Comparative Genomic Analysis of Malaria Mosquito Vector-Associated Novel Pathogen *Elizabethkingia anophelis*

Jeanette Teo[1,†], Sean Yang-Yi Tan[2,3,†], Yang Liu[2], Martin Tay[2,3], Yichen Ding[2], Yingying Li[3], Staffan Kjelleberg[2,4], Michael Givskov[2,5], Raymond T.P. Lin[1], and Liang Yang[2,3,*]

[1]Department of Laboratory Medicine, Microbiology Unit, National University Hospital, Singapore

[2]Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, Singapore

[3]School of Biological Sciences, Nanyang Technological University, Singapore

[4]Centre for Marine Bio-Innovation and School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, Australia

[5]Costerton Biofilm Center, Department of International Health, Immunology and Microbiology, Panum Institute, University of Copenhagen, Denmark

*Corresponding author: E-mail: yangliang@ntu.edu.sg.

[†]These authors contributed equally to this work.

Accepted: April 30, 2014

Data deposition: This project has been deposited at DDBJ/EMBL/GenBank as individual WGS bioprojects, with the following accession numbers: NUHP1 (ASYE00000000), NUHP2 (ASYF00000000), NUHP3 (ASYG00000000), NUH1 (ASYH00000000), NUH4 (ASYI00000000), NUH6 (ASYJ00000000), NUH11 (ASYK00000000).

Abstract

Acquisition of *Elizabethkingia* infections in intensive care units (ICUs) has risen in the past decade. Treatment of *Elizabethkingia* infections is challenging due to the lack of effective therapeutic regimens, leading to a high mortality rate. *Elizabethkingia* infections have long been attributed to *Elizabethkingia meningoseptica*. Recently, we used whole-genome sequencing to reveal that *E. anophelis* is the pathogenic agent for an *Elizabethkingia* outbreak at two ICUs. We performed comparative genomic analysis of seven hospital-isolated *E. anophelis* strains with five available *Elizabethkingia* spp. genomes deposited in the National Center for Biotechnology Information Database. A pan-genomic approach was applied to identify the core- and pan-genome for the *Elizabethkingia* genus. We showed that unlike the hospital-isolated pathogen *E. meningoseptica* ATCC 12535 strain, the hospital-isolated *E. anophelis* strains have genome content and organization similar to the *E. anophelis* Ag1 and R26 strains isolated from the midgut microbiota of the malaria mosquito vector *Anopheles gambiae*. Both the core- and accessory genomes of *Elizabethkingia* spp. possess genes conferring antibiotic resistance and virulence. Our study highlights that *E. anophelis* is an emerging bacterial pathogen for hospital environments.

Key words: *Elizabethkingia*, comparative genomics, pan/core-genomes.

Introduction

Elizabethkingia is a genus of aerobic, nonmotile, Gram-negative rods that is ubiquitous in nature. Members of this genus thrive in wet habitats and hospital settings, in particular water supplies and saline flushing solutions. Among the *Elizabethkingia* spp., the species *Elizabethkingia meningoseptica* is well established as a serious causative agent of neonatal meningitis and sepsis (Dooley et al. 1980), and a notable rise in *E. meningoseptica* nosocomial infections has been recorded in recent years. Treatment of *E. meningoseptica* infections is notoriously difficult, and there is a lack of effective specific therapeutic regimens (Hsu et al. 2011). The mortality rate of nosocomial infections caused by *E. meningoseptica* can reach as high as 52% in neonates (Bloch et al. 1997) and ranges from 23% (Teres 1974) to 33% (Bloch et al. 1997) in nonneonates. Hence, the acquisition of *E. meningoseptica* in intensive care units (ICUs) is used as a significant predictor of mortality (Teres 1974). Except for *E. meningoseptica*, it is rarely reported that other *Elizabethkingia* species can cause infections although *E. miricola* has been reported to be associated with sepsis (Green et al. 2008).

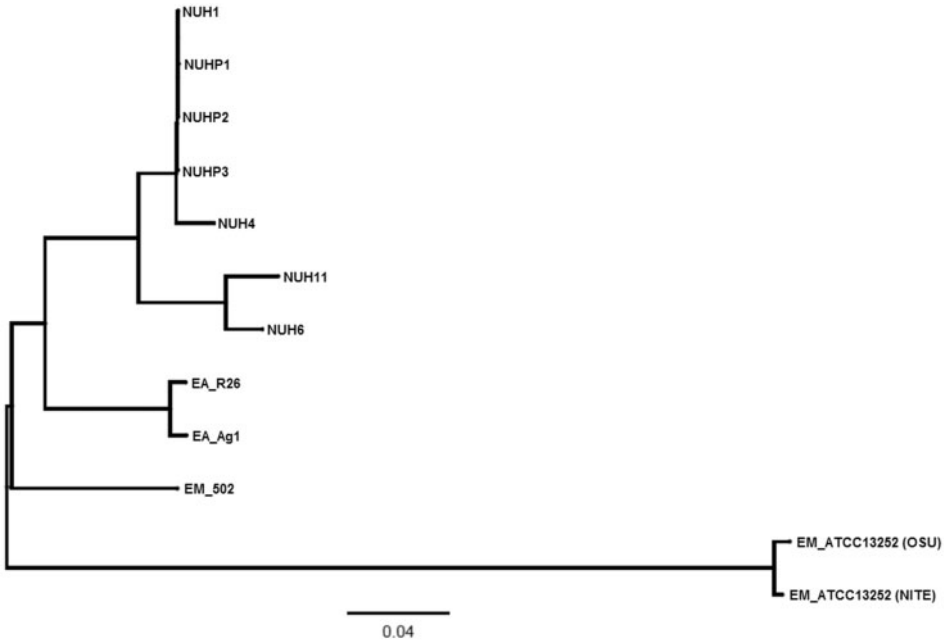

Fig. 1.—A phylogenetic tree showing the 12 *Elizabethkingia* spp. This phylogenetic tree was produced by pair-wise genome comparisons by Progressive Mauve.

Strains	NUH11	NUHP3	EM_502	NUHP2	EM_13253_OSU	EM_13253_NITE	NUH1	NUH4	NUHP1	EA_Ag1	EA_R26	NUH6
NUH11	0	20250	25450	20250	27815	27847	20248	20382	20239	24497	24555	365
NUHP3	20250	0	22306	24	8119	8151	38	178	29	21290	21361	20186
EM_502	25450	22306	0	22306	29825	29857	22316	22430	22285	26161	26229	25469
NUHP2	20250	24	22306	0	8119	8151	42	180	29	21288	21361	20186
EM_13253_OSU	27815	8119	29825	8119	0	46	8127	8264	8092	28843	28916	27749
EM_13253_NITE	27847	8151	29857	8151	46	0	8159	8296	8124	28873	28946	27781
NUH1	20248	38	22316	42	8127	8159	0	178	37	21298	21369	20184
NUH4	20382	178	22430	180	8264	8296	178	0	176	21414	21485	20316
NUHP1	20239	29	22285	29	8092	8124	37	176	0	21269	21342	20175
EA_Ag1	24497	21290	26161	21288	28843	28873	21298	21414	21269	0	188	24536
EA_R26	24555	21361	26229	21361	28916	28946	21369	21485	21342	188	0	24600
NUH6	365	20186	25469	20186	27749	27781	20184	20316	20175	24536	24600	0

Fig. 2.—SNP distance matrix among the 12 *Elizabethkingia* spp. SNP difference between each pair of *Elizabethkingia* spp. was calculated by using the snpTree web server.

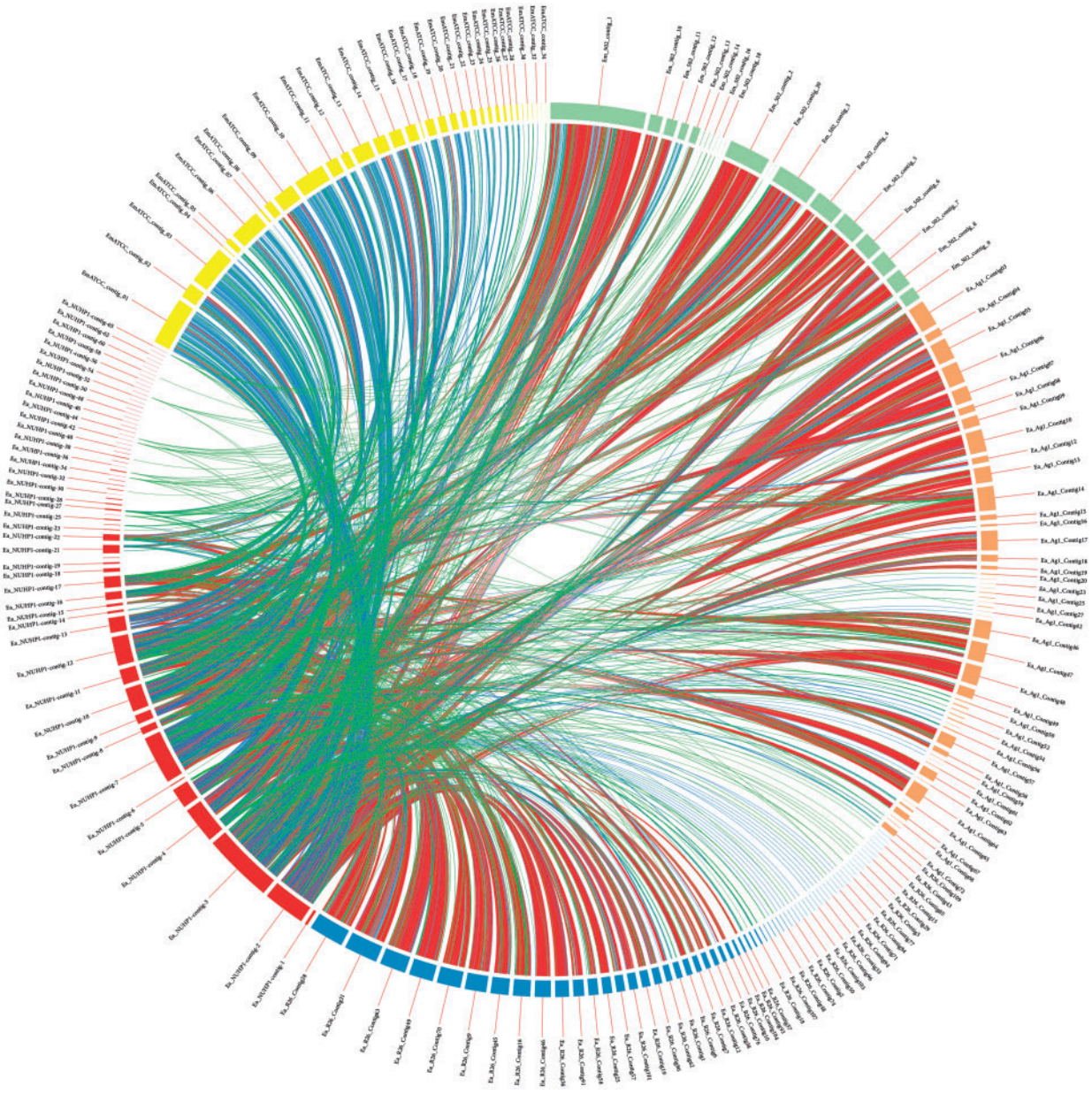

Fig. 3.—Sequence comparison by alignment. The *Elizabethkingia anophelis* NUHP1 strain (red arc) was aligned against: *E. anophelis* R26 strain (blue arc), *E. anophelis* Ag1 (orange arc), *E. meningoseptica* ATCC 12535 (NITE) (yellow arc), and *E. meningoseptica* 502 (light green arc). Colored links between contigs represent homologous regions spanning: 10^2–10^3 bp (green), 10^3–10^4 bp (blue), and above 10^4 bp (red). The presence of red links between the NUHP1 strain and *E. anophelis* R26, Ag1 contigs, and *E. meningoseptica* 502 contigs indicate a high degree of similarity between these genomes.

The limited genomic information available for *Elizabethkingia* hinders our understanding of the virulence mechanisms and molecular epidemiology of its member species. Recently, we used whole-genome sequencing to investigate an *Elizabethkingia* outbreak in two ICUs at the National University Hospital, Singapore (Balm et al. 2013). All the patient-associated *Elizabethkingia* strains and hand hygiene sink aerator-associated *Elizabethkingia* strains were isolated within a 1-month period at the ICUs. We found the outbreak agent to be a novel species—*E. anophelis* (Teo et al. 2013), usually found in the midgut microbiota of the malaria mosquito vector *Anopheles gambiae*. Here, we report comparative genomic analysis on the *Elizabethkingia* spp. to investigate their mechanism for virulence, stress response, and niche adaptation.

General Characteristics of the Outbreak *E. anophelis* Strains

The general genomic characteristics of the seven *Elizabethkingia* spp. strains derived from this study, three patient (NUHP1, NUHP2, and NUHP3) and four sink isolates (NUH1, NUH4, NUH6, and NUH11), obtained from the RAST server (Aziz et al. 2008) are presented in supplementary table S1, Supplementary Material online.

The Whole-Genome Shotgun (WGS) sequences of five previously sequenced *E. anophelis* strains Ag1 (Bioproject accession number, PRJNA80705) and R26 (Bioproject accession number, PRJNA178189), *E. meningoseptica* ATCC 12535 (NITE) (Bioproject accession number, PRJNA199489), *E. meningoseptica* ATCC 12535 (OSU) (Bioproject accession number, PRJNA198814), and *E. meningoseptica* 502 (Bioproject accession number, PRJNA176121) were also submitted to the RAST server; supplementary table S2, Supplementary Material online, shows the general characteristics of these strains. The genome sizes and GC content of the hospital-isolated strains are similar to the other *E. anophelis* strains and one of the *E. meningoseptica* strains (502) (supplementary tables S1 and S2, Supplementary Material online).

Comparative Genomic Analysis

Multiple genome alignment was performed by using Progressive Mauve (Darling et al. 2010) to compare all the genomes of the *Elizabethkingia* spp. The phylogenetic tree based on the multiple genome alignment showed that *E. anophelis* and *E. meningoseptica* genomes belong to distinct groups (fig. 1). Single-nucleotide polymorphism (SNP) differences between each pair of *Elizabethkingia* spp. was calculated using the snpTree web server (Leekitcharoenphon et al. 2012) and shown in figure 2. These results showed that the genomes of the patient isolates from the current outbreak (NUHP1, NUHP2, and NUHP3) were very similar to each other, NUH1 and NUH4, which suggests that the *E. anophelis* strains NUHP1, NUHP2, NUHP3, NUH1, and NUH4 may be clonal. The NUH6 and NUH11 strains may exist as a separate clone as they were found to have high-genomic similarity to each other but not to the other hospital isolates. The *E. anophelis* Ag1 and R26 strains formed a distinct group in the phylogenetic tree. Also, the *E. meningoseptica* ATCC 12535 (NITE) and ATCC 12535 (OSE) strains formed a distinct group in the phylogenetic tree, whereas the *E. meningoseptica* 502 formed yet another distinct group.

To reach a high-resolution comparison, multiple whole-genome sequence alignments were performed with CIRCOS v0.64 (Krzywinski et al. 2009), using the *E. anophelis* NUHP1 strain, *E. anophelis* Ag1 and R26 strains, and the *E. meningoseptica* ATCC 12535 (NITE) and 502 strains (fig. 3). The red links show a high number of large homologous regions (longer than 10,000 nucleotides) between *E. anophelis* NUHP1 strain and the *E. anophelis* Ag1 strain, *E. anophelis* R26 strain, and the *E. meningoseptica* 502 strain. The genome of the *E. meningoseptica* ATCC 12535 (NITE) is less close to the *E. anophelis* NUHP1 (fig. 3). This result correlates well with the phylogenetic tree result (fig. 1) and GC content (supplementary tables S1 and S2, Supplementary Material online) of the genomes of these *Elizabethkingia* spp.

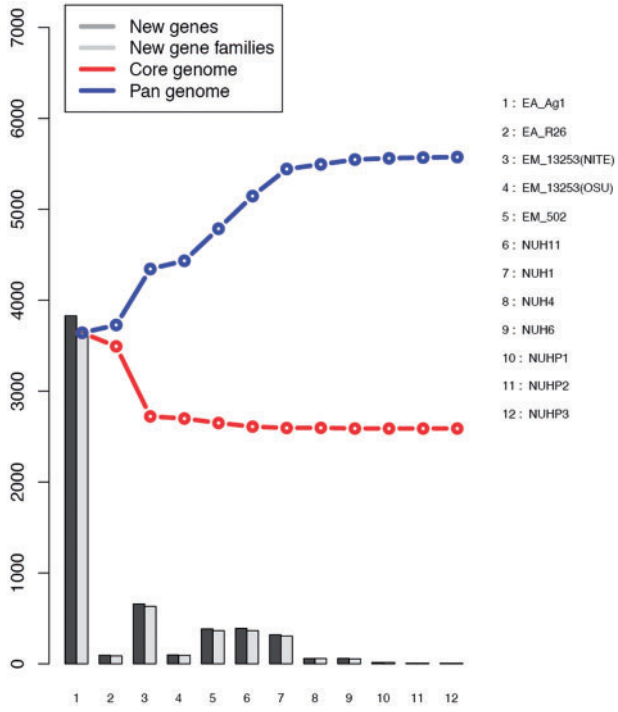

Fig. 4.—Curves for the core-genomes, pan-genomes, and numbers of new genes of the 12 *Elizabethkingia* spp.

Core/Pan-Genomic Analysis of *Elizabethkingia* spp.

We performed core/pan-genomic analysis of the *Elizabethkingia* spp. The results of all permutations of the order of addition for each of the 12 genomes are presented in figure 4. As expected, the number of genes in the core-genome initially decreases and that of the pan-genome initially increases with addition of each new genome sequence. Extrapolation of the curve indicates that the core-genome reaches a minimum of 2,589 "core" genes (fig. 4). In accordance with the core-genome size, the pan-genome reaches a maximum of 5,575 genes in total across 12 genomes (fig. 4).

Resistance and Virulence Profile of the *Elizabethkingia* spp.

The core/accessory genomes were searched against the Comprehensive Antibiotic Resistance Database (McArthur et al. 2013) and Virulence Factors of Pathogenic bacteria Database (VFDB) (Chen et al. 2012) to identify antibiotic resistant genes and virulence genes. Thirty percentage identity and

Table 1

Proteins Involved in Antibiotic Resistance Encoded from Core- and Accessory Genomes of the *Elizabethkingia* spp. Identified by BLAST Search against the Comprehensive Antibiotic Resistance Database

Database ID	% Identity	Antibiotic Resistance	Annotation
Core genome			
(AGly)ApmA:FN806789:2858–3682:822	44.71	Aminoglycosides	Aminocyclitol acetyltransferase. Confers apramycin resistance.
(Bla)AIM-1:AM998375:1173–2084:912	30.88	Beta-lactamases	Metallo-beta-lactamase AIM-1. Imipenemase.
(Bla)B-1:AF189298:1–750:750	94.35	Beta-lactamases	*Chryseobacterium meningosepticum* PINT class B carbapenemase BlaB-1 gene. *Elizabethkingia meningoseptica* class B carbapenemase BlaB-1
(Bla)Beta-lactamase_class-A:NC_010410:1803480–1804499:1020	35.09	Beta-lactamases	Beta-lactamase_class-A
(Bla)SFO-1:FJ848785:4719–5594:876	32.5	Beta-lactamases	AmpR of SFO-1; activator of ampA.
(Flq)OqxA:EU370913:46652–47827:1176	30.71	Fluoroquinolones	OqxA membrane-fusion protein. component of RND-type multidrug efflux pump that confers resistance to olaquindox
(Flq)OqxBgb:EU370913:47851–51003:3153	39.96	Fluoroquinolones	OqxB integral membrane protein. component of RND-type multidrug efflux pump that confers resistance to olaquindox
(Gly)VanH:DQ246438:124–1179:1056	33.65	Glycopeptides	VanH, D-lactate dehydrogenase.
(Gly)VanR-A:M97297:3976–4671:696	32.33	Glycopeptides	VanR.
(Gly)VanR-B:AY655721:69–728:660	34.15	Glycopeptides	VanRB, regulator protein.
(Gly)VanT:AF162694:3008–5104:2097	32.25	Glycopeptides	Serine racemase VanT. converts L-serine to D-serine; involved in vancomycin resistance
(Gly)VanW-B:AY655721:3069–3896:828	33.57	Glycopeptides	VanW.
(MLS)CarA:M80346:411–2066:1656	41.54	Macrolide-lincosamide-streptogramin	CarA, carbomycin resistance protein.
(MLS)CfrA:AM408573:10028–11077:1050	31.9	Macrolide-lincosamide-streptogramin	Cfr, rRNA methylase, mediates the PhLOPSA resistance phenotype
(Tet)otrA:X53401:349–2341:1992	43.66	Tetracyclines	OtrA, oxytetracycline resistance
(Tmt)Dfr16:AF077008:115–558:474	34.03	Trimethoprim	DHFRXVI, trimethoprim resistant dihydrofolate reductase
Accessory genome			
(AGly)Aac3-Ig:CP000282:2333620–2334096:477	33.56	Aminoglycosides	Sde_1840,Gentamicin 3′-N-acetyltransferase
(AGly)Aac6:DQ302723:81–482:402	31.09	Aminoglycosides	Aac(6′), aminoglycoside-6′-N-acetyltransferase
(Bla)ACC-3:AF180957:1–1131:1131	30.56	Beta-lactamases	ACC-3, AMPC cephalosporinase precursor protein ACC-3
(Bla)IND-7:AB529520:1–720:720	72.73	Beta-lactamases	BlaIND-7, metallo-beta-lactamase IND-7
(Bla)OCH-7:AJ295345:1–1173:1173	30.15	Beta-lactamases	Bla OCH-7, beta-lactams hydrolysis
(Flq)OqxBgb:EU370913:47851–51003:3153	31.64	Fluoroquinolones	pOLA52_67, OqxB integral membrane protein, component of RND-type multidrug efflux pump that confers resistance to olaquindox
(Gly)VanRc3:AY033764:3846–4541:696	30.34	Glycopeptides	VanRc3
(Gly)VanR-M:FJ349556:982–1680:699	32.35	Glycopeptides	Van operon, response regulator
(MLS)CarA:M80346:411–2066:1656	34.88	Macrolide-lincosamide-streptogramin	Carbomycin resistance protein (carA)
(MLS)LmrA:X59926:318–1763:1446	30.43	Macrolide-lincosamide-streptogramin	LmrA gene for lincomycin resistance protein

(continued)

Table 1 Continued

Database ID	% Identity	Antibiotic Resistance	Annotation
(MLS)MefB:FJ196385:11084–12313:1230	36.18	Macrolide-lincosamide-streptogramin	Macrolide efflux pump.
(MLS)MsrE:JF769133:7246–8721:1476	36.96	Macrolide-lincosamide-streptogramin	Macrolide efflux protein.
(MLS)OleB:L36601:1421–3130:1710	37.04	Macrolide-lincosamide-streptogramin	ATP-binding protein. oleandomycin resistance and secretion
(MLS)TlrC:M57437:277–1923:1647	30.77	Macrolide-lincosamide-streptogramin	Tylosin resistance protein (tlrC) gene
(MLS)VatF:AF170730:70–735:666	31.21	Macrolide-lincosamide-streptogramin	Streptogramin A acetyl transferase (sat) gene. Confers resistance to class A streptogramins
(MLS)vgaa-LC:DQ823382:1–1569:1569	31.11	Macrolide-lincosamide-streptogramin	Lincosamide-streptogramin A resistance protein (vga(A)LC) gene
(Rif)Arr7:FN397623:1189–1641:453	41.73	Rifampicin	ADP-ribosyltransferase. Resistance to rifampin.
(Tet)OtrB:AF079900:40–1733:1692	30.27	Tetracyclines	Tetracycline efflux protein (otrB) gene
(Tet)TetX:M37699:586–1752:1167	59.36	Tetracyclines	Transposon Tn4351 tetracycline resistance protein (tetX) gene

expectation value < 1e-5 were used as a threshold when performing the BLASTP searches because the genomes of *Elizabethkingia* spp. are very new and highly likely to not have been included in any of these databases before. Sixteen and 19 antibiotic-resistant genes were identified from the core- and accessory genomes of *Elizabethkingia* spp., respectively, which cover genes conferring resistance to aminoglycosides, beta-lactamases, fluoroquinolones, glycopeptides, macrolide-lincosamide-streptogramin, tetracyclines, trimethoprim, and rifampicin (table 1). These genes correlate with the reported antibiotic resistant profiles of *Elizabethkingia* spp. (Hsu et al. 2011). The patient-isolated NUHP1, NUHP2, and NUHP3 strains are resistant to tetracycline (minimum inhibitory concentration, MIC = 64 μg/ml), ciprofloxacin (MIC = 16 μg/ml), erythromycin (MIC > 512 μg/ml), ceftazidime (MIC = 64 μg/ml), tobramycin (MIC > 512 μg/ml), and vancomycin (MIC = 32 μg/ml). Some of the antibiotic resistance genes might be nonfunctional. A complete genome sequence is required for intact analysis of these resistance gene operons.

In our study, 146 and 70 virulence genes were identified from the core- and accessory genomes of *Elizabethkingia* spp., respectively, which include genes involved in lipopolysaccharide biosynthesis, iron siderophore synthesis, heme uptake, transposase synthesis, alginate synthesis, and so on (supplementary table S3, Supplementary Material online).

Functional Classification of Genes Only Belongs to *E. meningoseptica* or *E. anophelis*

To gain knowledge about the difference in metabolic capacity between *E. meningoseptica* and *E. anophelis*, we enriched genes that exist only in *E. meningoseptic*a but not in *E. anophelis* (referred to as EM only) and genes that exist only in *E. anophelis* but not in *E. meningoseptica* (EA only) based on BLAST analysis. There were 842 genes unique to the five *E. meningoseptica* genomes and 1,416 genes unique to the seven *E. anophelis* genomes. These unique genes were then classified according to their predicted functional role (fig. 5). When compared with the EA-only genes, the EM-only genes are enriched in predicted proteins belonging to Clusters of Orthologous Group (COG) category G (carbohydrate transport and metabolism), H (coenzyme transport and metabolism), I (lipid transport and metabolism), K (transcription), R (general function prediction only), S (function unknown), and T (signal transduction mechanisms) (fig. 5). Conversely, the EA-only genes are enriched in C (energy production and conversion), L (replication, recombination, and repair), and P (inorganic ion transport and metabolism) (fig. 5).

In conclusion, our study revealed the emergence of a novel pathogen *E. anophelis* in the hospital environment. *Elizabethkingia anophelis* is well known to be a dominant species in the gut microbiota of the malaria mosquito vector *A. gambiae* (Dong et al. 2009; Boissiere et al. 2012;

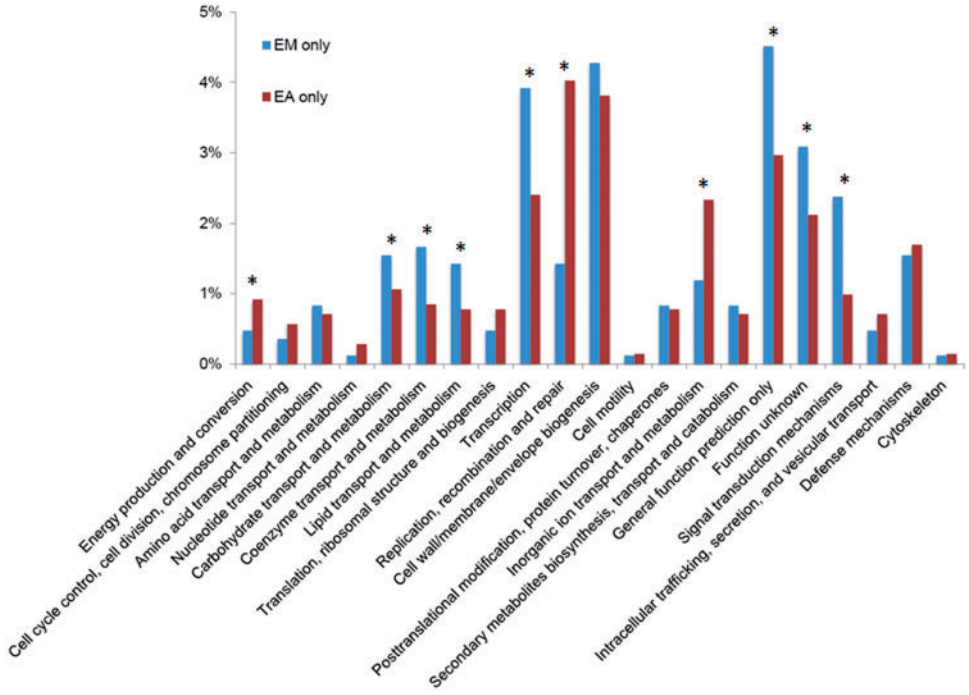

Fig. 5.—The identified EM-only and EA-only proteins were assigned to Clusters of Orthologous Groups (COGs). The *y* axis indicates the percentage of genes in a specific function cluster out of the total numbers of EM-only and EA-only proteins, respectively. *The abundances of specific function clusters were compared statistically as described in Rodriguez-Brito et al. (2006) and Allen et al. (2009) using a subsample size of 500 and 1,000 bootstrap replicates at a statistical confidence of 99%.

Osei-Poku et al. 2012). *Elizabethkingia* species in the midgut of the malaria vector may modulate the anti-*Plasmodium* effects of the host's immune genes, thus prolonging the life span of the *Plasmodium*-infected malaria mosquito vector (Dong et al. 2009). The genome content and organization of *E. anophelis* is similar to, yet distinct from the well-known *E. meningoseptica*, which often causes high mortality among hospital acquired infections. Our study suggests that the mosquito vector might be a potential mobile reservoir of antibiotic and virulence genes for emerging bacterial pathogens. However, we should notice that the core- and pan-genome analysis is based on only a small group of genomes. More genomes of the *Elizabethkingia* spp. are required for sophisticated comparative genomic analysis. Further studies will be carried out to comparatively investigate the pathogenesis mechanisms employed by *E. anophelis* and *E. meningoseptica* in causing human infections.

Materials and Methods

Ethics Statement

Ethical approval was not required for the study because it was done as part of surveillance and management of healthcare-associated infection.

Genome Sequencing, Assembly, and Comparative Genomic Analyses

The genomes of seven *Elizabethkingia* spp. strains: Three patient isolates (NUHP1, NUHP2, and NUHP3) and four environmental isolates (NUH1, NUH4, NUH6, and NUH11) were sequenced in this study. Whole-genome DNA of these *E. anophelis* strains were purified using QIAamp DNA Mini Kit (QIAgen) and sequenced on an Illumina MiSeq platform generating 150-bp-long paired-end reads. Reads were assembled into contigs using de novo assembly in CLCBio's Genomics Workbench NGS suite (CLCBio, version 6.0.3) with default settings. Average genomic coverages across the strains ranged from 111 to 160-fold. The assembled genomes were compared with the five available *Elizabethkingia* spp. genomes deposited in the National Center for Biotechnology Information (NCBI) Database (supplementary table S2, Supplementary Material online).

Multiple genome alignment was used to compare the genomes of the *Elizabethkingia* spp. by using Progressive Mauve with match seed weight 15, min Locally Collinear Block weight 45, minimum island size 50, maximum backbone gap size 50, and minimum backbone size 50 (Darling et al. 2010). Phylogenetic tree diagrams were prepared using the software FigTree ver 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/, last accessed May 9, 2014). SNP differences

between each pair of *Elizabethkingia* spp. was calculated using the snpTree web server for assembled genomes with minimum coverage 10 and minimum distance between SNPs 10 (Leekitcharoenphon et al. 2012). Contigs of the NUHP1 genome were aligned with the three other *Elizabethkingia* species to identify homologous sequences (above 100 bp in length and at least 80% homology), using BLASTn as implemented in NCBI BLAST+ v. 2.2.8 (Camacho et al. 2009). A visual representation linking homologous regions between NUHP1 and the three strains was constructed with CIRCOS v. 0.64 (Krzywinski et al. 2009).

Core/Pan-Genomic Analysis

Core- and pan-genomes were calculated, and curves for pan-genome and core-genome were generated by the CMG-biotools package with BLAST cutoff of 50% identity and 50% coverage of the longest gene (Vesth et al. 2013). The identified core- and accessory genomes were BLAST searched against the Comprehensive Antibiotic Resistance Database (McArthur et al. 2013) and VFDB (Chen et al. 2012) to identify antibiotic resistant genes and virulence genes by using Bio-Edit (Ibis Biosciences, Carlsbad, CA, http://www.mbio.ncsu.edu/bioedit/bioedit.html, last accessed May 9, 2014) (minimum 30% identity with *E* value $< 1e^{-5}$).

EM-only proteins and EA-only proteins were enriched by the CMG-biotools package (Vesth et al. 2013). The identified EM-only proteins and EA-only proteins were assigned to COGs, and their abundances compared statistically as described in Rodriguez-Brito et al. (2006) and Allen et al. (2009) using a subsample size of 500 and 1,000 bootstrap replicates at a statistical confidence of 99%.

Nucleotide Sequence Accession Numbers

Each of the seven outbreak *E. anophelis* genomes were deposited at DDBJ/EMBL/GenBank as individual WGS bioprojects. The respective accession numbers of the seven *E. anophelis* genomes are NUHP1 (ASYE00000000), NUHP2 (ASYF00000000), NUHP3 (ASYG00000000), NUH1 (ASYH00000000), NUH4 (ASYI00000000), NUH6 (ASYJ00000000), NUH11 (ASYK00000000), respectively.

Supplementary Material

Supplementary tables S1–S3 are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

Acknowledgments

We thank Dr. Daniela Drautz, Mr. Yap Zhei Hwee and Rikky Wenang Purbojati for helping with genome sequencing experiment. This research is supported by the National Research Foundation and Ministry of Education Singapore under its Research Centre of Excellence Programme and the Start-up Grants (M4330002.C70) from Nanyang Technological University, Singapore. This work was supported by grants from the Danish Council for Strategic Research (M.G.)

Literature Cited

Allen MA, et al. 2009. The genome sequence of the psychrophilic archaeon, *Methanococcoides burtonii*: the role of genome evolution in cold adaptation. ISME J. 3:1012–1035.

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

Balm MND, et al. 2013. Bad design, bad practices, bad bugs—frustrations in controlling an outbreak of *Elizabethkingia meningoseptica* in intensive care units. J Hosp Infect. 85:134–140.

Bloch KC, Nadarajah R, Jacobs R. 1997. *Chryseobacterium meningosepticum*: an emerging pathogen among immunocompromised adults. Report of 6 cases and literature review. Medicine 76: 30–41.

Boissiere A, et al. 2012. Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. PLoS Pathog. 8:e1002742.

Camacho C, et al. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421.

Chen L, Xiong Z, Sun L, Yang J, Jin Q. 2012. VFDB 2012 update: toward the genetic diversity and molecular evolution of bacterial virulence factors. Nucleic Acids Res. 40:D641–D645.

Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5: e11147.

Dong Y, Manfredini F, Dimopoulos G. 2009. Implication of the mosquito midgut microbiota in the defense against malaria parasites. PLoS Pathog 5:e1000423.

Dooley JR, Nims LJ, Lipp VH, Beard A, Delaney LT. 1980. Meningitis of infants caused by *Flavobacterium meningosepticum*: report of a patient and analysis of 63 infections. J Trop Pediatr 26:24–30.

Green O, Murray P, Gea-Banacloche JC. 2008. Sepsis caused by *Elizabethkingia miricola* successfully treated with tigecycline and levofloxacin. Diagn Microbiol Infect Dis 62:430–432.

Hsu MS, et al. 2011. 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. 30:1271–1278.

Krzywinski M, et al. 2009. Circos: an information aesthetic for comparative genomics. Genome Res. 19:1639–1645.

Leekitcharoenphon P, et al. 2012. snpTree—a web-server to identify and construct SNP trees from whole genome sequence data. BMC Genomics 13(Suppl 7), S6.

McArthur AG, et al. 2013. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother. 57:3348–3357.

Osei-Poku J, Mbogo CM, Palmer WJ, Jiggins FM. 2012. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. Mol Ecol 21:5138–5150.

Rodriguez-Brito B, Rohwer F, Edwards RA. 2006. An application of statistics to comparative metagenomics. BMC Bioinformatics 7:162.

Teo J, et al. 2013. The first case of intensive care unit outbreak by the novel agent *Elizabethkingia anophelis*. Lancet 382:855–856.

Teres D. 1974. ICU-acquired pneumonia due to *Flavobacterium meningosepticum*. J Am Med Assoc 228:732.

Vesth T, Lagesen K, Acar O, Ussery D. 2013. CMG-biotools, a free workbench for basic comparative microbial genomics. PLoS One 8:e60120.

Associate editor: B. Venkatesh