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ORIGINAL RESEARCH

Molecular Characteristics and Antimicrobial Susceptibility Profiles of *Elizabethkingia* Clinical Isolates in Shanghai, China

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Purpose: To investigate molecular characteristics and antimicrobial susceptibility profiles of clinical isolates of *Elizabethkingia* in Shanghai, China.

Methods: *Elizabethkingia* isolates were collected in a university-affiliated hospital in 2012–2015 and 2017–2018. They were re-identified to species level by 16S rRNA gene and species-specific gene sequencing. Antimicrobial susceptibility testing, screening for metallo-beta-lactamase production, identification of antimicrobial resistance genes and pulsed-field gel electrophoresis (PFGE) were performed.

Results: Among 52 *Elizabethkingia* isolates, *E. anophelis* was the most prevalent species (67.3%), followed by *E. meningoseptica* (26.9%). High carriage rates of bla_{CME}, bla_{BlaB} and bla_{GOB} genes were consistent with the poor in vitro activity of most β-lactams including carbapenems. Nevertheless, β-lactamase inhibitors increased susceptibility rates significantly for cefoperazone and piperacillin. Susceptibility rates for minocycline, tigecycline, rifampin and levofloxacin were 100%, 78.8%, 76.9% and 71.2%, respectively. Ser83Ile or Ser83Arg substitution in the DNA gyrase A unit was associated with resistance to fluoroquinolones. MIC_{50}/MIC_{90} values of vancomycin and linezolid were 16/16 mg/L and 16/32 mg/L, respectively. Molecular typing showed twenty-one different types of PFGE and more than one indistinguishable isolates were observed in each of the eight subtypes.

Conclusion: Tetracyclines, tigecycline, β-lactam/β-lactamase inhibitor combinations, rifampin and fluoroquinolones demonstrated high rates of in vitro activity against clinical isolates of *Elizabethkingia*. Both genetic diversity and clonality were observed from this health-care facility. Our report provides potential alternative treatment options for *Elizabethkingia* infections.

Keywords: *Elizabethkingia*, antimicrobial susceptibility, molecular typing, multidrug resistance, resistant mechanism

Introduction

The genus *Elizabethkingia* is an infrequent Gram-negative non-fermenting bacillus and has recently emerged as a cause of life-threatening infections in humans, including meningitis, bacteremia, pneumonia and urinary tract infection.[1–3] Importantly, neonatal meningitis was the most common presentation of *Elizabethkingia* infection in children while a variety of clinical manifestations were reported in immunocompromised patients.[4] Furthermore, an increasing number of global cases of *Elizabethkingia* infection in recent years showed high morbidity and mortality, which reinforced the significance of early identification and treatment.[4]

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Elizabethkingia has undergone several taxonomic changes since the first description in 1959. It was reclassified from genus *Flavobacterium* in 1994 and from genus *Chryseobacterium* in 2005.[5] In addition to *E. meningoseptica* and *E. miricola*, several new species have been proposed in the last decade, including *E. anophelis*,[6] *E. bruuniana, E. ursingii*, and *E. occulta*.[7] *E. anophelis* and *E. meningoseptica* are most common among them.

The data from the SENTRY Antimicrobial Surveillance Program showed that *C. meningoseptica* represented only 0.1% (24/18,569) of the non-fermentative gram-negative bacilli in North America, Latin America, Europe and the Asia-Pacific region from 1997 to 2001.[8] Despite the overall low isolation rate of clinical strains, healthcare–associated outbreaks attributable to *Elizabethkingia* species have been reported in Singapore,[9] England[10] and Taiwan[11] since 2012. Moreover, two large-scale outbreaks were identified in the United States from 2014 to 2016, one causing significant mortality (6/10), and the second involving 65 individuals and resulting in 20 deaths (https://www.cdc.gov/*Elizabethkingia*/outbreaks/). Notably, the second outbreak occurred primarily in community settings, with the source of infection unclear.[12]

Elizabethkingia-related infections are complicated by the biofilm formation,[13] intracellular invasion,[14] and multi-drug resistance of the strains, and thus one needs to be cautious in selecting appropriate antimicrobial drugs. *Elizabethkingia* isolates display intrinsic resistance to multiple β-lactams as a result of Ambler class A serine extended-spectrum β-lactamase (ESBL) gene bla_{CME}[15] and two chromosomal Ambler class B metallo-β-lactamase (MBL) genes, bla_{BlaB} and bla_{GOB}.[16] It also confers resistance to quinolones due to mutations in DNA gyrase and/or topoisomerase IV genes.[17]

Given the limited epidemiological data on clinical isolates of *Elizabethkingia* in China, we investigated the molecular characteristics and antimicrobial susceptibility profiles of *Elizabethkingia* isolates in a university-affiliated hospital in Shanghai, China.

Materials and Methods

Identification of *Elizabethkingia* and Clinical Information of Patients

Non-duplicate isolates of *Elizabethkingia* were collected from a 1216-bed university-affiliated adult hospital in 2012–2018 with the exception of 2016 when isolates were missing. *Elizabethkingia* strains were preliminarily identified in the clinical laboratory from various clinical samples, such as specimens from respiratory tract, blood, urine, bile, exudate and indwelling needle. They were all included except those missing or dead. The hosts were inpatients and outpatients aged 18 years and older, and the departments included geriatrics, surgery, intensive care unit (ICU), neurology, infectious disease, general practice, hematology and thoracic surgery (Table 1).

Table 1 Characteristics of 52 Patients with *Elizabethkingia* Colonization or Infection

Age (years)	
Range	18–96
Mean±SD	64±21
Gender, n (%)	
Male	36 (69.2)
Female	16 (30.8)
Hospitalization duration (days), mean±SD	39±40
Comorbidity, n (%)	
Hypertension	18 (34.6)
Diabetes mellitus	7 (13.5)
Chronic obstructive pulmonary disease	6 (11.5)
Cardiovascular disease	5 (9.6)
End-stage renal disease	4 (7.7)
Mechanical ventilation, n (%)	29 (55.8)
Indwelling device, n (%)	38 (73.1)
Central venous catheter	28 (53.8)
Nasogastric tube	22 (42.3)
Urinary catheter	20 (38.5)
Surgical puncture or drain	20 (38.5)
Surgery, n (%)	20 (38.5)
Transplantation	5 (9.6)
Chemoradiotherapy, n (%)	3 (5.8)
Ward, n (%)	
Geriatrics	14 (26.9)
Neurosurgery	9 (17.3)
Surgery	7 (13.5)
Intensive care unit	7 (13.5)
Neurology	5 (9.6)
Infectious disease	4 (7.7)
General practice	2 (3.8)
Hematology	2 (3.8)
Thoracic surgery	1 (1.9)
Outpatient	1 (1.9)
Site of isolation, n (%)	
Respiratory tract	45 (86.5)
Blood	2 (3.8)
Urine	2 (3.8)
Bile	1 (1.9)
Exudate	1 (1.9)
Indwelling needle	1 (1.9)

The isolates were re-identified to species level by PCR amplification and sequencing of the 16S rRNA gene followed by analysis using the EzTaxon server (http://www.ezbiocloud.net/, reference sequence: *E. anophelis* strain R26, GenBank accession number NZ_CP023401; *E. meningoseptica* type strain 13253, NZ_ASAN01000081; *E. miricola* DSM 14571, NZ_VNHK00000000.1; *E. ursingii* G4122, NZ_LNOK01000028),[18,19] and by species-specific primers (*E. anophelis*-specific primers targeted lipid A-disaccharide synthase gene; *E. meningoseptica*-specific primers targeted a putative sodium-proton antiporter; *E. miricola* cluster-specific primers targeted urease gene *ureG*).[20,21] The indistinguishable *E. miricola* cluster isolates were further confirmed by RNA polymerase subunit gene (*rpoB*) sequencing.[21]

Medical records of patients were retrospectively reviewed to acquire clinical information.

Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations (MICs) of antimicrobial agents were determined using the broth microdilution method and interpreted on the basis of the Clinical and Laboratory Standards Institute (CLSI) criteria for other non-*Enterobacteriaceae* for most of the antibiotics.[22] The US FDA susceptibility breakpoints for *Enterobacteriaceae* were extrapolated for tigecycline. For rifampin, vancomycin and linezolid, the breakpoints for *Staphylococcus* spp. were applied.[23,24]

EDTA Combination Disk Test (EDT)

Imipenem discs and imipenem/0.5 M EDTA combination discs were used for the detection of the MBL phenotype as described previously.[25] The test was considered to be positive if the diameter of the inhibition zone of the imipenem/EDTA disc was 7 mm larger than that of the imipenem disc alone.[25]

Identification of β-Lactamase Genes and Mutations in the Quinolone Resistance-Determining Regions (QRDRs)

All isolates were screened for ESBL gene bla_{CME} and MBL genes, bla_{BlaB} and bla_{GOB}, as described previously.[15,16,26] Mutations in the QRDRs of *gyrA, gyrB, parC* and *parE* genes were determined by PCR amplification and sequencing. Alignment was performed with the respective reference sequences in the GenBank database (NCBI reference sequence: *E. anophelis* strain NUHP1, NZ_CP007547.1; *E. meningoseptica* strain G4076, NZ_CP016376.1; *E. miricola* strain EM798-26, NZ_CP023746.1).[17]

Molecular Typing

Pulsed-field gel electrophoresis (PFGE) was performed with CHEF Mapper XA system (Bio-Rad, USA). The genomic DNA of *Elizabethkingia* was prepared in agarose blocks and digested with restriction enzyme XhoI. *Salmonella enterica* serotype Braenderup H9812, as a molecular size marker, was digested with XbaI. The DNA fragments were separated at 6.0 V/cm, 120° angle, temperature of 14°C, and switch times of 1 to 18 s for a total run time of 18 hrs. PFGE band profiles were analyzed using BioNumerics version 7.6 software. The similarity matrix was calculated by Dice's coefficients with 1.5% optimization and 1.5% band matching tolerance, and a dendrogram was generated using the unweighted pair group method with arithmetic averages (UPGMA). Isolates with ≥95%, 85–95% and <85% similarity were considered as a PFGE subtype, a PFGE type and a different type, respectively.

Results

Identification and Prevalence of *Elizabethkingia* Isolates

A total of 57 (0.6%) non-duplicate *Elizabethkingia* isolates were collected among 8804 gram-negative non-fermenting bacillus isolates from a university-affiliated Hospital in 2012–2015 and 2017–2018.

Fifty-two isolates were confirmed to be *Elizabethkingia* isolates using 16S rRNA gene sequencing, including 35 (67.3%) isolates of *E. anophelis*, 14 (26.9%) isolates of *E. meningoseptica* and 2 (3.8%) isolates of *E. miricola*. One isolate had 99.6% similarity with *E. ursingii* strain G4122 and 99.3% similarity with *E. miricola* strain DSM 14,571 with 16S rRNA gene sequencing, but 99.9% similarity with *E. miricola* strain F13 (accession no: NZ_CP040450.1) with further *rpoB* gene sequencing. Therefore, this isolate was identified as *E. miricola*. All of the isolates were further confirmed with species-specific gene sequencing, which was consistent with the identification with 16S rRNA gene and *rpoB* gene sequencing.

Most of the *Elizabethkingia* strains were isolated from the respiratory tract (45/52, 86.5%), followed by blood (2/52, 3.8%) and urine (2/52, 3.8%). An increase in numbers was observed from 6 (11.5%) isolates in 2012 to 12 (23.1%) isolates in 2017 and 21 (40.4%) in 2018 (Figure 1).

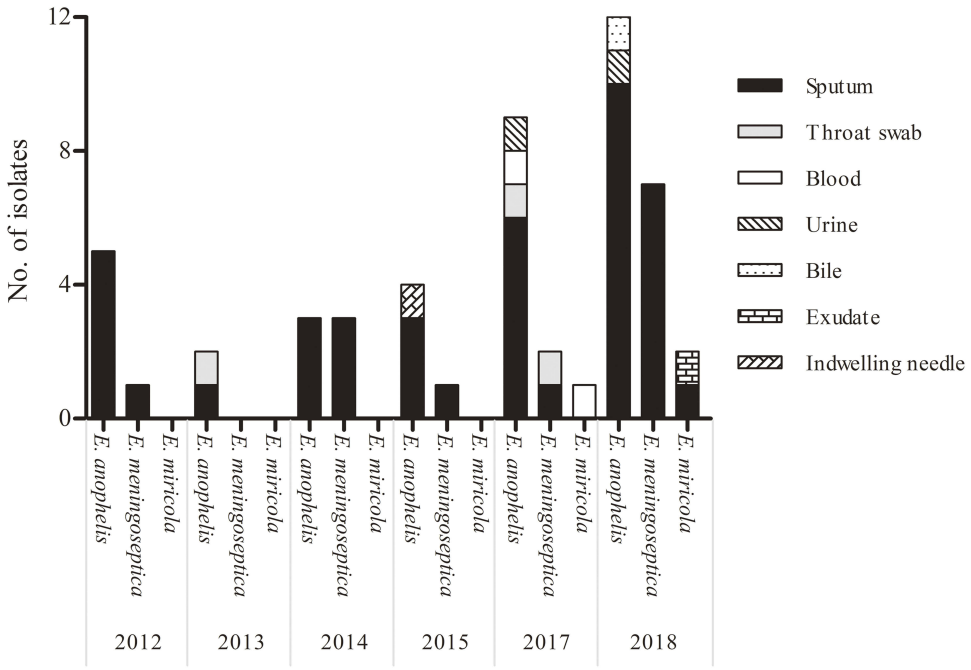


Figure 1 The distribution of 52 *Elizabethkingia* isolates according to the year and site of isolation.

Additionally, ten samples showed concomitant isolates of other bacterial species such as *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* in sputum.

Clinical Characteristics of Patients

Of the 52 patients, 51 were hospitalized patients and one was outpatient (Table 1). Male accounted for 69.2% (36/52) while female constituted 30.8% (16/52). The mean age was 64 years old, with the range of 18–96 years old. Prolonged hospital stays (≥2 weeks) was observed in 46 patients. Geriatrics department was the most common ward (14/52, 26.9%), followed by neurosurgery department (9/52, 17.3%), surgery department (7/52, 13.5%) and ICU (7/52, 13.5%). Comorbidity was identified in all hospitalized patients, with hypertension as the most prevalent underlying disease (18/52, 34.6%) followed by diabetes mellitus (7/52, 13.5%), chronic obstructive pulmonary disease (6/52, 11.5%), cardiovascular disease (5/52, 9.6%) and end-stage renal disease (4/52, 7.7%). Besides, 20 (38.5%) patients had a history of surgery before *Elizabethkingia* was identified, and 5 (9.6%) underwent transplantation while 3 (5.8%) received chemoradiotherapy. Twenty-nine (55.8%) patients received mechanical ventilation and 38 (73.1%) patients had medical devices indwelled. The overall in-hospital mortality rate was 13.5% (7/52).

Antimicrobial Susceptibility Pattern

Multidrug resistance was observed in all 52 *Elizabethkingia* isolates (Table 2). All of them were resistant to aztreonam, ceftazidime, colistin, and all were non-susceptible to cefepime, carbapenems (imipenem and meropenem) and vancomycin. They also exhibited high resistance rates to linezolid, gentamicin, amikacin and trimethoprim-sulfamethoxazole (96.2%, 96.2%, 86.5% and 63.5%, respectively). Low susceptibility rates were observed for cefoperazone (5.8%) and piperacillin (46.2%) in contrast to the increased susceptibility when they were in combination with β-lactamase inhibitors, namely cefoperazone-sulbactam and piperacillin-tazobactam (88.5% and 86.5%, respectively).

The susceptibility rates of *Elizabethkingia* isolates to ciprofloxacin, levofloxacin and rifampin were 50.0%, 71.2% and 76.9%, respectively. Minocycline was more active rather than doxycycline and tigecycline (susceptible rates, 100% versus 96.2% and 78.8%, respectively). MIC_{50}/MIC_{90} values of vancomycin and linezolid against *Elizabethkingia* isolates were 16/16 mg/L and 16/32 mg/L, respectively.

MBL Phenotype and Genotype of *Elizabethkingia* Isolates

EDTA combination testing was positive for 52 *Elizabethkingia* isolates, suggesting the capability of MBL

Table 2 Antimicrobial Susceptibilities of 52 *Elizabethkingia* Isolates Determined by the Broth Microdilution Method

Antimicrobial Agents	Breakpoint (mg/L)			MIC (mg/L)			Number (%) of Isolates		
	S	I	R	Range	50%	90%	S	I	R
Piperacillin	≤16	32–64	≥128	8 to >128	32	64	24 (46.2)	23 (44.2)	5 (9.6)
Piperacillin-tazobactam	≤16/4	32/4–64/4	≥128/4	0.25/4 to >128/4	4/4	32/4	45 (86.5)	7 (13.5)	0 (0.0)
Cefoperazone	≤16	32	≥64	16 to >128	32	>128	3 (5.8)	25 (48.1)	24 (46.2)
Cefoperazone-sulbactam	≤16/8	32/16	≥64/32	2/1–128/64	8/4	64/32	46 (88.5)	1 (1.9)	5 (9.6)
Ceftazidime	≤8	16	≥32	64 to >128	>128	>128	0 (0.0)	0 (0.0)	52 (100.0)
Cefepime	≤8	16	≥32	16 to >128	32	>128	0 (0.0)	8 (15.4)	44 (84.6)
Aztreonam	≤8	16	≥32	>128	>128	>128	0 (0.0)	0 (0.0)	52(100.0)
Imipenem	≤4	8	≥16	8 to >128	16	128	0 (0.0)	6 (11.5)	46 (88.5)
Meropenem	≤4	8	≥16	8 to >128	32	>128	0 (0.0)	4 (7.7)	48 (92.3)
Colistin	≤2	–	≥4	>128	>128	>128	0 (0.0)	0 (0.0)	52 (100.0)
Amikacin	≤16	32	≥64	16 to >128	64	>128	4 (7.7)	3 (5.8)	45 (86.5)
Gentamicin	≤4	8	≥16	4 to >128	64	>128	1 (1.9)	1 (1.9)	50 (96.2)
Ciprofloxacin	≤1	2	≥4	0.25 to >128	1	128	26 (50.0)	6 (11.5)	20 (38.5)
Levofloxacin	≤2	4	≥8	0.25–64	1	32	37 (71.2)	1 (1.9)	14 (26.9)
Rifampin	≤1	2	≥4	0.5–64	1	8	40 (76.9)	6 (11.5)	6 (11.5)
Trimethoprim-sulfamethoxazole	≤2/38	-	≥4/76	1/19–16/304	4/76	8/152	19 (36.5)	0 (0.0)	33 (63.5)
Tigecycline	≤2	4	≥8	1–32	4	8	41 (78.8)	8 (15.4)	3 (5.8)
Doxycycline	≤4	8	≥16	1–8	2	4	50 (96.2)	2 (3.8)	0 (0.00)
Minocycline	≤4	8	≥16	0.25–4	0.5	1	52 (100.0)	0 (0.0)	0 (0.0)
Vancomycin	≤4	8–16	≥32	8–32	16	16	0 (0.0)	12 (23.1)	40 (76.9)
Linezolid	≤4	-	≥8	4–128	16	32	2 (3.8)	0 (0.0)	50 (96.2)

Notes: In the combinations, the concentration of tazobactam was 4 mg/L constant. The ratio of cefoperazone to sulbactam was 2:1, and the ratio of trimethoprim to sulfamethoxazole was 1:19.
Abbreviations: MIC, minimal inhibitory concentration; S, susceptible; I, intermediate; R, resistant.

production. This was consistent with the results that these 52 isolates were non-susceptible to imipenem.

A total of 51 *Elizabethkingia* carried β-lactamase genes. Specifically, 49 *Elizabethkingia* isolates harbored the bla_{BlaB} gene, 28 carried bla_{GOB} gene and 36 had bla_{CME} gene. Twenty-six *Elizabethkingia* isolates harbored the above three genes (Table 3). Two isolates were negative for MBL genotype detection.

Single-nucleotide mutations in QRDR region of the *gyrA* gene were observed in 13 isolates which led to amino acid substitutions Ser83Ile in nine isolates and Ser83Arg in 4 isolates, conferring resistance to ciprofloxacin and levofloxacin. No non-synonymous alternations were detected in *gyrB, parC* or *parE*. One isolate of *E. anophelis* did not have amino acid substitutions in the QRDR region of the *gyrA, gyrB, parC* or *parE*, but showed high MICs of ciprofloxacin and levofloxacin (both 16 mg/L).

Table 3 Distribution of β-Lactamase Genes in 52 *Elizabethkingia*

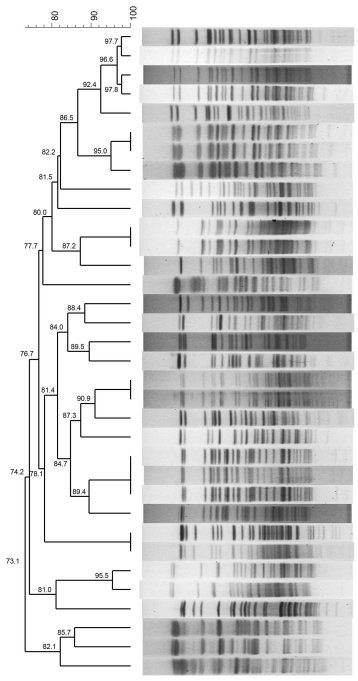
β-Lactamase Gene	No. of Isolates	EDTA Combination Disk Test
Negative	1	+
bla_{CME}	1	+
bla_{BlaB}	14	+
bla_{CME} + bla_{BlaB}	8	+
bla_{CME} +bla_{GOB}	1	+
bla_{BlaB} + bla_{GOB}	1	+
bla_{CME} + bla_{BlaB}+ bla_{GOB}	26	+

Genetic Relatedness and Retrospective Analysis

One isolate was resistant to XhoI digestion. The remaining 51 *Elizabethkingia* isolates were clustered into 21 different PFGE types designated A-T (Figure 2). Specifically, 34 *E. anophelis* isolates were grouped into 14 clusters, 14 *E. meningoseptica* isolates into 4 clusters while 3 *E. miricola* isolates into 3 clusters.

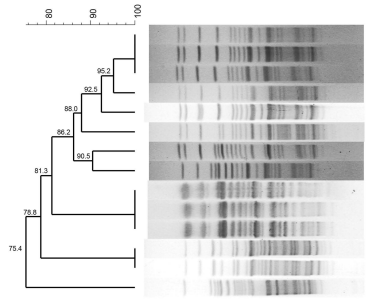
Of all PFGE types, A and O types were the most frequent containing 8 isolates each. *E. anophelis* isolates in A type accounted for 23.5% (8/34), while *E. meningoseptica* isolates in O type made up 57.1% (8/14). Above all, there were indistinguishable isolates sharing 100% relatedness in each

A



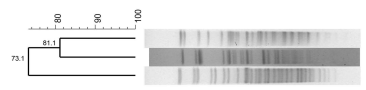
Isolate ID	PFGE type/ subtype	Gender/age (years)	Ward	Collection date (DD/MM/YY)	Source	PIP	TZP	CFP	CSL	AMK	LVX	RIF	SXT	TGC
Ean042	A/A1	M/62	Surgery	29/05/2014	Sputum	S	S	I	S	R	R	S	R	S
Ean082	A/A1	F/57	Neurology	17/01/2018	Sputum	S	S	I	S	R	R	S	S	S
Ean006	A/A1	M/79	Infectious disease	29/06/2017	Urine	S	S	R	R	R	R	R	R	S
Ean097	A/A1	F/75	Surgery	10/07/2018	Sputum	I	S	R	I	R	R	R	S	S
Ean051	A/A2	M/71	Neurosurgery	19/07/2013	Sputum	S	S	I	S	R	R	S	S	S
Ean103	A/A3	F/51	Surgery	09/08/2018	Sputum	S	S	R	R	R	R	R	S	S
Ean104	A/A3	M/56	Surgery	15/08/2018	Sputum	S	S	R	R	R	R	R	S	S
Ean117	A/A3	M/46	Surgery	01/11/2018	Bile	S	S	R	R	R	R	R	S	S
Ean012	B/B	M/36	Hematology	16/11/2017	Throat swab	R	I	I	S	R	R	S	R	I
Ean026	C/C	M/62	General practice	16/07/2015	Indwelling needle	I	S	I	S	R	S	S	S	S
Ean095	D/D1	M/63	Neurosurgery	05/07/2018	Sputum	S	S	S	S	R	S	S	S	S
Ean096	D/D1	M/54	Neurosurgery	06/07/2018	Sputum	S	S	I	S	R	S	S	R	S
Ean070	D/D2	M/73	Neurology	28/10/2012	Sputum	S	S	I	S	R	S	S	R	S
Ean106	E/E	F/63	Neurosurgery	23/08/2018	Sputum	I	S	I	S	R	S	S	R	S
Ean009	F/F1	M/78	Surgery	25/08/2017	Sputum	S	S	I	S	R	S	S	R	S
Ean045	F/F2	M/95	Geriatrics	16/10/2014	Sputum	I	S	I	S	R	S	I	S	I
Ean011	G/G1	F/54	Neurosurgery	10/10/2017	Sputum	I	S	I	S	S	S	S	R	S
Ean052	G/G2	F/42	Hematology	21/07/2013	Throat swab	R	S	R	S	I	S	S	R	S
Ean003	H/H1	F/74	General practice	27/03/2017	Sputum	I	I	I	S	R	S	S	R	S
Ean004	H/H1	M/93	Geriatrics	13/04/2017	Sputum	I	S	I	S	R	S	S	R	S
Ean040	H/H2	F/39	Infectious disease	28/08/2014	Sputum	I	S	I	S	R	S	S	R	I
Ean068	H/H3	M/49	Neurosurgery	12/10/2012	Sputum	R	I	R	S	R	R	S	R	S
Ean061	I/I1	M/90	Geriatrics	20/02/2012	Sputum	I	S	R	S	S	S	I	R	S
Ean062	I/I1	M/87	Geriatrics	23/04/2012	Sputum	I	S	R	S	R	S	I	R	S
Ean063	I/I1	M/96	Geriatrics	25/04/2012	Sputum	I	S	R	S	S	S	I	R	S
Ean005	I/I2	F/61	Intensive care unit	14/05/2017	Sputum	I	S	I	S	R	S	S	S	S
Ean021	J/J	M/57	Intensive care unit	14/01/2015	Sputum	I	S	I	S	R	S	S	R	S
Ean022	J/J	M/23	Intensive care unit	22/01/2015	Sputum	I	S	I	S	R	S	S	S	S
Ean016	K/K	M/75	Neurosurgery	28/11/2017	Blood	R	I	R	S	R	S	I	R	I
Ean093	K/K	M/47	Intensive care unit	10/06/2018	Sputum	I	S	I	S	R	S	S	R	S
Ean027	L/L	M/61	Thoracic surgery	30/11/2015	Sputum	S	S	I	S	R	S	S	R	S
Ean113	M/M1	F/60	Neurology	12/10/2018	Sputum	I	I	I	S	R	R	S	R	S
Ean116	M/M2	M/45	Neurosurgery	17/10/2018	Sputum	S	S	S	S	R	S	S	R	S
Ean107	N/N	M/79	Intensive care unit	25/08/2018	Urine	I	S	S	S	R	S	S	R	R

B



Isolate ID	PFGE type/ subtype	Gender/age (years)	Ward	Collection date (DD/MM/YY)	Source	PIP	TZP	CFP	CSL	AMK	LVX	RIF	SXT	TGC
Eme025	O/O1	M/91	Geriatrics	27/05/2015	Sputum	S	S	R	S	R	S	S	S	S
Eme041	O/O1	M/92	Geriatrics	16/01/2014	Sputum	S	S	R	S	R	S	S	S	I
Eme047	O/O1	M/84	Geriatrics	21/12/2014	Sputum	S	S	R	S	R	S	S	S	I
Eme001	O/O1	M/96	Geriatrics	18/03/2017	Sputum	S	S	R	S	R	S	S	S	I
Eme100	O/O2	M/88	Geriatrics	18/07/2018	Sputum	I	S	R	S	R	S	S	R	S
Eme064	O/O3	M/61	Intensive care unit	26/04/2012	Sputum	S	S	R	S	I	S	S	R	S
Eme008	O/O4	F/24	Outpatient	27/07/2017	Throat swab	I	I	R	R	R	R	R	S	S
Eme044	O/O5	M/22	Neurology	11/09/2014	Sputum	S	S	I	S	I	S	S	R	R
Eme105	P/P	F/91	Geriatrics	22/08/2018	Sputum	S	S	R	S	R	I	S	S	R
Eme111	P/P	F/71	Geriatrics	14/09/2018	Sputum	S	S	R	S	R	S	S	R	S
Eme118	P/P	F/95	Geriatrics	03/11/2018	Sputum	S	S	R	S	R	S	S	S	S
Eme098	Q/Q	F/56	Infectious disease	12/07/2018	Sputum	I	S	R	S	R	R	S	R	S
Eme101	Q/Q	M/54	Infectious disease	26/07/2018	Sputum	I	S	R	S	R	R	S	R	S
Eme081	R/R	M/91	Geriatrics	15/01/2018	Sputum	S	S	I	S	R	S	S	R	I

C



Isolate ID	PFGE type/ subtype	Gender/age (years)	Ward	Collection date (DD/MM/YY)	Source	PIP	TZP	CFP	CSL	AMK	LVX	RIF	SXT	TGC
Emi099	S/S	M/55	Surgery	14/07/2018	Exudate	R	I	R	S	R	S	I	R	S
Emi007	T/T	M/18	Neurosurgery	10/07/2017	Blood	S	S	I	S	R	S	S	S	S
Emi088	U/U	F/43	Intensive care unit	26/04/2018	Sputum	I	S	I	S	R	S	S	R	S

Figure 2 Dendrogram of PFGE patterns of 52 *Elizabethkingia* isolates using the BioNumerics software. (**A**) Thirty-four *E. anophelis* isolates; (**B**) Fourteen *E. meningoseptica* isolates; (**C**) Three *E. miricola* isolates.

Abbreviations: M, male; F, female; PIP, piperacillin; TZP, piperacillin-tazobactam; CFP, cefoperazone; CSL, cefoperazone-sulbactam; AMK, amikacin; LVX, levofloxacin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline; S, susceptible; I, intermediate; R, resistant.

of 8 clusters (A3, D1, H1, I1, J, O1, P and Q subtypes), indicating clonal spread in this health-care facility. All of the identical clones in respective subtypes were collected from the sputum samples of patients in the same wards within 6 months, except that 2 indistinguishable isolates in the H1 subtype were recovered in departments of geriatrics and general practice, respectively. Meanwhile, the geriatrics department had the most isolates with identical clones (H1, I1, O1, P subtypes) spanning from 2012 to 2018. Similar antimicrobial susceptibility patterns of the above identical isolates were detected within subtypes. Mechanical ventilation and indwelling devices were also present among patients who had A3, D1, J, O1, P and Q subtypes. On the other hand, no epidemiological relationship was observed for closely related clones at 95% genetic similarity in the A1 and K subtypes. Possibly related strains in F, G and M types also exhibited variation of

spacial or temporal distribution, which were clustered for ≥85% similarity values.

Discussion

Six species of the genus *Elizabethkingia* (*E. anophelis, E. meningoseptica, E. miricola, E. bruuniana, E. ursingii* and *E. occulta*) are emerging in children and immunocompromised patients with the growing emphasis on the pathogenicity for former four species.[1,18] Nowadays, development of microbiological identification techniques makes it possible to recognize several emerging unusual bacteria that cause diseases mainly in immunocompromised patients. Actually, traditional identification systems with inferior discrimination power for infrequent species could lead to the misidentification, misdiagnosis, failure of therapy as well as underestimation of the incidence of the infection in the past.[4] Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) Vitek mass spectrometry (VMS) and molecular identification techniques (16S rRNA, *rpoB* gene sequencing and whole-genome sequencing) emerge as advantageous tools for accurate identification of microbe with excellent discrimination, especially infrequent opportunistic bacteria, such as *Elizabethkingia* species,[27] *Cardiobacterium hominis*[28] and *Kocuria marina*.[29]

In this study, patients with comorbidity, indwelling devices, mechanical ventilation and prolonged hospital stays were vulnerable to *Elizabethkingia* colonization or infection. Geriatrics, neurosurgery, surgery and ICU departments were the top four departments. Prior studies also found that *E. meningoseptica* strains were commonly isolated from ICU in India[30] and Taiwan.[31] These data consistently suggest that *Elizabethkingia* has a predilection for infecting immunocompromised patients.

E. anophelis was the most prevalent species of *Elizabethkingia* followed by *E. meningoseptica*, while *E. miricola* isolates were rare in the collection. In fact, more evidence indicated the predominance of *E. anophelis*, rather than *E. meningoseptica*, among *Elizabethkingia* in clinical settings.[18,20] In this study, we also found that the identification for Emi007 was ambiguous with 16S rRNA gene sequencing, which could be partly explained by the existence of multiple copies with different sequences and the hypervariable regions of 16S rRNA.[32] It has been pointed out that the *rpoB* gene is a single copy gene and has a higher resolution of phylogenetic evolution than the 16S rRNA gene; thus, it could accurately distinguish *Elizabethkingia* strains at the species level.[7,32] PFGE profiles showed finer resolution of clonal relationships, indicating that Emi007 was genetically more related to Emi099 than Emi088 with a similarity of 81.1%, thereby supporting its identity as *E. miricola.*

Genetic diversity and clonal dissemination were both suggested by PFGE typing. Although the genotypes of *E. anophelis* isolates were highly diverse, clonal spread was observed in several pairs of patients in the same or different departments. *E. meningoseptica* isolates from 2012 to 2018 were genetically homogenous (8/14 isolates in O type, 3/14 in P type, 2/14 in Q type) indicating clonal expansion and persistence in recent years. Previous reports have found that *Elizabethkingia* acquisition might be associated with water sources or water-related equipment, such as sinks and hand hygiene sink aerator within the hospital environment.[33] Clonal dissemination could be mediated by the hands of hospital staff or patients.[34] Therefore, it is necessary to reinforce hand hygiene and environmental cleaning when this genus is detected in the hospital.

The treatment for *Elizabethkingia* infection is challenging because *Elizabethkingia* isolates tend to display inherent resistance to antimicrobial agents, including aminoglycosides, most β-lactams and colistin. Multiple resistance genes and drug efflux systems in *Elizabethkingia* have been demonstrated by genomic and proteomic analysis.[35,36] In this study, the high carriage rate of bla_{CME}, bla_{BlaB} and bla_{GOB} genes was consistent with the broad-spectrum resistance to β-lactams including carbapenems. Nevertheless, piperacillin-tazobactam and cefoperazone-sulbactam were active in vitro against *Elizabethkingia*, in accordance with the results of a previous study where both combinations showed reasonable in vitro activity (70–85% and 65–80% of susceptibility rate, respectively) against 170 clinical *Elizabethkingia* isolates in China.[37] Furthermore, treatment with combination therapy of piperacillin-tazobactam and trimethoprim-sulfamethoxazole or a fluoroquinolone was reported to be effective in paediatric patients with *E. meningoseptica* infections, with variable susceptibility of strains to piperacillin-tazobactam (100%), trimethoprim-sulfamethoxazole (78.6%) and fluoroquinolones (33.3–87.5%).[38] The restoration of activity with β-lactam inhibitors may be explained by the production of ESBLs genes such as bla_{CME} especially when MBLs were expressed at a low level, along with decreased outer membrane permeability involved in the carbapenem-resistant *Elizabethkingia* isolates.[39] Despite the favorable activity of β-lactam/β-lactamase inhibitor combinations as a part of the combination regimen, there was little evidence over their use as monotherapy for patients. Therefore, their clinical efficacy needs further evaluation.

The reports varied on the susceptibility of *Elizabethkingia* isolates against fluoroquinolones.[32] Favored in vitro activity of fluoroquinolones was observed in this study, and the susceptibility rate for levofloxacin was higher than that for ciprofloxacin. By contrast, fluoroquinolones exhibited poor activity against *E. anophelis* and *E. meningoseptica* while all *E. miricola* isolates were susceptible to levofloxacin and moxifloxacin in a hospital in South Korea.[18] In another report, a significant difference was noted between the susceptibility rates of *Elizabethkingia* against ciprofloxacin and levofloxacin (9.8% and 52.2%, respectively).[40] One possible explanation for such discrepancy was that levofloxacin containing the C-8 methoxy group exerted stronger antibacterial activity against fluoroquinolone-resistant bacteria that harbored *gyrA* mutation.[41,42]

Successful treatment has been described using fluoroquinolones for *Elizabethkingia* infection. In a retrospective clinical study, the quinolone group achieved a higher microbiological cure rate, higher clinical success rate and lower 14-day in-hospital mortality rate in patients with *E. meningoseptica* bacteraemia than the non-fluoroquinolone group did.[43] In addition, higher 14-day mortality in patients with bacteraemia caused by levofloxacin-resistant *E. meningoseptica* compared with those with levofloxacin-susceptible strains was reported in Taiwan.[44] Consequently, while it may be suitable to consider fluoroquinolones as a choice of empirical antimicrobial therapy for *Elizabethkingia* infection, early identification of fluoroquinolone resistance in *Elizabethkingia* isolates is of significant importance in tackling this multidrug-resistance pathogen.

We found the Ser83Ile or Ser83Arg substitution in GyrA in 13 fluoroquinolone-resistant clinical *Elizabethkingia* isolates, which was in accordant with previous reports.[17] A study in Taiwan identified that non-synonymous alterations of additional sites in GyrA (positions 95 and 102) and GyrB (positions 425, 452, and 470) involved levofloxacin non-susceptibility as well.[40] The AcrAB efflux pump also played a role in mediating fluoroquinolone resistance by increased expression in *Elizabethkingia* isolates.[25]

It remains controversial whether antibiotics active against Gram-positive cocci, such as vancomycin and linezolid, are useful in the fight against *Elizabethkingia*. In this study, MIC_{50}/MIC_{90} values of vancomycin against *Elizabethkingia* isolates were 16/16 mg/L, as shown in a previous study.[45] Interestingly, clinical cure was noted in patients with *Elizabethkingia* infection despite high MIC of vancomycin against *Elizabethkingia* (≥16 mg/L).[23,46] Given the above conflicting data and scant information in literature, it may be necessary to establish the criteria for determining the in vitro antimicrobial susceptibility against *Elizabethkingia*.

Minocycline, doxycycline, tigecycline and rifampin were active against *Elizabethkingia* in vitro in this study. It is interesting that tigecycline, the derivative of minocycline, showed inferior antimicrobial activity, as reported in Taiwan.[25] Overall, taking into account their limitation (poor distribution in tissue, for example), different pharmacokinetics characteristics and scanty supporting clinical evidence, their roles in treating patients with invasive *Elizabethkingia* infections need further evaluation.

Conclusion

Elizabethkingia isolates were still rare in this clinical setting since they tended to occur in immunocompromised patients and had a risk of misidentification through conventional methods. *E. anophelis* was the most prevalent species among them, most of which exhibited phylogenetic diversity. Putative circulating clones were discovered in *E. anophelis* and *E. meningoseptica*, indicating a potential risk of further dissemination. *Elizabethkingia* isolates displayed multidrug resistance characteristics. However, tetracyclines, tigecycline, β-lactam/β-lactamase inhibitor combinations, rifampin and fluoroquinolone demonstrated appealing in vitro activity against *Elizabethkingia*, and further clinical studies may be needed to determine their potential role in treating *Elizabethkingia* infection.

Ethics Approval

Verbal informed consent of patients was obtained after the approval of the Ethics Committee of Huashan Hospital, Fudan University, China (approval number: KY2019-544). The patient data were analyzed in anonymity.

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Disclosure

The authors report no conflicts of interest in this work.

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