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Comparison of Whole-Genome Sequences for Three Species of the *Elizabethkingia* Genus

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Abstract

Background: There are increasing researches on whole-genome sequences for clinical strains of *Elizabethkingia* genus which can cause severe infection in humans, while few studies on the comparative genomics of species in the *Elizabethkingia* genus in China have been conducted.

Methods: The *Elizabethkingia* genus, isolated in a tertiary hospital of Beijing, China, were re-identified and analyzed through *in silico* DNA-DNA hybridization (DDH), whole-genome sequence-based phylogeny. Antibiotic resistance genes, antimicrobial resistance-associated proteins, virulence factors were identified, and clusters of orthologous groups were evaluated by Kyoto Encyclopedia of Genes and Genomes (KEGG). The clinical information of patients infected by these organisms was collected and the characteristics were analyzed.

Results: There were three species among 20 clinical isolates of *Elizabethkingia* genus: *E. meningoseptica*, *E. anophelis* and *E. miricola*. *E. anophelis* accounted for the majority. *E. meningoseptica* exhibited higher GC content and possessed carbapenemase-encoding genes of $bla_{\text{GOB-16}}$ and $bla_{\text{B-12}}$ while *E. anophelis* carried genes of $bla_{\text{CME-1}}$. Multiple kinds of antimicrobial resistance-associated proteins were predicted and the virulence factors about adherence, biofilm formation, iron and magnesium uptake, stress adaptation, and immune evasion were discovered. Among 2622 clusters of core genomes identified from the three species of the *Elizabethkingia* genus, the majority of genes were metabolism-related. Pan genome displayed an upregulation, while the core genome displayed a downregulation with the addition of new genes for the 20 *Elizabethkingia* strains.

Conclusions: The composition was different in antimicrobial resistance-related, virulence-related and metabolism-related genes depending upon species of *Elizabethkingia*. An adaptive evolution of *Elizabethkingia* to environmental change including hospital settings has been developed.

Background

Elizabethkingia genus, a group of non-motile aerobic gram-negative rods bacterium, exists widely in water and soil [1–8]. Species of *Elizabethkingia meningoseptica* was first identified by King in 1959, named as *Flavobacterium meningosepticum*, and was categorized into C*hryseobacterium* genus. According to 16S ribosomal RNA (rRNA) gene sequence in 2005, Kim *et al*. reclassified *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* into the genus of *Elizabethkingia* [9, 10]. *E. anophelis* was isolated from the midgut of the mosquito *Anopheles gambiae* in McCarthy Island of Gambia, Africa in 2011 [11]. In 2017, three novel species were proposed and added in *Elizabethkingia* genus including *E. bruuniana*, *E. ursingii*, and *E. occulta* [12].

The *Elizabethkingia* are found to cause severe neonatal meningitis, nosocomial pneumonia, endocarditis, and bacteremia, especially in immuno-compromised patients [13–15]. Recently, infections caused by *E. anophelis* are life threatening and have been constantly reported worldwide [2–8]. A severe community-

associated outbreak caused by *E. anophelis* occurred in Wisconsin in 2016, which led to deaths of 18 patients as reported [4, 6, 8].

Whole-genome sequencing has been considered as a powerful technique in molecular microbiology, by which comprehensive information about the drug resistance and virulence factors can be provided, host-pathogen interaction and host-environment reaction can also be predicted [16]. In this study, genomic features as well as clinical characteristics of 20 *Elizabethkingia* strains were investigated based on our previous antimicrobial susceptibility testing [17].

Results

Identification of Elizabethkingia **species by whole-genome sequence**

There were 14 isolates of *E. anophelis*, 5 isolates of *E. meningoseptica* and 1 isolate of *E. miricola* among 20 nonduplicated *Elizabethkingia* isolates according to the results of whole-genome comparison as shown in Fig. 1. The heat map displayed a clear delineation of three species in the *Elizabethkingia* genus. Whole-genome sequence-based phylogenetic tree of the 20 *Elizabethkingia* isolates and the reference strains by the Reference Sequence Alignment based Phylogeny Builder (REALPHY) were presented (Fig. 2). The difference was clear among 3 species of the *Elizabethkingia* strains. The phylogenetic distance between *E. miricola* and *E. anophelis* was closer than that between *E. miricola* and *E. meningoseptica*.

Statistically significant difference was not found in genome size between *E. meningoseptica* and *E. anophelis*. There was also no statistically significance among *Elizabethkingia* species in total contig number, N50 contig length, N90 contig length, gene number or pseudogene number. GC base content was detected more in *E. meningoseptica* than *in E. anophelis* ($p < 0.001$) (Table 1).

Table 1
General features of 20 *Elizabethkingia* genomes

General features	All (n = 20)	*E. meningoseptica* (n = 5)	*E. anophelis* (n = 14)	*E. miricola* (n = 1)	*p*-Value (EME vs EAN)
Size (Mb)	3.85 ± 0.11	3.84 ± 0.13	3.85 ± 0.11	3.96	0.888
GC%	35.83 ± 0.34	36.39 ± 0.13	35.64 ± 0.08	35.86	< 0.001
Total contig number (> 500 bp)	27.2 ± 20.83	17.40 ± 6.54	32.28 ± 22.77	5	0.174
N50 contig length (bp)	642861.10 ± 531516.71	528116.60 ± 99490.75	573562.21 ± 465037.96	2,186,768	0.843
N90 contig length (bp)	173608.35 ± 155732.98	142947.60 ± 18984.20	145764.64 ± 106998.21	716,724	0.955
Gene number	3606.05 ± 93.76	3577.20 ± 121.76	3606.71 ± 81.03	3741	0.547
Pseudogene number	2.5 ± 4.64	0.80 ± 0.84	2.00 ± 3.37	18	0.450

EME: *Elizabethkingia meningoseptica*; EAN: *Elizabethkingia anophelis*

Identification of antibiotic resistance genes in Elizabethkingia species

As we can see from Table 2 and Fig. 3, resistance genes of 6 classes of antibiotics including beta-lactamase, sulfonamides, macrolides, tetracyclines, aminoglycosides and glycopeptides from 20 isolates of *Elizabethkingia* were identified. All reference strains and clinical isolates possessed antibiotic efflux pump gene of *adeF*. Several antibiotic resistance genes existed in specific species of *Elizabethkingia*, such as bla_{GOB-16} and bla_{B-12} in *E. meningoseptica*, bla_{CME-1} in *E. anophelis* and bla_{GOB-13} and bla_{B-6} in *E. miricola*.

Table 2
Antibiotic resistance genes of the 20 clinical *Elizabethkingia* isolates and reference strains

Antibiotic resistance genes	*E. meningoseptica*		*E. anophelis*		*E. miricola*		*p*-Value
	ATCC 13253	Clinical isolates (n = 5)	R26	Clinical isolates (n = 14)	GTC 862	Clinical isolates (n = 1)	(EME vs EAN)
Beta-lactamase							
bla_{GOB-9}	-	0	+	50% (7)	-	0	0.076
bla_{GOB-10}	-	0	-	42.86% (6)	-	0	0.124
bla_{GOB-11}	-	0	-	0	+	0	0.526
bla_{GOB-12}	-	0	-	7.14% (1)	-	0	0.684
bla_{GOB-13}	-	0	-	0	-	100% (1)	0.526
bla_{GOB-16}	+	100% (5)	-	0	-	0	< 0.001
bla_{B-2}	-	0	-	7.14% (1)	+	0	0.684
bla_{B-6}	-	0	-	0	-	100% (1)	0.526
bla_{B-11}	-	0	+	85.71% (12)	-	0	0.001
bla_{B-12}	+	100% (5)	-	0	-	0	< 0.001
bla_{B-14}	-	0	-	7.14% (1)	-	0	0.684
bla_{CME-1}	-	0	-	100% (14)	+	0	< 0.001
$bla_{OXA-347}$	-	0	-	7.14% (1)	-	0	0.684
Sulfonamides							
sul2	-	0	-	28.57% (4)	-	0	0.292
Macrolides							
ermF	-	40% (2)	-	7.14% (1)	-	0	0.155
ereD	-	20% (1)	-	35.71% (5)	-	0	0.516
Tetracyclines							
tetX	-	40% (2)	-	14.28% (2)	-	0	0.226

+ or - represented reference strains with or without antibiotic resistance genes

Antibiotic resistance genes	*E. meningoseptica*		*E. anophelis*		*E. miricola*		*p*-Value (EME vs EAN)
	ATCC 13253	Clinical isolates (n = 5)	R26	Clinical isolates (n = 14)	GTC 862	Clinical isolates (n = 1)	
Aminoglycosides							
aadS	-	20% (1)	-	78.57% (11)	-	0	0.020
Glycopeptides							
vanW	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Resistance-nodulation-cell division (RND) antibiotic efflux pump							
adeF	+	100% (5)	+	100% (14)	+	100% (1)	0.526
+ or - represented reference strains with or without antibiotic resistance genes							

Prediction of antimicrobial resistance-associated proteins in Elizabethkingia **species**

The antimicrobial resistance-associated proteins of *E. meningoseptica* and *E. anophelis* were predicted as in Table 3.

Table 3
Antimicrobial resistance-associated proteins in 20 clinical *Elizabethkingia* isolates and reference strains

Class of Antimicrobial Resistance	*E. meningoseptica*		*E. anophelis*		*E. miricola*		*p*-Value (EMEas EAN)
	ATCC 13253	Clinical isolates (n = 5)	R26	Clinical isolates (n = 14)	GTC 862	Clinical isolates (n = 1)	
beta-lactamase							
beta-lactamase	+	100% (5)	+	100% (14)	+	100% (1)	0.526
BLI	+	80% (4)	+	92.86% (13)	+	100% (1)	0.468
beta-lactamase ClassC	+	100% (5)	+	14.28% (2)	+	100% (1)	0.001
Metallo-beta-lactamase family protein	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Multidrug resistance efflux pumps							
CmeB	+	100% (5)	+	100% (14)	+	100% (1)	0.526
TolC	-	0	-	0	+	0	0.526
MATE family of MDR efflux pumps	+	100% (5)	+	100% (14)	+	100% (1)	0.526
AcrB	+	0	+	0	+	0	0.526
Multiple antibiotic resistance MAR locus							
MarA/MarB/MarC	-	100% (5)	-	100% (14)	+	100% (1)	0.526
Multidrug resistance, tripartite systems							
MFP	+	100% (5)	+	100% (14)	+	100% (1)	0.526
IM	+	100% (5)	+	100% (14)	+	100% (1)	0.526
OM	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Resistance to macrolides							

+ or - represented reference strains with or without antimicrobial resistance-associated proteins; BLI: Beta-lactamase superfamily I; MFP: Membrane fusion protein; IM: Inner membrane; OM: Outer membrane

Class of Antimicrobial Resistance	*E. meningoseptica*		*E. anophelis*		*E. miricola*		*p*-Value (EMEas EAN)
	ATCC 13253	Clinical isolates (n = 5)	R26	Clinical isolates (n = 14)	GTC 862	Clinical isolates (n = 1)	
Macrolide-efflux protein	-	0	-	100% (14)	-	100% (1)	< 0.001
Erythromycin esterase homolog	-	100% (5)	-	0	-	0	< 0.001
Erythromycin resistance methylase B	-	0	-	0	-	0	0.526
Resistance to tetracyclines							
Tetracycline efflux protein TetA	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Resistance to aminoglycosides							
AadA1	-	0	-	0	-	0	0.526
Aminoglycoside phosphotransferase	-	0	-	0	-	0	0.526
Kanamycin kinase	-	40% (2)	-	21.43% (3)	-	0	0.418
Resistance to sulfonamides							
Sulfonamide-resistant dihydropteroate synthase gene family	-	0	-	0	-	0	0.526
Resistance to vancomycin							
VanW	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Resistance to fluoroquinolones–DNA gyrase							
GyrA, GyrB	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Topoisomerase IV							
ParC, ParE	+	100% (5)	+	100% (14)	+	100% (1)	0.526

+ or - represented reference strains with or without antimicrobial resistance-associated proteins; BLI: Beta-lactamase superfamily I; MFP: Membrane fusion protein; IM: Inner membrane; OM: Outer membrane

Prediction of virulence factors in Elizabethkingia **species**

The potential virulence factors of the three *Elizabethkingia* species were shown in Table 4 and Fig. 3. We noticed that some virulence factors existed in all clinical and reference strains, such as hsp60, streptococcal enolase, exopolysaccharide, Mg^{2+} transport, EF-Tu, catalase, and peroxidase, while some existed differently upon species, i.e., phospholipase D, isocitrate lyase and lipopolysaccharide existed mostly in *E. meningoseptica*, and O-antigen mostly in *E. anophelis*.

Table 4
Virulence factors and their associated genes in three *Elizabethkingia* species and reference strains

Virulence factors	Genes	*E. meningoseptica*		*E. anophelis*		*E. miricola*		*p*-Value
		ATCC 13253	Clinical isolates (5)	R26	Clinical isolates (14)	GTC 862	Clinical isolates (1)	(EME vs EAN)
Adherence								
Hsp60	*htpB*	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Polar flagella	*flmH*	-	0	-	0	+	0	0.526
Biofilm formation								
AdeFGH efflux pump (cation/multidrug efflux pump)	*adeG*	+	100% (5)	-	92.86% (13)	+	0	0.684
Enzyme								
Phospholipase C	*plcA*	+	60% (3)	+	100% (14)	-	0	0.067
Phospholipase D	*pld*	+	100% (5)	-	7.14% (1)	-	0	< 0.001
Streptococcal enolase	*eno*	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Immune evasion								
Exopolysaccharide	*galE/pgi*	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Iron uptake								
Heme biosynthesis	*hemL*	+	100% (5)	-	85.71% (12)	+	0	0.648
Acid resistance								
Urease	*ureB/ureG*	+	20% (1)	-	28.57% (4)	+	100% (1)	0.709
Lipid and fatty acid metabolism								
Isocitrate lyase	*icl*	+	100% (5)	-	21.43% (3)	-	0	0.004
Pantothenate synthesis	*panD*	-	0	-	0	-	0	0.526

+ or - represented reference strains with or without virulence factors and associated genes

Virulence factors	Genes	*E. meningoseptica*		*E. anophelis*		*E. miricola*		*p*-Value
		ATCC 13253	Clinical isolates (5)	R26	Clinical isolates (14)	GTC 862	Clinical isolates (1)	(EME vs EAN)
Magnesium uptake								
Mg^{2+} transport	*mgtB/mgtE*	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Other adhesion-related proteins								
EF-Tu	*tuf*	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Secretion system								
Type III secretion system effectors	*hopJ1*	-	0	-	0	-	0	0.526
Stress adaptation								
Catalase-peroxidase	*katG*	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Catalase (KatAB)	*katA*	+	100% (5)	+	100% (14)	+	100% (1)	0.526
Macrophage inducible genes								
Mig-5	*mig-5*	-	0	-	0	-	0	0.526
Serum resistance and immune evasion								
Lipopolysaccharide	*wbtI*	+	100% (5)	-	14.28% (2)	-	0	0.001
O-antigen	*fcl*	-	20% (1)	-	85.71% (12)	-	100% (1)	0.007
+ or - represented reference strains with or without virulence factors and associated genes								

Analysis of Kyoto encyclopedia of genes and genomes, clusters of orthologous groups of Elizabethkingia **species**

Through the functional analysis of the Clusters of Orthologous Groups (COGs) in genomes of the 20 *Elizabethkingia* (Fig. 4), core genomes were found to be mostly related to metabolism, while majority of accessory and unique gene families involved in information storage and processing, R (general function prediction only) occupied the most, followed by K (transcription) and M (cell wall/membrane/envelope biogenesis), otherwise D (cell cycle control, cell division, and chromosome partitioning) accounted for the least (Fig. 4A and 4B). Detailed information about COG distribution among components of gene families revealed that core genomes took 70.8% of J (translation, ribosomal structure, and biogenesis), and

accessory genomes occupied 49.0%, most in T (Signal transduction mechanisms), the unique genomes possessed 58.8%, the most in V (defense mechanisms).

Analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) distribution on 20 *Elizabethkingia* strains (Fig. 5) demonstrated that functional genomes of metabolism accounted for the most (Fig. 5A). Among these genomes, carbohydrate metabolism occupied the most, followed by amino acid metabolism (Fig. 5B). Further analysis indicated that core genes accounted for 32.3% in carbohydrate metabolism, accessory genes and unique genes accounted for 41.0% and 26.7%, respectively.

Analysis of core and pan genome

A flower plot of core and pan genome analysis presented 2622 core genes, 686 to 1119 accessory genes and 0 to 415 unique genes (Fig. 6 and Table S1). The pan genome displayed an upregulation from 3561 to 6596, while the core genome displayed a downregulation from 3134 to 2440 (Fig. 7 and Table S2).

Clinical Characteristics of Elizabethkingia Infections

As to the source of these 20 *Elizabethkingia* strains, specimens from respiratory tract accounted for the most (65%), followed by the exudate (15%), blood (10%), urine (5%) and bile (5%). Both isolates of *E. meningoseptica* and *E. anophelis* were mostly originated from respiratory tract. *E. anophelis* was the only species isolated from blood and *E. miricola* was the only species isolated from the urine, as shown in Table S3. Table 5 showed the clinical characteristics of patients infected of *E. meningoseptica*, *E. anophelis* and *E. miricola*.

Table 5
Characteristics, clinical information and outcome of patients with *Elizabethkingia* infections

Characteristics	% (n = 20)	% (Number) of Isolates			*p*-Value
		E. meningoseptica (n = 5)	E. anophelis (n = 14)	E. miricola (n = 1)	(EME vs EAN)
Sex					
Male	80% (16)	100% (5)	71.43% (10)	100% (1)	
Female	20% (4)	0	28.57% (4)	0	0.575
Age					
Range (year)	42–93	53–88	42–93	59	
Median (year)	70	62	76		
Hospitalization duration					
Range (day)	2-227	21–83	2-227	13	
Median (day)	42	53	35		
Ward					
Intensive care unit	45% (9)	20% (1)	57.14% (8)	0	0.153
Respiratory	20% (4)	40% (2)	14.28% (2)	0	0.226
Emergency	15% (3)	0	21.43% (3)	0	0.508
Surgery	15% (3)	40% (2)	0	100% (1)	0.067
Neurology	5% (1)	0	7.14% (1)	0	0.684
Comorbidity					
Cardiovascular disease	70% (14)	60% (3)	71.43% (10)	100% (1)	0.637
Hypertension	65% (13)	60% (3)	64.28% (9)	100% (1)	0.865
Cerebral infarction	45% (9)	40% (2)	50% (7)	0	0.701
Tumor	45% (9)	80% (4)	28.57% (4)	100% (1)	0.046
Diabetes mellitus	40% (8)	40% (2)	42.86% (6)	0	0.912
Chronic obstructive pulmonary disease	20% (4)	20% (1)	21.43% (3)	0	0.946

N%: Percentage of neutrophils

Characteristics	% (n = 20)	% (Number) of Isolates			*p*-Value
		E. meningoseptica (n = 5)	E. anophelis (n = 14)	E. miricola (n = 1)	(EME vs EAN)
End-stage renal disease	20% (4)	20% (1)	21.43% (3)	0	0.946
Laboratory data					
White blood cell count (cells/mm^3)	9354 ± 5037	6998 ± 2994	10,118 ± 5610	10,440	0.258
N (%)	80.3 ± 10.43	86.640 ± 10.523	77.414 ± 9.731	89	0.092
Hemoglobin (g/dL)	95.95 ± 21.11	110.200 ± 18.593	90.357 ± 20.720	103	0.077
Outcome					
Survived	80% (16)	100% (5)	71.43% (10)	100% (1)	0.292
Dead	20% (4)	0	28.57% (4)	0	0.292
N%: Percentage of neutrophils					

Discussion

The role of whole-genome sequencing in species discrimination of Elizabethkingia genus

The traditional DDH was used to be one of the most important criterion for discrimination of bacterial species. Recently, *in silico* DDH has been deemed as a more accurate substitution for traditional DDH [18]. In our study, genome-to-genome distance calculator (GGDC) plus *in silico* DDH was used to reveal the delineation of *Elizabethkingia* species. The 20 clinical strains were initially identified as *E. meningoseptica*, however, they were found to be *E. anophelis* (14 strains), *E. meningoseptica* (5 strains) and *E. miricola* (1 strain). *E. anophelis* turned out to be the majority species of the *Elizabethkingia* genus (70%, 14/20), consistent with previous research [12, 16, 19].

Comparative genome analysis of antibiotic resistance genes in Elizabethkingia genus

E. meningoseptica differed from *E. anophelis* or *E. miricola* in many aspects as our comparative genome analysis demonstrated. For instance, genomes of *E. meningoseptica* possessed more GC content than those of *E. anophelis* and *E. miricola*. As to antibiotic resistance genes, bla_{GOB-16} and bla_{B-12}

carbapenemase-encoding genes were more in *E. meningoseptica*, while bla_{CME-1} was more in *E. anophelis*. bla_{GOB-9} and bla_{GOB-10} also existed in *E. anophelis* (Fig. 3). bla_{GOB-13} and bla_{B-6} were identified in *E. miricola*. Extended-spectrum serine-beta-lactamase carboxymethyl ether (CME) (class D) and two unrelated wide-spectrum metallo-beta-lactamases (MBLs), BlaB (subclass B1) and GOB (subclass B3) belong to the family of beta-lactamases. Due to the divergence of CME and MBLs among *Elizabethkingia* species, it might be a potential evidence for species discrimination to analyze the homology of different phylogenetic cluster.

The relationship of antibiotic resistance genes, antimicrobial resistance-associated proteins and antimicrobial phenotype

Elizabethkingia isolates have been reported to be resistant to varieties of antimicrobial agents, not only most beta-lactams or beta-lactams/beta-lactamase inhibitors, but also aminoglycosides, macrolides, tetracycline, vancomycin, and carbapenems. While *Elizabethkingia* were susceptible to piperacillin, piperacillin-tazobactam, minocycline, fluoroquinolones, tigecycline, and trimethoprim-sulfamethoxazole [2–5]. Studies revealed that antibiotic susceptibility patterns of *Elizabethkingia* were closely related to species and geographical locations [20].

According to our previous study, our clinical strains of *Elizabethkingia* genus possessed high level of multi-drug resistance [17]. The present study demonstrated that proteins resistant to beta-lactamases, vancomycin, tetracyclines, quinolones, macrolides, and multidrug resistance efflux pumps were identified in our clinical strains, while proteins resistant to aminoglycosides and sulfonamides were absent. The coincidence rate between antimicrobial resistance genes and phenotype of beta-lactamases was 100%. Although antimicrobial resistance genes of sulfonamides, macrolides, tetracyclines and aminoglycosides were absent, these strains of *Elizabethkingia* possessed resistance phenotype to them. Interestingly, TetA, a kind of protein in charge of tetracycline efflux, was found in our clinical *Elizabethkingia* strains, but these strains were 100% susceptible to minocycline (Table S4). It has been reported that vancomycin was used to treat neonatal meningitis with *E. meningoseptica* successfully [21], however, all of the 20 clinical *Elizabethkingia* strains presented minimum inhibitory concentration (MIC) of vancomycin more than 8 μg/ml [22], the similar results were also discovered in other studies [19, 20]. *vanW*, a vanB-type glycopeptide resistance gene, was identified in all the three *Elizabethkingia* species. The exact function of *vanW* still remained uncertain, but an involvement of its mutations has been observed in the regulation of resistance to teicoplanin [23]. Hence, the use of glycopeptide should be cautious in treating infection by *Elizabethkingia* [19, 20].

Comparative genome analysis of virulence factors predicted among Elizabethkingia species

Bacterial virulence factors are essential for pathogenesis, in our study, exopolysaccharide, heme biosynthesis, urease, and Mg^{2+} transport were predicted in three species of 20 *Elizabethkingia* strains (Table 4 and Fig. 3), while they existed only in *E. miricola* GTC 862^T by Liang *et al*. [16]. The *adeG* gene existed in 18 strains of *Elizabethkingia* (Table 4), it was related to biofilm which could cause bacteria to adhere to the medical devices and resist against disinfectant [24–26].

Virulence factors about lipid and fatty acid metabolism, serum resistance and immune evasion and phospholipase D were found mostly in *E. meningoseptica*, that might be the reason why *E. meningoseptica* tends to cause more neonatal meningitis and sepsis. It was not clear whether O-antigen involved in immune evasion played a role in outbreaks largely triggered by *E. anophelis*. There were less categories of virulence factors in *E. miricola*, which could explain why *E. miricola* caused occasional clinical infection case reports [27].

COGs and KEGG analysis of Elizabethkingia genus

COGs, clusters of orthologous groups, that involve species-related genes evolving from a common gene, remain the original function during the evolution process. Detection of COGs and prediction of their functions are of fundamental importance in many fields, particularly in pathogenic analysis with new sequence and function of intracellular survival related to COGs with “information storage and processing” [28, 29]. Both COGs and KEGG analysis of *Elizabethkingia* genus indicated that function of metabolism occupied the largest part, most of which were linked to carbohydrate metabolism.

Pan genome proposed by Tettelin *et al*. was introduced to discriminate genomes, to investigate the core (conserved), accessory (dispensable), and unique (strain-specific) genes, to trace horizontal gene-flux among strains, and to acquire information about species evolution [30]. The only strain of *E. miricola* possessed most unique genomes among these 20 clinical *Elizabethkingia* strains, indicating a higher degree of species evolution than other species. Further study on more strains are needed to explore the complicated species evolution of *Elizabethkingia* genus.

Clinical characteristics of Elizabethkingia infections

Chronic underlying illnesses, such as cardiovascular disease, hypertension, diabetes mellitus, malignancy, and liver cirrhosis were common in most patients with *Elizabethkingia* infections [3–5, 31, 32]. Although mortality rate of *E. anophelis* and *E. meningoseptica* was 24–34% and 30% reported by Lin *et al*. (2018) and Lin *et al*. (2019) respectively [5, 19], the death rate of patients in our study was lower than that. We also found that among mortality-affecting factors, cerebral infarction was an independent risk factor (Table S5). Therefore, we suggest that more attention should be paid on patients with cerebral infarction and *Elizabethkingia* infection in order to reduce mortality.

Conclusions

A genomic comparative analysis as well as clinical characteristics of 20 clinical strains among three *Elizabethkingia* species were conducted. The results provided information to better understand the drug resistance, virulence, gene evolution from the point of genome structure of this pathogen.

Methods

Strains

The 20 clinical isolates of *Elizabethkingia* species were isolated from patients in Chinese PLA General Hospital (Beijing, China) between 2014 and 2018. They were initially identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) system (bioMérieux, Marcy l'Etoile, France) and stored at -80°C. The sequences for reference strains of *E. meningoseptica* ATCC 13253^T, *E. anophelis* R26^T and *E. miricola* GTC 862^T were originated from GenBank accession no AJ704540, EF426425 and AB071953 respectively.

DNA preparation and sequencing

Columbia Agar with 5% sheep blood was used for subculture of stocked bacteria. The total DNA of fresh colonies on Mueller Hinton Agar was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Nanodrop2000 UV-Vis Spectrophotometer (Thermo scientific, Massachusetts, United States) was used for measuring the concentration of genomic DNA. The genomic DNA of these 20 clinical isolates were sequenced by Illumina HiSeq-PE150 (Illumina, San Diego, CA, USA), and then assembled into contiguous sequences by short oligonucleotide analysis package (SOAP) *de novo* with short and low-coverage contigs filtered out [33].

Species identification and whole-genome phylogenetic analysis

Reference strains of *E. meningoseptica* ATCC 13253^T, *E. miricola* GTC 862^T and *E. anophelis* R26^T were selected for whole-genome comparison with each species. Online pipeline Reference Sequence Alignment based Phylogeny Builder (REALPHY) and the Center for Genomic Epidemiology (CGE) Pipeline (http://www.genomicepidemiology.org/) were employed to construct phylogenetic tree and to confirm the species of *Elizabethkingia* [22]. *In silico* DNA-DNA hybridization (DDH) values were calculated using Genome-to-Genome Distance Calculator (GGDC, http://ggdc.dsmz.de/home.php), formula 2 was adopted to analyze the results according to manufacturer's suggestion, 70% was used as criteria for species delimitation [34,35]. The heat map was generated through https://discover.nci.nih.gov/cimminer/ [16].

Annotation and analysis of 20 strains of *Elizabethkingia* Genome

The assembled genome sequences of the 20 *Elizabethkingia* isolates were submitted to the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) and the Rapid Annotations using Subsystem Technology (RAST) server (http://rast.nmpdr.org/) [36-38]. Antibiotic resistance genes were analyzed through CGE Pipeline and https://ardb.cbcb.umd.edu/ [39]. The antimicrobial resistance-associated proteins of the 20 *Elizabethkingia* clinical isolates as well as 3 reference strains were predicted by the RAST Prokaryotic Genome Annotation Server [37,38]. The virulence factors were revealed through http://www.mgc.ac.cn/VFs/ [40].

Clusters of orthologous groups, Kyoto encyclopedia of genes and genomes and pan genome analysis

Analysis of core (conserved), accessory (dispensable), and unique (strain-specific) genes was performed with the use of the Bacterial Pan Genome Analysis Tool (BPGA) [41]. The BPGA was also used to generate

pan genome and core genome phylogenies and to access the Clusters of Orthologous Groups (COGs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database [41].

Data Analysis

Clinical information was authorized and obtained from clinical medical record system of Chinese PLA General Hospital. The clinical and whole-genome sequenced data were analyzed with SPSS version 23.0 (IBM, Armonk, NY, USA). Categorical variables were calculated with the chi-squared test or Fisher exact test as appropriate. Continuous variables were illustrated with average ± standard deviation (SD) and calculated using the independent-sample t test or nonparametric Wilcoxon rank sum test according to normal distribution. Univariate analyses with the two-tailed *p*-value were conducted to examine the variables for mortality. $p < 0.05$ was of statistical significance.

Abbreviations

DDH: DNA-DNA hybridization; KEGG: Kyoto encyclopedia of genes and genomes; CGE: Center for genomic epidemiology; REALPHY: Reference sequence alignment based phylogeny builder; COGs: Clusters of orthologous groups; GGDC: Genome-to-genome distance calculator; CME: Carboxymethyl ether; MBLs: Metallo-beta-lactamases; MIC: Minimum inhibitory concentration; MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SOAP: Short oligonucleotide analysis package; NCBI: National Center for Biotechnology Information; PGAP: Prokaryotic genome annotation pipeline; RAST: Rapid annotations using subsystem technology; BPGA: Bacterial pan genome analysis; SD: Standard deviation; EME: *Elizabethkingia meningoseptica*; EAN: *Elizabethkingia anophelis*; RND: Resistance-nodulation-cell division; BLI: Beta-lactamase superfamily I; MFP: Membrane fusion protein; IM: Inner membrane; OM: Outer membrane

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for Publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this article and its supplementary information files.

Competing interests

The authors declare no competing interests.

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Authors' contributions

DXS designed and organized this study, revised the manuscript, CY performed most of the experiments, analyzed the sequence and wrote the manuscript, ZL collected strains, and performed the antimicrobial susceptibility testing, SY analyzed the sequence, KY, XL assembled and annotated the DNA short reads. All authors have read and approved the final manuscript.

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References

1. Loch TP, Faisal M. Emerging *flavobacterial* infections in fish: A review. J Adv Res. 2015;6(3):283-300.

2. Breurec S, Criscuolo A, Diancourt L, et al. Genomic epidemiology and global diversity of the emerging bacterial pathogen *Elizabethkingia anophelis*. Sci Rep. 2016;6:30379.
3. Lau SK, Chow WN, Foo CH, et al. *Elizabethkingia anophelis* bacteremia is associated with clinically significant infections and high mortality. Sci Rep. 2016;6:26045.
4. Perrin A, Larsonneur E, Nicholson AC, et al. Evolutionary dynamics and genomic features of the *Elizabethkingia anophelis* 2015 to 2016 Wisconsin outbreak strain. Nat Commun. 2017;8:15483.
5. Lin JN, Lai CH, Yang CH, Huang YH, Lin HH. Clinical manifestations, molecular characteristics, antimicrobial susceptibility patterns and contributions of target gene mutation to fluoroquinolone resistance in *Elizabethkingia anophelis*. J Antimicrob Chemother. 2018;73(9):2497-2502.
6. Snesrud E, McGann P, Walsh E, et al. Clinical and Genomic Features of the First Cases of *Elizabethkingia anophelis* Infection in New York, Including the First Case in a Healthy Infant Without Previous Nosocomial Exposure. J Pediatric Infect Dis Soc. 2019;8(3):269-271.
7. Teo J, Tan SY, Tay M, et al. First case of *E anophelis* outbreak in an intensive-care unit. Lancet. 2013;382(9895):855-856.
8. Navon L, Clegg WJ, Morgan J, et al. Notes from the Field: Investigation of *Elizabethkingia anophelis* Cluster - Illinois, 2014-2016. MMWR Morb Mortal Wkly Rep. 2016;65(48):1380-1381.
9. KING EO. Studies on a group of previously unclassified bacteria associated with meningitis in infants. Am J Clin Pathol. 1959;31(3):241-247.
10. Kim KK, Kim MK, Lim JH, Park HY, Lee ST. Transfer of Chryseobacterium meningosepticum and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. Int J Syst Evol Microbiol. 2005;55(Pt 3):1287-1293.
11. Kämpfer P, Matthews H, Glaeser SP, Martin K, Lodders N, Faye I. Elizabethkingia anophelis sp. nov., isolated from the midgut of the mosquito Anopheles gambiae [published correction appears in Int J Syst Evol Microbiol. 2012 Apr;62(Pt 4):1016]. Int J Syst Evol Microbiol. 2011;61(Pt 11):2670-2675.
12. Nicholson AC, Gulvik CA, Whitney AM, 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* nov., *Elizabethkingia ursingii* sp. nov., and *Elizabethkingia occulta* sp. nov. Antonie Van Leeuwenhoek. 2018;111(1):55-72.
13. Moore LS, Owens DS, Jepson A, et al. Waterborne *Elizabethkingia meningoseptica* in Adult Critical Care. Emerg Infect Dis. 2016;22(1):9-17.
14. Hsu MS, Liao CH, Huang YT, et al. Clinical features, antimicrobial susceptibilities, and outcomes of *Elizabethkingia meningoseptica* (*Chryseobacterium meningosepticum*) bacteremia at a medical center in Taiwan, 1999-2006. Eur J Clin Microbiol Infect Dis. 2011;30(10):1271-1278.
15. Jean SS, Lee WS, Chen FL, Ou TY, Hsueh PR. *Elizabethkingia meningoseptica*: an important emerging pathogen causing healthcare-associated infections. J Hosp Infect. 2014;86(4):244-249.
16. Liang CY, Yang CH, Lai CH, Huang YH, Lin JN. Comparative Genomics of 86 Whole-Genome Sequences in the Six Species of the *Elizabethkingia Genus* Reveals Intraspecific and Interspecific Divergence. Sci Rep. 2019;9(1):19167.

17. Liu Z, Wang LF, Ye LY, Shen DX. Analysis of drug resistance and homology of *Elizabethkingia meningoseptica*. Acad J Clin PLA Med Sch. 2020;41(3). (in Chinese)
18. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol. 2007;57(Pt 1):81-91.
19. Lin JN, Lai CH, Yang CH, Huang YH. Comparison of Clinical Manifestations, Antimicrobial Susceptibility Patterns, and Mutations of Fluoroquinolone Target Genes between *Elizabethkingia meningoseptica* and *Elizabethkingia anophelis* Isolated in Taiwan. J Clin Med. 2018;7(12):538.
20. Han MS, Kim H, Lee Y, et al. Relative Prevalence and Antimicrobial Susceptibility of Clinical Isolates of *Elizabethkingia* Species Based on 16S rRNA Gene Sequencing. J Clin Microbiol. 2016;55(1):274-280.
21. Tai IC, Liu TP, Chen YJ, Lien RI, Lee CY, Huang YC. Outbreak of *Elizabethkingia meningoseptica* sepsis with meningitis in a well-baby nursery. J Hosp Infect. 2017;96(2):168-171.
22. Wailan AM, Paterson DL, Kennedy K, Ingram PR, Bursle E, Sidjabat HE. Genomic Characteristics of NDM-Producing *Enterobacteriaceae* Isolates in Australia and Their blaNDM Genetic Contexts. Antimicrob Agents Chemother. 2015;60(1):136-141.
23. Santona A, Paglietti B, Al-Qahtani AA, et al. Novel type of VanB2 teicoplanin-resistant hospital-associated *Enterococcus faecium*. Int J Antimicrob Agents. 2014;44(2):156-159.
24. Chen S, Soehnlen M, Blom J, Terrapon N, Henrissat B, Walker ED. Comparative genomic analyses reveal diverse virulence factors and antimicrobial resistance mechanisms in clinical *Elizabethkingia meningoseptica* strains. PLoS One. 2019;14(10):e0222648.
25. Janda JM, Lopez DL. Mini review: New pathogen profiles: *Elizabethkingia anophelis*. Diagn Microbiol Infect Dis. 2017;88(2):201-205.
26. Jacobs A, Chenia HY. Biofilm formation and adherence characteristics of an *Elizabethkingia meningoseptica* isolate from Oreochromis mossambicus. Ann Clin Microbiol Antimicrob. 2011;10:16.
27. Teo J, Tan SY, Liu Y, et al. Comparative genomic analysis of malaria mosquito vector-associated novel pathogen *Elizabethkingia anophelis*. Genome Biol Evol. 2014;6(5):1158-1165.
28. Tekaia F. Inferring Orthologs: Open Questions and Perspectives. Genomics Insights. 2016;9:17-28.
29. Yang X, Wang J, Bing G, et al. Ortholog-based screening and identification of genes related to intracellular survival. Gene. 2018;651:134-142.
30. Tettelin H, Masignani V, Cieslewicz MJ, et al. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial "pan-genome". Proc Natl Acad Sci U S A. 2005;102(39):13950-13955.
31. Huang YC, Lin YT, Wang FD. Comparison of the therapeutic efficacy of fluoroquinolone and non-fluoroquinolone treatment in patients with *Elizabethkingia meningoseptica* bacteraemia. Int J Antimicrob Agents. 2018;51(1):47-51.
32. Huang YC, Huang YW, Lin YT, Wang FD, Chan YJ, Yang TC. Risk factors and outcome of levofloxacin-resistant *Elizabethkingia meningoseptica* bacteraemia in adult patients in Taiwan. Eur J Clin Microbiol Infect Dis. 2017;36(8):1373-1380.

33. Honaas LA, Wafula EK, Wickett NJ, et al. Selecting Superior De Novo Transcriptome Assemblies: Lessons Learned by Leveraging the Best Plant Genome. PLoS One. 2016;11(1):e0146062.
34. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics. 2013;14:60.
35. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA. 2009;106(45):19126-19131.
36. Tatusova T, DiCuccio M, Badretdin A, et al. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 2016;44(14):6614-6624.
37. Aziz RK, Bartels D, Best AA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75.
38. Overbeek R, Olson R, Pusch GD, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014;42(Database issue):D206-D214.
39. Liu B, Pop M. ARDB–Antibiotic Resistance Genes Database. Nucleic Acids Res. 2009;37(Database issue):D443-D447.
40. 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. 2016;44(D1):D694-D697.
41. Chaudhari NM, Gupta VK, Dutta C. BPGA- an ultra-fast pan-genome analysis pipeline. Sci Rep. 2016;6:24373.

Figures

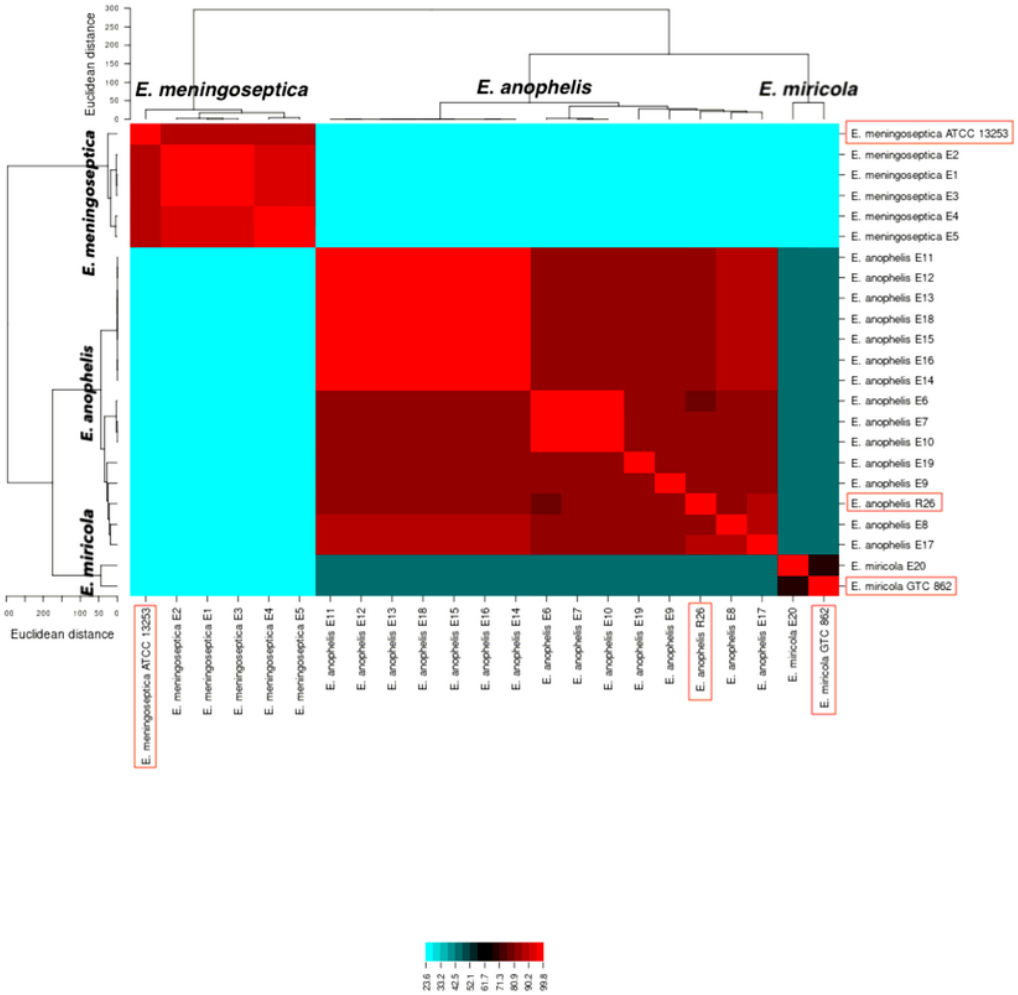

Figure 1

Comparison of general genomic features among Elizabethkingia species Notes of figure 1: Red rectangles represented the reference strains of E. meningoseptica ATCC 13253T, E. anophelis R26T and E. miricola GTC 862T.

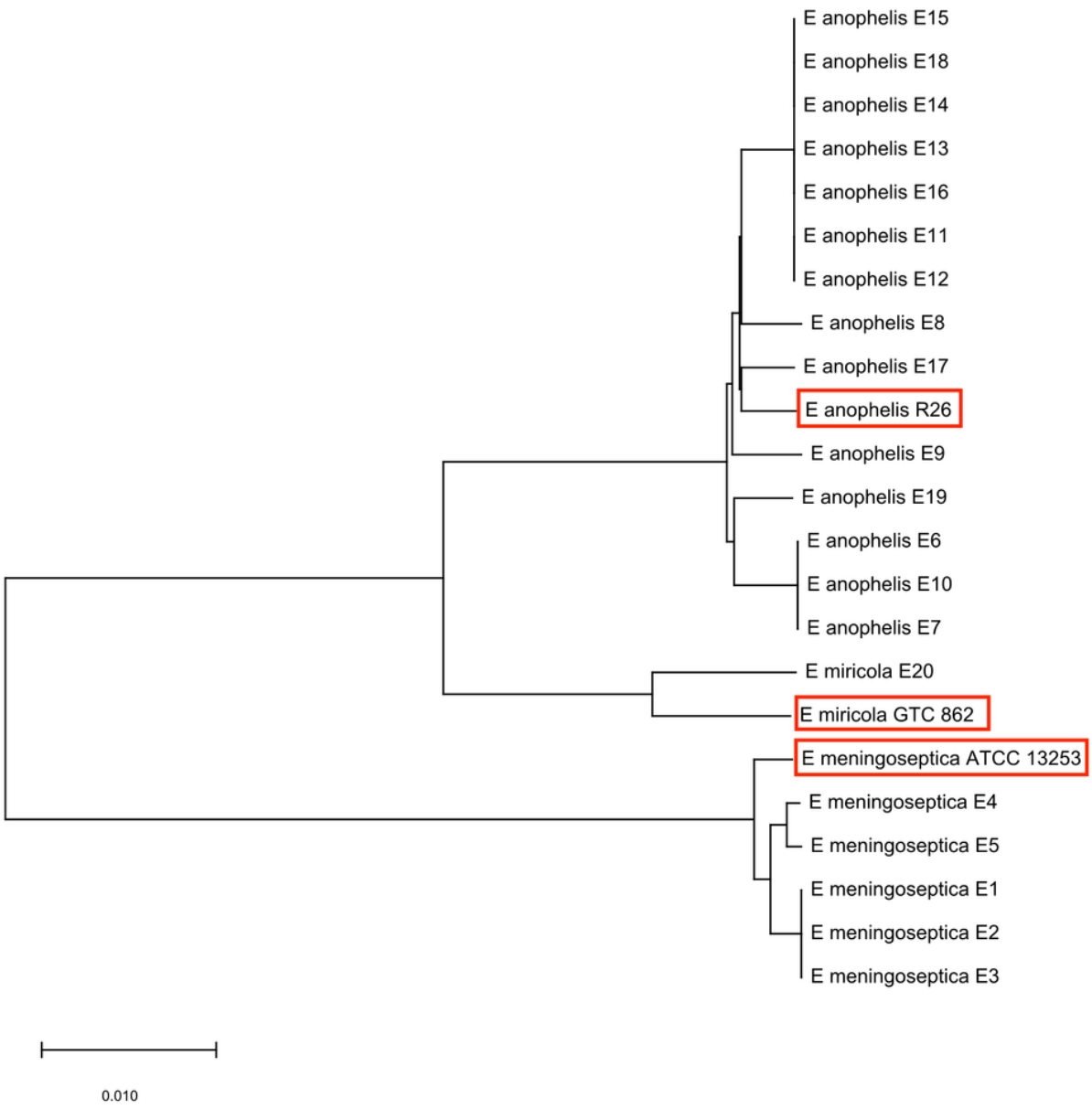

Figure 2

The whole-genome sequence-based phylogenetic tree of the 20 Elizabethkingia isolates Notes of figure 2: Red rectangles represented the reference strains of E. meningoseptica ATCC 13253T, E. anophelis R26T and E. miricola GTC 862T.

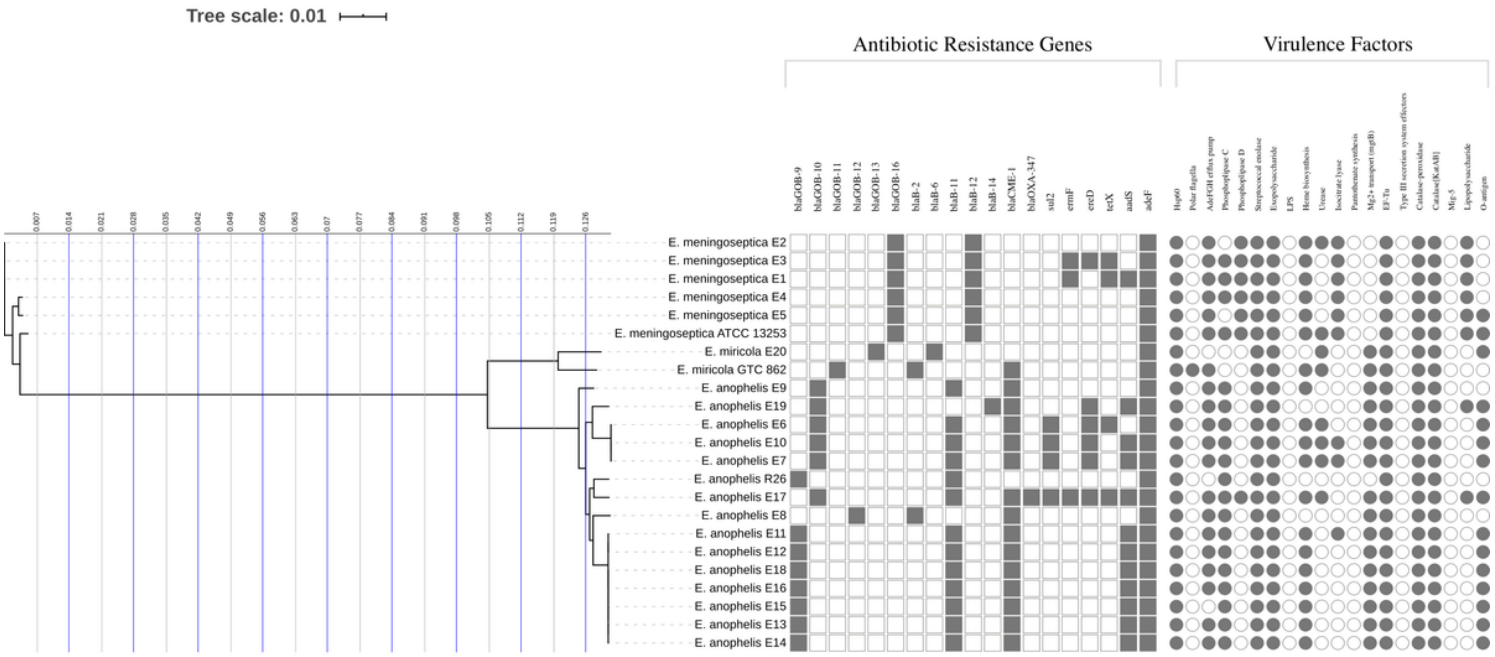

Figure 3

The phylogenetic distance, antibiotic resistance genes and virulence factors based on whole-genome sequence Notes of figure 3: Black or white square represented with or without antibiotic resistance genes; Black or white circle represented with or without virulence factors

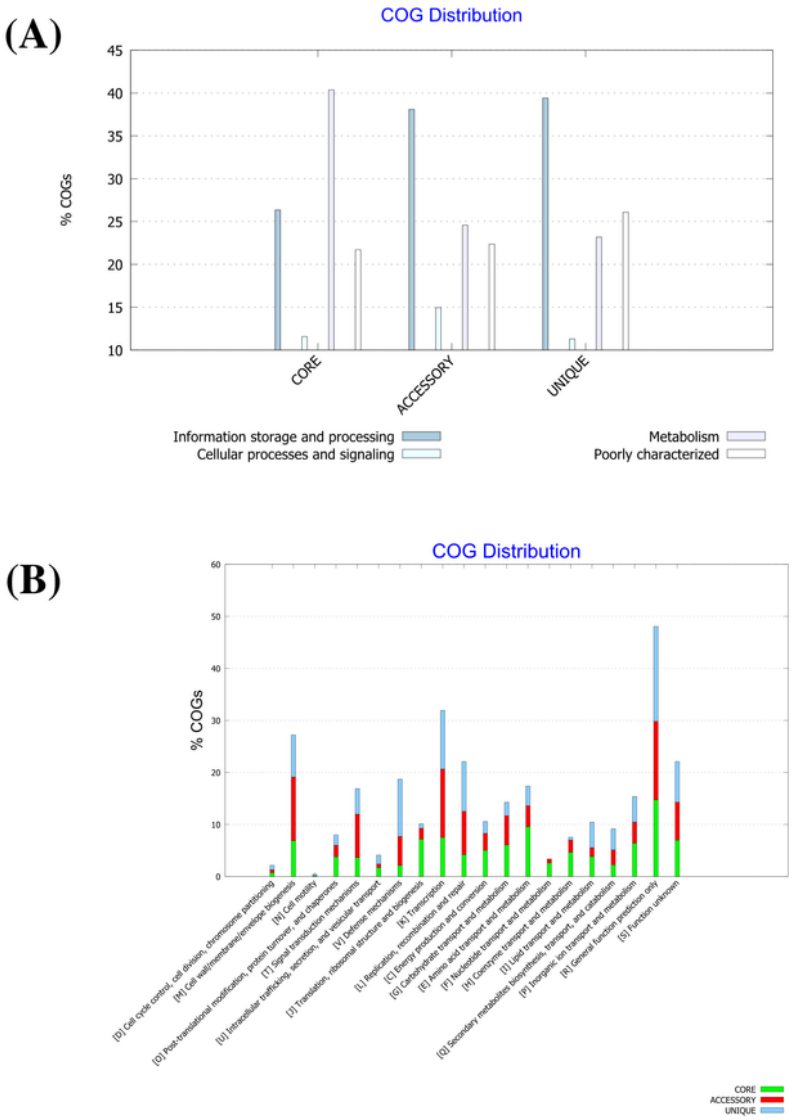

Figure 4

Functional analysis of the COGs in genomes of the 20 Elizabethkingia (A): Distribution of functional COGs in core, accessory and unique genome; (B): Detailed distribution of COGs with their functions

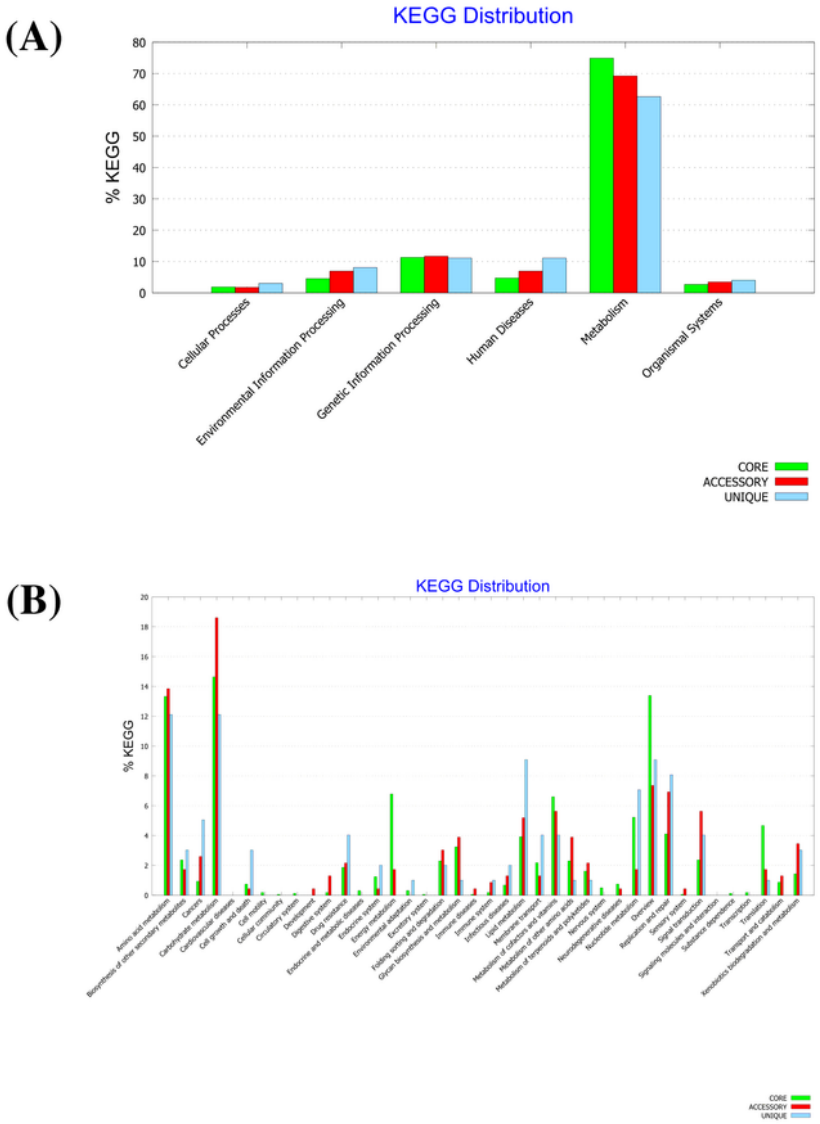

Figure 5

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the 20 Elizabethkingia strains (A): Distribution of functional KEGG in core, accessory and unique genome; (B): Detailed distribution of KEGG with their functions

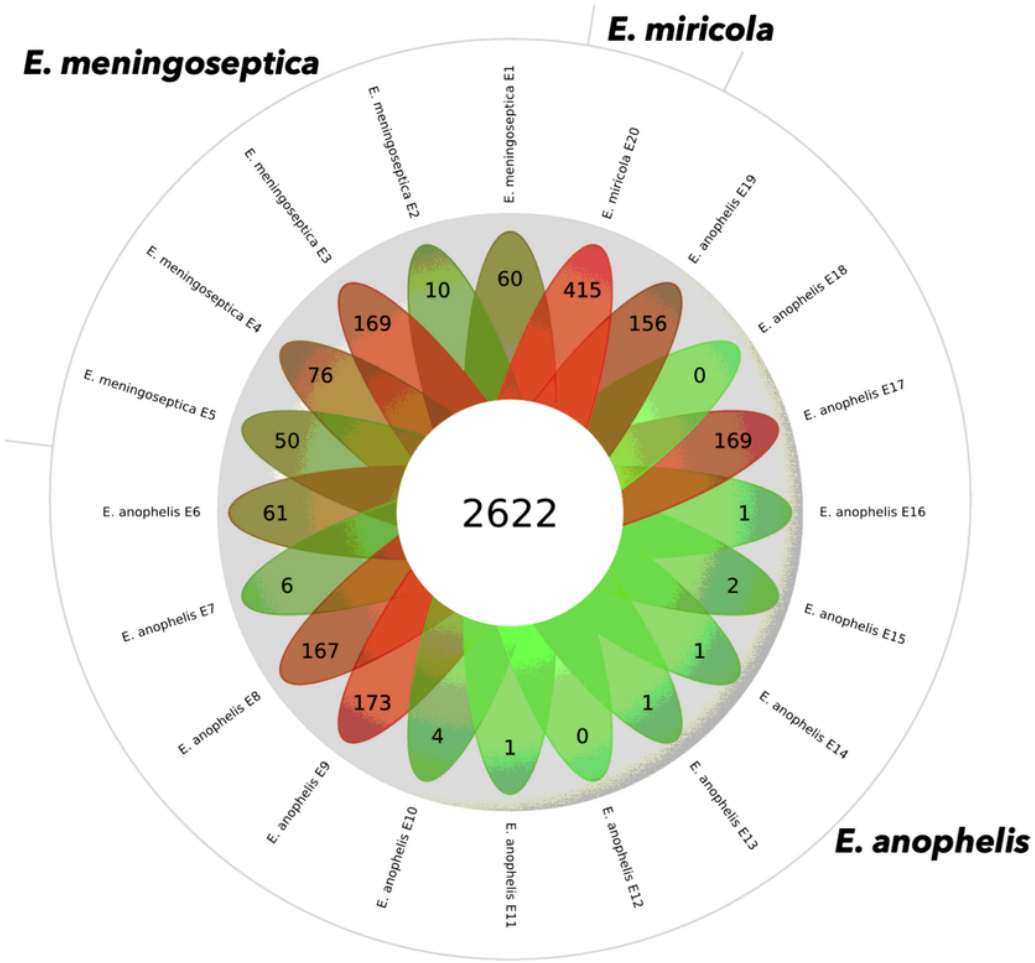

Figure 6

Flower plot of core and pan genome analysis for 20 Elizabethkingia strains

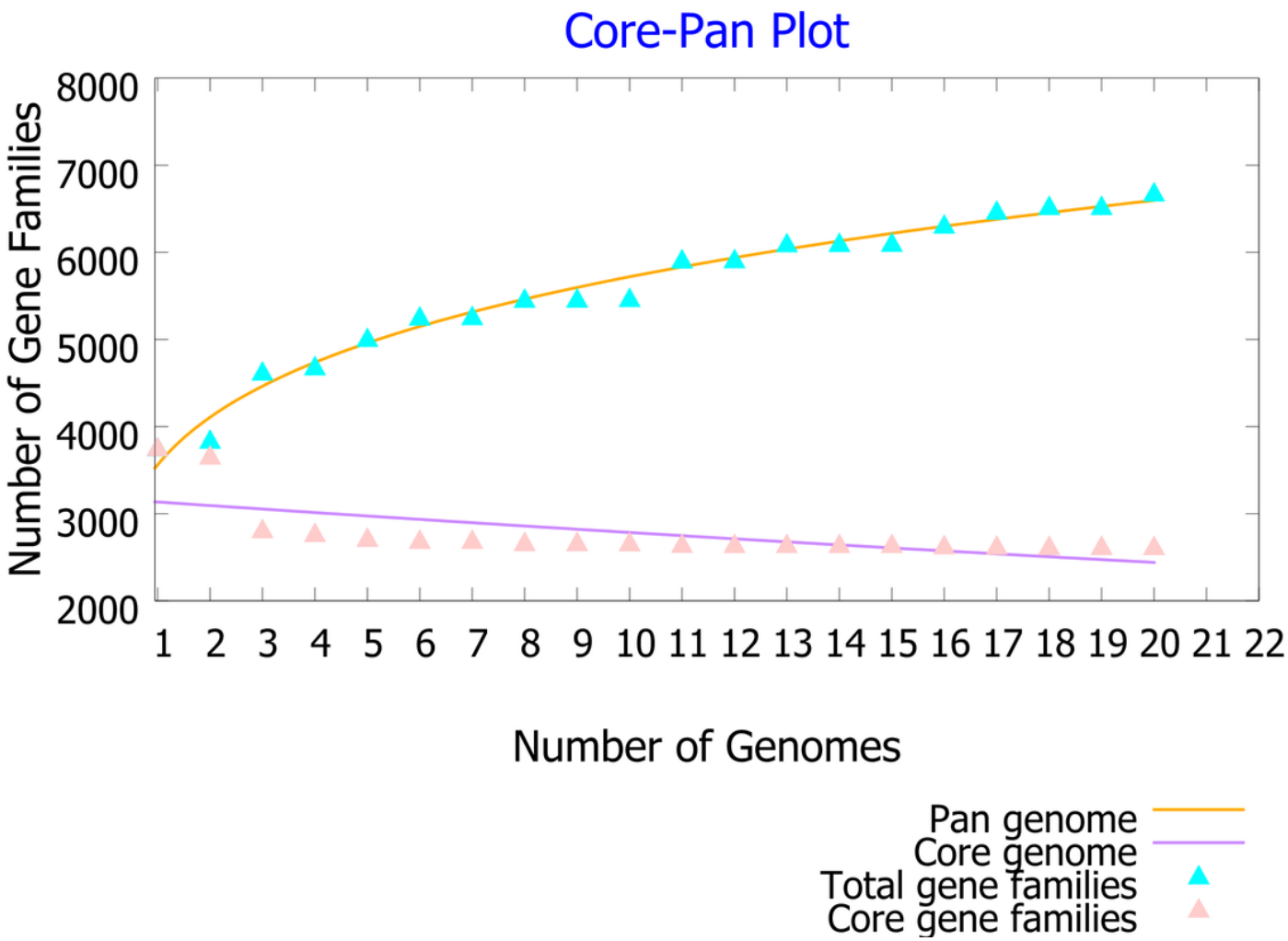

Figure 7

Core-pan plot of the 20 Elizabethkingia strains

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- WholegenomesequenceofE.miricolaE20assembled.txt
- WholegenomesequenceofE.miricolaE20assembled.txt
- WholegenomesequenceofE.miricolaE20assembled.txt
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