*\(^5,12\).

We previously published the draft whole-genome sequence of *E. anophelis* strain EM361-97 isolated in Taiwan (GenBank accession number, LWDS00000000.1)\(^14\). The whole-genome sequence could provide insights into the characteristics of the putative virulence factors, pathogenesis, and drug resistance of microorganisms.

\(^1\)Department of Critical Care Medicine, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan. \(^2\)Division of Infectious Diseases, Department of Internal Medicine, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan. \(^3\)School of Medicine, College of Medicine, I-Shou University, Kaohsiung, Taiwan. \(^4\)Department of Biological Science and Technology, Meiho University, Pingtung, Taiwan. Correspondence and requests for materials should be addressed to J.-N.L. (email: jinoli@kmu.edu.tw)
Comparison of genomes among different strains can be used in the analyses of phylogenetic relationships and epidemiological features. However, there has been little research investigating the genomic characteristics, global epidemiology, and genomic diversity of *E. anophelis*. In this study, we analysed the genomic features of *E. anophelis* strain EM361-97. We also compared the genomics and investigated the phylogenetic relationships with other strains of *E. anophelis* from other world regions.

Materials and Methods

Ethics and experimental biosafety statements. This study was approved by the Institutional Review Board of E-Da Hospital (EMRP-105-134). The need for patient’s informed consent was waived by the Institutional Review Board of E-Da Hospital as the retrospective analysis of anonymously clinical data posed no more than minimal risk of harm to subjects and involved no procedures for which written consent was normally required outside of the research context. The experiments in this study were approved by the Institutional Biosafety Committee of E-Da Hospital. All experiments were performed in accordance with relevant guidelines and regulations.

Isolate of *E. anophelis*. *Elizabethkingia anophelis* strain EM361-97 was isolated from the blood of a 46-year-old male patient with advanced nasopharyngeal carcinoma and lung cancer. During admission, the patient suffered from pneumonia, respiratory failure, and profound shock. He initially received empirical antibiotics with levofloxacin. Unfortunately, the patient died several days after this infection. One blood culture from the patient yielded a gram-negative bacillus that was initially identified as *E. meningoseptica* using API/ID32 GN (bioMérieux S.A., Marcy l’Etoile, France) by the clinical microbiology laboratory. This isolate was named strain EM361-97 and was stored at −80 °C as a glycerol stock for further experiments. We re-identified this isolate as *E. anophelis* using 16S ribosomal RNA (rRNA) gene sequencing as previously published. The minimum inhibitory concentration (MIC) of this isolate was examined using the broth microdilution method. The susceptibilities were determined according to the interpretative standards for “other non-Enterobacteriaceae” as suggested by the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Whole-genome sequencing and genome annotation of *E. anophelis* EM361-97. The deoxyribonucleic acid (DNA) of this isolate was prepared using a Wizard Genomic DNA Purification Kit according to the manufacturer’s instructions (Promega, WI, USA). The genome was sequenced using an Illumina HiSeq. 2000 Sequencing Platform (Illumina, CA, USA). The short reads were assembled and optimized according to paired-end and overlap relationship via mapping reads to contig using SOAP de novo v. 2.04. The assembled genome was then submitted to the NCBI Prokaryotic Genome Annotation Pipeline and the Rapid Annotations using the Whole-Genome Sequencing Platform (RAST) Prokaryotic Genome Annotation Server (http://rast.nmpdr.org/) for gene function annotations.

The graphical map of the circular genome was generated using the CGView Server (http://stothard.afns.ualberta.ca/cgview_server/) for the genome of *E. anophelis*. The total length of the draft genome was 4,084,052 bp, with a mean GC content of 35.7%. This genome contained 3,774 genes that made up 87.9% of genome. The genomic features of *E. anophelis* strain EM361-97 were determined according to the interpretive standards for “other non-Enterobacteriaceae” as suggested by the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Comparative genomic analysis. For comparison, the genome sequences of 34 available, nonduplicated, different genome sequences of *E. anophelis* in GenBank were downloaded from the National Center for Biotechnology Information (NCBI) genome sequence repository (https://www.ncbi.nlm.nih.gov/genome/). The genome-wide comparison and annotation of clusters of orthologous groups (COGs) were generated using the web server OrthoVenn. The average nucleotide identity (ANI) values between two genome sequences were calculated using the original ANI function of OrthoANI. The heat maps were generated using Cimminer.

The in silico DNA-DNA hybridization (DDH)-analogous values between different strains were calculated using the Genome-to-Genome Distance Calculator (GGDC) 2 (http://ggdc.dsmz.de/distcalc2.php). A 70% similarity of in silico DDH value represents the cut-off value for species boundaries. The phylogenetic tree was constructed using Cimminer.

Data Availability. The names of organisms, strains, biosample numbers, bioproject numbers, assembly numbers, isolated origins, and release dates of bacteria used in this study are shown in Supplementary Table S1.

Results and Discussion

General genome description of *E. anophelis* EM361-97. The statistics of assembly and annotation are shown in Table 1. The total length of the draft genome was 4,084,052 bp, with a mean GC content of 35.7%. This genome contained 3,774 genes that made up 87.9% of genome. The genomic features of *E. anophelis* EM361-97 are shown in Fig. 1. The number of tandem repeat sequence was 108. The assembly contained 18 scaffolds, 27 contigs, 3,743 coding sequences (CDSs), 53 minisatellite DNAs, 26 microsatellite DNAs, 51 transfer RNAs (tRNAs), and 15 rRNAs (Fig. 1A).

The genome of *E. anophelis* strain EM361-97 analysed by the RAST Server revealed 356 subsystems that could be classified into 27 categories (Fig. 1B). Among these, the “amino acid and derivatives” subsystem accounted for the largest number of 319 CDSs, followed by carbohydrate metabolism (268 CDSs), protein metabolism (220 CDSs), and RNA metabolism (121 CDSs). Regarding the 88 CDSs in the “virulence, disease, and defense” subsystem, 12 were related to invasion and intracellular resistance, and 76 were associated with resistance to antibiotics and toxic compounds. The
high number of antibiotic resistance-associated CDSs suggests that *E. anophelis* EM361-97 might be resistant to multiple antibiotics.

**Orthologous genes.** Orthologous genes are clusters of genes in different species that have evolved by vertical descent from a single ancestral gene. A genome-wide comparison of orthologous clusters in different isolates provides insight into the gene structure, gene function, and molecular evolution of genomes. The COGs analysis of strain EM361-97 was compared with the other four genomes isolated from the USA (strains CSID_301518368 and 3375), Africa (strain V0378064 [E18064]), and Singapore (strain NUHP1) (Fig. 2). The analysis shows that *E. anophelis* EM361-97 contained 3,611 proteins, 3,324 COGs, and 234 singletons. Among the 3,324 COGs in strain EM361-97, 2,988 COGs were shared by all five strains, and 11 COGs were only present in the strain EM361-97 genome. The unique COGs existing in EM361-97 involved genes functioning with transferase activity, cofactor binding, oxidoreductase activity, nucleotide binding, fatty acid elongation, and 3-oxoacyl-[acyl-carrier-protein] reductase (NADPH) activity. The representative meanings of these singular genes in *E. anophelis* EM361-97 are not clear. Further investigations to understand the features of these unique genes in *E. anophelis* EM361-97 are warranted.
Genomic comparison among *Elizabethkingia* species. The genomic comparison among *E. anophelis* EM361-97, *E. anophelis* R26, *E. meningoseptica* KC1913, *E. miricola* GTC 862, *E. bruuniana* G0146, *E. ursingii* G4122, and *E. occulta* G4070 implemented using the RAST/SEED Server is shown in Fig. 3A. The genome of *E. anophelis* EM361-97 was apparently closer to that of *E. anophelis* R26 than the other *Elizabethkingia* species. The evolutionary relatedness among these strains was measured by *in silico* DDH-analogous values (Fig. 3B). The DDH value between *E. anophelis* EM361-97 and *E. anophelis* R26 was 82%. In contrast, the DDH value between *E. anophelis* EM361-97 and *E. meningoseptica* KC1913 was only 24.2%.

Genus *Elizabethkingia* previously comprised four species, namely *E. meningoseptica*, *E. miricola*, *E. anophelis*, and *E. endophytica*. However, the strain of *E. endophytica* was re-identified as an additional strain of *E. anophelis* based on *in silico* DDH of whole-genome sequencing (77% DDH value with regard to *E. anophelis* strain R26)\(^2\). Recently, Nicholson *et al.* proposed three novel *Elizabethkingia* species, *Elizabethkingia bruuniana* sp. nov., *Elizabethkingia ursingii* sp. nov., and *Elizabethkingia occulta* sp. nov. Our study showed that strain EM361-97
belonged to \textit{E. anophelis}, with a DDH value of 82% between \textit{E. anophelis} EM361-97 and the type strain of \textit{E. anophelis} R26\(^5\). In addition, \textit{Elizabethkingia meningoseptica} KC1913\(^5\) demonstrated a relatively large phylogenetic distance from other strains of \textit{Elizabethkingia}. These findings are consistent with the previous report of the taxonomic classification in genus \textit{Elizabethkingia}\(^5\).

**Whole-genome phylogenetic analysis of \textit{E. anophelis}**. The phylogeny of the 34 available strains of \textit{E. anophelis} based on ANI is shown in Fig. 4. The phylogenetic analysis revealed that \textit{E. anophelis} EM361-97 was a sister group to \textit{E. anophelis} EMS-007, which was isolated from a patient with T-cell non-Hodgkin’s lymphoma in China. The sister group of \textit{E. anophelis} strains EM361-97 and FMS-007 was a clade sister of strains Po0527107 (E27017) and V0378064 (E18064) isolated from two neonates with meningitis in the Central African Republic\(^6\). The seven strains isolated from Singapore were divided into two clusters (NUH1, NUH2, NUH3, NUH4, and NUH6, NUH11). The 13 strains isolated from the USA clustered in four groups, and the four strains that caused the outbreak of \textit{E. anophelis} infection in Wisconsin (strains CSID_3015183678, CSID_3015183681, CSID_3015183684, CSID_3000521207) were in the same clade.

**Virulence factors**. \textit{Elizabethkingia anophelis} infections in humans have shown a mortality rate of 24% to 60%\(^5,6,11\), and this high mortality rate may be in part correlated with the virulence of this pathogen and also the preexisting conditions of the patients (e.g., old age, neonates, and immunosuppression). In this study, homologs of 25 virulence factors were identified in \textit{E. anophelis} EM361-97 using VFDB\(^22,23\) (Supplementary Table S2). These virulence factors included products of the capsule, lipopolysaccharide, endopeptidase, lipid biosynthesis and metabolism, magnesium transport protein, macrophage infectivity, heat shock protein, catalase, peroxidase, superoxide dismutase, two-component regulatory system, and others.

According to the VFDB classification scheme, virulence factors are divided into offensive, defensive, nonspecific, and virulence-associated regulatory genes\(^22\). In our study, 13 of 25 pathogen-associated virulence factors homologs were identified to play offensive functional roles, eight were associated with defensive functions, three were nonspecific virulence factors, and one was related to regulation of virulence-associated genes. In strains Po0527107 (E27017) and V0378064 (E18064), Breurec et al.\(^1\) identified several offensive virulence factors that were found in strain EM361-97, including \textit{clpC, kdtB, pilR, sodB, gae, bplC, katA, clpP, fleQ, and htpB}. These virulence factors were also detected in the Wisconsin strains\(^1\). Pathogenic genomes were identified to have more offensive virulence factors, such as toxin and type III/IV secretion systems, than non-pathogenic genomes. In contrast, defensive, nonspecific, and regulatory virulence factors, such as iron uptake, motility, and antimphagocytosis, were found more frequently in non-pathogenic genomes than in pathogenic genomes\(^11\). Ho Sui et al.\(^3\) carried out a large-scale study to analyse the virulence factors of multiple bacteria and found over-presentation of offensive virulence factors, such as type III/IV secretion systems or toxins, within genomic islands of invasive pathogens. The manifestation of many offensive virulence factors in \textit{E. anophelis} suggests this microorganism may severely damage the host. However, this hypothesis lacks validity. More experiments are warranted to test the hypothesis of offensive virulence factors in \textit{E. anophelis}.

**Antimicrobial resistance and associated genes of \textit{E. anophelis} EM361-97**. The MIC and susceptibility of \textit{E. anophelis} EM361-97 are shown in Table 2. This isolate was only susceptible to piperacillin-tazobactam.
and minocycline. The MIC of tigecycline was 2 mg/L. However, there are no interpretive criteria of the susceptibility for *E. anophelis* to tigecycline in the CLSI and European Committee on Antimicrobial Susceptibility Testing.

Little information is known about the antimicrobial susceptibility of *E. anophelis*. Han et al. reported the susceptibilities of 51 *E. anophelis* isolates from South Korea. The susceptibility rates to piperacillin-tazobactam, piperacillin, levofloxacin, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole were 92%, 82%, 29%, 22%, 22%, and 22%, respectively. All the isolates were resistant to ceftazidime and imipenem. However, the MICs of minocycline and tigecycline were not examined in that study. Perrin et al. used the disk diffusion method to examine antimicrobial susceptibilities of 29 *E. anophelis* isolates in the Wisconsin outbreak. Most of these isolates were resistant to ceftazidime, imipenem, amikacin, tobramycin, gentamicin, but susceptible to cefepime, piperacillin, piperacillin-tazobactam, ciprofloxacin, and levofloxacin. Minocycline was also not tested in the study of Perrin et al. The antibiogram of isolates in the Wisconsin outbreak was different from that of isolates in Singapore by macrolides and isepamycin.

Gene functions annotated using the RAST/SEED Server recognised 76 genes of *E. anophelis* EM361-97 that were related to antibiotic resistance, including 12 for β-lactamase resistance, one for vancomycin resistance (vanW), four for fluoroquinolone resistance (parC, parE, gyrA, gyrB), nine for the membrane component of the tripartite multidrug resistance system, and 16 for multidrug resistance efflux pumps (six CmeB, one TolC, two MATE family efflux pumps, five OML, and two AcrB) (Table 2). The protein function annotations based on UniProtKB/Swiss-Prot demonstrated a number of proteins that played the role of antibiotic resistance, including multidrug resistance proteins (MdtA, MdtB, MdtC, MdtD, MdtE, MdtK, MdtL), probable multidrug resistance protein EmrK, multidrug export protein EmrA, macrolide export protein MacA, macrolide export ATP-binding/permease protein MacB, multidrug resistance outer membrane protein MdtQ, outer membrane efflux protein MepC, carbapenem antibiotics biosynthesis protein CarD, β-lactamase (BRO-1, 2), multidrug efflux pump subunit AcA, lincomycin resistance protein, DNA gyrase subunit A and subunit B, erythromycin resistance ATP-binding protein MsrA, and vancomycin B-type resistance protein VanW (Table 2). A replacement of serine by isoleucine at position 83 of DNA gyrase subunit A (Ser83Ile; AGC → ATC) was identified in *E. anophelis* strain EM361-97. Perrin et al. also found the same mutation of DNA gyrase subunit A in the Wisconsin outbreak strain CSID_3000521792. These findings are in agreement with the resistance of these two strains to fluoroquinolones.

Figure 4. The phylogenetic tree of the 34 available strains of *E. anophelis* in GenBank based on average nucleotide identity (ANI) values. The phylogenetic analysis revealed that *E. anophelis* EM361-97 was a sister group to *E. anophelis* FMS-007, which was a clade sister of strains Po0527107 (E27017) and V0378064 (E18064) isolated in the Central African Republic.
## Table 2. The minimum inhibitory concentration, susceptibility, and genes associated with antibiotic resistance in *E. anophelis* EM361-97. MIC, minimum inhibitory concentration. ¹Associated with multidrug resistance: membrane component of tripartate multidrug resistance system, multidrug resistance efflux pumps (CmeB, ToIC, MATE family efflux pump, OML, AcrA, AcrB), outer membrane efflux protein BepC, multidrug resistance proteins (MdtA, MdtB, MdtC, MdtD, MdtE, MdtK, MdtL), multidrug resistance protein EmrK, multidrug export protein EmrA, multidrug resistance outer membrane protein MdtQ, ABC transporter, MFS transporter, transcription-repair coupling factor, acriflavine resistance protein, and isoleucine-tRNA ligase. ²Susceptibility was determined according to the interpretive standards for other non-*Enterobacteriaceae* of CLSI.

| Antibiotic Group¹ | Antibiotics | MIC | Interpretation¹ | Resistant Gene/Protein/Mechanism¹ |
|--------------------|-------------|-----|-----------------|-----------------------------------|
| **Penicillins**    | Piperacillin | 32  | I               |                                    |
| **β-lactam/β-lactamase inhibitor combinations** | Piperacillin-tazobactam | 16/4 | S                |                                    |
| **Cephalosporins** | Cefazidime   | >16 | R               |                                    |
|                   | Cefepime     | 32  | R               |                                    |
|                   | Ceftiraxone  | >32 | R               |                                    |
| **Monobactams**    | Aztreonam    | >16 | R               |                                    |
| **Carbapenems**    | Imipenem     | >8  | R               | Carbenem antibiotics biosynthesis protein CarbD |
|                   | Meropenem    | >8  | R               |                                    |
| **Aminoglycosides**| Gentamicin   | >8  | R               | Resistance-nodulation-cell division transporter system Multidrug resistance efflux pump Aminoglycoside subclass B3 metallo-β-lactamase |
|                   | Tobramycin   | >8  | R               |                                    |
|                   | Amikacin     | >32 | R               | N-acetyltransferase APH(3') family aminoglycoside O-phosphotransferase elongation factor Tu |
| **Tetracyclines**  | Tetracycline | >8  | R               | Tetracycline resistance protein TetX Major facilitator superfamily transporter Tetracycline efflux pump NAPD-requiring oxidoreductase |
|                   | Minocycline  | <1  | S               |                                    |
|                   | Tigecycline  | 2   | R               |                                    |
| **Fluoroquinolones**| Ciprofloxacin | >2  | R               | DNA gyrase subunit A and subunit B Topoisomerase IV |
|                   | Levofoxacin  | >8  | R               |                                    |
| **Folate pathway inhibitors** | Trimethoprim - sulfamethoxazole | >4/76 | R               | Group A drug-sensitive dihydrofolate reductase sulfonamide-resistant dihydropteroate synthase Sdh1 |
| **Macrolides**     | —            | —   | —               | Macrolide export protein MacA, MacB, Erythromycin resistance ATP binding protein MsrA |
| **Vancomycin**     | —            | —   | —               | Vancomycin B-type resistance protein VanW |
| **Clindamycin**    | —            | —   | —               | Lincosycin resistance protein |
| **Chloramphenicol**| —            | —   | —               | Resistance-nodulation-cell division transporter system Multidrug resistance efflux pump Group B chloramphenicol acetyltransferase (tetracycline resistant) |

### Conclusions

In this work, the genomic features of the *E. anophelis* strain EM361-97 were constructed and compared with the genomes of other *Elizabethkingia* strains. Functional studies of this pathogen are required to validate these findings.

### References

1. Henriques, I. S. *et al.* Prevalence and diversity of carbapenem-resistant bacteria in untreated drinking water in Portugal. *Microb. Drug Resist.* 18, 531–537 (2012).
2. da Silva, P. S. L. & Pereira, G. H. *Elizabethkingia meningoseptica*: Emergent bacteria causing pneumonia in a critically ill child. *Pediatr. Int.* 55, 231–234 (2013).
3. Jean, S. S., Lee, W. S., Chen, F. L., Ou, T. Y. & Hsueh, P. R. *Elizabethkingia meningoseptica*: an important emerging pathogen causing healthcare-associated infections. *J. Hosp. Infect.* 86, 244–249 (2014).
4. King, E. O. Studies on a group of previously unclassified bacteria associated with meningitis in infants. *Am. J. Clin. Pathol.* 31, 241–247 (1959).
5. CDC. Recent Outbreaks, *Elizabethkingia*. [https://www.cdc.gov/elizabethkingia/outbreaks/](https://www.cdc.gov/elizabethkingia/outbreaks/) (2016).
6. Navon, L. *et al.* Notes from the Field: Investigation of *Elizabethkingia anophelis* cluster - Illinois, 2014–2016. *MMWR.* 65, 1380–1381 (2016).
7. Breurec, S. *et al.* Genomic epidemiology and global diversity of the emerging bacterial pathogen *Elizabethkingia anophelis*. *Sci. Rep.* 6, 30379 (2016).
8. Kämpfer, P. *et al.* *Elizabethkingia anophelis* sp. nov., isolated from the midgut of the mosquito *Anopheles gambiae*. *Int. J. Syst. Evol. Microbiol.* 61, 2670–2675 (2011).
9. Frank, T. *et al.* First case of *Elizabethkingia anophelis* meningitis in the Central African Republic. *Lancet* 381, 1876 (2013).
10. Teo, J. *et al.* First case of *E anophelis* outbreak in an intensive-care unit. *Lancet* 382, 855–856 (2013).
11. Lau, S. K. P. *et al.* *Elizabethkingia anophelis* bacteremia is associated with clinically significant infections and high mortality. *Sci. Rep.* 6, 26045 (2016).
12. Perrin, A. et al. Evolutionary dynamics and genomic features of the Elizabethkingia anophelis 2015 to 2016 Wisconsin outbreak strain. *Nat. Commun.*, 8, 15483 (2017).

13. Wisconsin Department of Health Services. Elizabethkingia. [https://www.dhs.wisconsin.gov/disease/elizabethkingia.htm](https://www.dhs.wisconsin.gov/disease/elizabethkingia.htm) (2017).

14. Lin, J. N., Yang, C. H., Lai, C. H., Huang, Y. H. & Lin, H. H. Draft genome sequence of *Elizabethkingia anophelis* strain EM361-97 isolated from the blood of a cancer patient. *Genome Announc.* 4, 1 (2016).

15. Hantsis-Zacharov, E., Shakéd, T., Senderovich, Y. & Halpern, M. *Chryseobacterium oranimense* sp. nov., a psychrotolerant, proteolytic and lipolytic bacterium isolated from raw cow’s milk. *Int. J. Syst. Evol. Microbiol.* 58, 2635–2639 (2008).

16. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. M100-S26 (Clinical and Laboratory Standards Institute, 2016).

17. Li, R., Li, Y., Kristiansen, K. & Wang, J. SOAP: short oligonucleotide alignment program. *Bioinform.* 24, 713–714 (2008).

18. Tatusova, T. et al. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* 44, 6614–6624 (2016).

19. Aziz, R. K. et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9, 75 (2008).

20. Overbeek, R. et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res.* 42, D206–214 (2014).

21. Grant, J. R. & Stothard, P. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Res.* 36, W181–184 (2008).

22. Chen, L. et al. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res.* 33, D325–328 (2005).

23. Chen, L., Zheng, D., Liu, B., Yang, J. & Jin, Q. VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. *Nucleic Acids Res.* 44, D694–697 (2016).

24. Liu, B. & Pop, M. ARDB–Antibiotic Resistance Genes Database. *Nucleic Acids Res.* 37, D443–447 (2009).

25. Wang, Y., Coleman-Derr, D., Chen, G. & Gu, Y. Q. OrthoVenn: a web server for genome wide comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Res.* 43, W78–84 (2015).

26. Lee, I., Kim, Y. O., Park, S. C. & Chun, J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int. J. Syst. Evol. Microbiol.* [https://doi.org/10.1099/ijsem.0.000760] [Epub ahead of print] (2015).

27. Meier-Kolthoff, J. P. et al. Antibiotic Resistance Genes Database. *Nucleic Acids Res.* 40, D360–364 (2012).

28. Overbeek, R., Boetius, A., Fonstein, M., Klenk, H. P. & Goerke, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14, 60 (2013).

29. Kämpfer, P., Busse, H. I., McNicol, J. A. & Glaeser, S. P. *Elizabethkingia endophytica* sp. nov., isolated from Zea mays and emended description of *Elizabethkingia anophelis* Kämpfer et al. 2011, *Int. J. Syst. Evol. Microbiol.* 65, 2187–2193 (2015).

30. Ho Sui, S. J., Fedynak, A., Hsiao, W. W. L., Langille, M. G. I. & Brinkman, F. S. L. The association of virulence factors with genomic context: identification of key factors that are not conserved across species. *Nucleic Acids Res.* [Epub ahead of print] (2017).

31. Nicholson, A. C. et al. Revisiting the taxonomy of the genus *Elizabethkingia* using whole-genome sequencing: *Elizabethkingia endophytica* Kämpfer et al. 2015 is a later subjective synonym of *Elizabethkingia anophelis* Kämpfer et al. 2011. *Int. J. Syst. Evol. Microbiol.* 66, 4535–4539 (2016).

32. Nicholsom, A. C. et al. Revisiting the taxonomy of the genus *Elizabethkingia* using whole-genome sequencing, optical mapping, and MALDI-TOF, along with proposal of three novel *Elizabethkingia* species: *Elizabethkingia bruuniana* sp. nov., *Elizabethkingia ursingii* sp. nov., and *Elizabethkingia occulta* sp. nov. *Antonie Van Leeuwenhoek* [Epub ahead of print] (2017).

33. Overbeek, R., Boetius, A., Fonstein, M., Klenk, H. P. & Goerke, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14, 60 (2013).

34. Lee, I., Kim, Y. O., Park, S. C. & Chun, J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int. J. Syst. Evol. Microbiol.* [https://doi.org/10.1099/ijsem.0.000760] [Epub ahead of print] (2015).

35. Nicholason, A. C. et al. Revisiting the taxonomy of the genus *Elizabethkingia* using whole-genome sequencing, optical mapping, and MALDI-TOF, along with proposal of three novel *Elizabethkingia* species: *Elizabethkingia bruuniana* sp. nov., *Elizabethkingia ursingii* sp. nov., and *Elizabethkingia occulta* sp. nov. *Antonie Van Leeuwenhoek* [Epub ahead of print] (2017).

36. Che, D., Hasan, M. S. & Chen, B. Identifying pathogenicity islands in bacterial pathogenomics using computational approaches. *Pathogens* 3, 36–56 (2014).

37. Ho Sui, S. J., Fedynak, A., Hsiao, W. W. L., Langille, M. G. I. & Brinkman, F. S. L. The association of virulence factors with genomic islands. *PLoS One* 4, e8094 (2009).

38. EUCAST: Clinical breakpoints. [http://www.eucast.org клиничные пороги] (2017).

39. Han, M. S. et al. Relative prevalence and antimicrobial susceptibility of clinical isolates of *Elizabethkingia* species based on 16S rRNA gene sequencing. *J. Clin. Microbiol.* 55, 274–280 (2017).

40. Teo, J. et al. Comparative genomic analysis of malaria mosquito vector-associated novel pathogen *Elizabethkingia anophelis*. *Genome Biol. Evol.* 6, 1158–1165 (2014).

Acknowledgements

This work was supported by grants EDP10105082 from E-Da Hospital and MOST 105-2314-B-214-008 and 106-2314-B-214-009-MY2 from the Ministry of Science and Technology, Taiwan.

Author Contributions

All authors provided significant contributions, and all authors are in agreement regarding the content of the manuscript. Conception/design: Jiun-Nong Lin and Hsi-Hsun Lin; provision of study materials: Chung-Hsu Lai; collection and assembly of data: Jiun-Nong Lin, Chung-Hsu Lai, Chih-Hui Yang, and Yi-Han Huang; data analysis and interpretation: all authors; manuscript writing: Jiun-Nong Lin and Chih-Hui Yang; and final approval of the manuscript: all authors.

Additional Information

Supplementary information accompanies this paper at [https://doi.org/10.1038/s41598-017-14841-8](https://doi.org/10.1038/s41598-017-14841-8).

Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2017