

Elizabethkingia bruuniana Infections in Humans, Taiwan, 2005–2017

Jiun-Nong Lin, Chung-Hsu Lai, Chih-Hui Yang,
Yi-Han Huang, Hsi-Hsun Lin

Author affiliations: E-Da Hospital, Kaohsiung, Taiwan (J.-N. Lin, C.-H. Lai, Y.-H. Huang); I-Shou University, Kaohsiung (J.-N. Lin, C.-H. Lai, Y.-H. Huang); Meiho University, Pingtung, Taiwan (C.-H. Yang); Taipei Veterans General Hospital, Taipei, Taiwan (H.-H. Lin)

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Using 16S rRNA and *rpoB* gene sequencing, we identified 6 patients infected with *Elizabethkingia bruuniana* treated at E-Da Hospital (Kaohsiung, Taiwan) during 2005–2017. We describe patient characteristics and the molecular characteristics of the *E. bruuniana* isolates, including their MICs. Larger-scale studies are needed for more robust characterization of this pathogen.

The *Elizabethkingia* genus comprises gram-negative, aerobic, nonmotile, nonspore-forming, nonfermenting rod-shaped bacteria (*I*). This genus previously comprised *E. meningoseptica*, *E. miricola*, and *E. anophelis*. In August 2017, Nicholson et al. proposed adding 3 new species, namely *E. bruuniana*, *E. ursingii*, and *E. occulta*, to this genus (*I*). However, little information exists about these species. In this study, we report the clinical characteristics and demographics of a group of patients with *E. bruuniana* infection in Taiwan and the molecular features of their *E. bruuniana* isolates.

We conducted this study at E-Da Hospital, a 1,000-bed university-affiliated medical center in Kaohsiung, Taiwan; this study was approved by the institutional review board of the hospital (no. EMRP-106-105). We searched the hospital database to identify microbial cultures performed during January 2005–December 2017 that yielded *Elizabethkingia*. The isolates were initially identified by staff in the clinical microbiology laboratory using API/ID32 phenotyping kits or VITEK MS (both from bioMérieux, <https://www.biomerieux.com>). We re-identified these species as *Elizabethkingia* using both 16S rRNA and *rpoB* gene sequencing. The primers and methods we used for amplification and sequencing of the 16S rRNA and *rpoB* genes were described previously (*1,2*). We compared the assembled 16S rRNA gene sequences with the nucleotide sequences of *Elizabethkingia*-type strains present in GenBank. We considered isolates with $\geq 99.5\%$ similarity in the 16S rRNA gene sequence

members of the same species, as recommended in a previous study (*3*). We constructed a phylogenetic tree using the *rpoB* genes of the isolates exhibiting $\geq 99.5\%$ 16S rRNA gene sequence identity with the *E. bruuniana* type strain G0146^T. We calculated the average nucleotide identity using OrthoANI (*4*) and computed in silico DNA–DNA hybridization (DDH) using the Genome-to-Genome Distance Calculator (*5*), using the average nucleotide identity value of $\geq 95\%$ and the DDH value of $\geq 70\%$ separately as criteria for species delineation (*4,5*). We sequenced the quinolone resistance–determining regions of DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) to look for mutations associated with resistance (Appendix Table, <https://wwwnc.cdc.gov/EID/article/25/7/18-0768-App1.pdf>).

For the 13-year period, we found 103 nonduplicate *Elizabethkingia* isolates in the database of the clinical microbiology laboratory. Among these, 8 isolates shared $\geq 99.5\%$ 16S rRNA gene sequence identity with *E. bruuniana* G0146^T, and an *rpoB* gene–based phylogenetic analysis revealed that 6 of the 8 isolates were more closely related to *E. bruuniana* G0146^T (Appendix Figure 1). We previously published the complete whole-genome sequence of 1 of these 6 isolates, EM798-26 (GenBank accession no. CP023746) (*6*). Using 16S rRNA gene sequence analysis, we initially identified this isolate as *E. miricola*. Average nucleotide identity analysis demonstrated that EM798-26 and *E. bruuniana* G0146^T share 97.7% whole-genome similarity (Appendix Figure 2). Using in silico DDH analysis, we predicted a DDH value of 81.7% for EM798-26 and *E. bruuniana* G0146^T (Appendix Figure 3). These results support that EM798-26 and the other 5 isolates (EM20-50, EM455-89, EM828-05, EM863-68, and EM891-63) are *E. bruuniana*.

These 6 isolates were collected from 6 (4 male and 2 female) patients (Table) with a mean age of 71.7 (SD ± 11) years. The sources of isolation included bronchoalveolar lavage fluid ($n = 2$), blood ($n = 2$), urine ($n = 1$), and the tip of the central venous catheter ($n = 1$). All infections were healthcare associated. Two patients had septic shock, and all patients had ≥ 1 concurrent medical condition, such as hypertension, diabetes mellitus, or a malignancy. Antimicrobial therapy included piperacillin/tazobactam, trimethoprim/sulfamethoxazole, levofloxacin, or tigecycline, either singly or in combination. None of the patients died of *E. bruuniana* infection.

Most *E. bruuniana* isolates were resistant to β -lactams, β -lactam and lactamase inhibitors, carbapenems, aminoglycosides, and trimethoprim/sulfamethoxazole (Table). All isolates were susceptible to minocycline, 4 (67%) to tigecycline and levofloxacin, and 2 (33%) to ciprofloxacin. The antimicrobial susceptibility patterns we found are similar to those of other *Elizabethkingia* spp. identified

Table. Characteristics of patients infected with *Elizabethkingia bruniana*, Taiwan, 2005–2017, and antimicrobial susceptibility of the *E. bruniana* isolates*

Category	Patient no./isolate no.					
	No. 1/EM20-50	No. 2/EM455-89	No. 3/EM798-26	No. 4/EM828-05	No. 5/EM863-68	No. 6/EM891-63
Patient characteristics						
Year of illness	2005	2011	2015	2016	2016	2017
Age, y/sex	67/F	62/F	81/M	60/M	88/M	72/M
Site of microbe isolation	Urine	CVC tip	Blood	Blood	BAL fluid	BAL fluid
Clinical manifestations	Urinary tract infection	Septic shock	Primary bacteremia	Primary bacteremia	Pneumonia	Pneumonia, septic shock
Underlying conditions	Cervical cancer, hypertension	Maxillary osteosarcoma, hypothyroidism, hypertension	Lymphoma, chronic kidney disease	Brain meningioma, stroke, hypertension	Liver cirrhosis, hypertension, CHF	Esophageal cancer, diabetes mellitus, hypertension
Treatment	TMP/SMX	TZP	Levofloxacin	TZP, levofloxacin	Levofloxacin	Tigecycline, levofloxacin
Outcome	Survived	Survived	Survived	Survived	Survived	Survived
MIC, mg/L†						
Piperacillin	64	64	>64	64	>64	64
TZP	32/4	>128/4	32/4	32/4	64/4	128/4
Ceftazidime	>16	>16	>16	>16	>16	>16
Cefepime	>32	>32	>32	>32	>32	>32
Ceftriaxone	>32	>32	>32	>32	>32	>32
Aztreonam	>16	>16	>16	>16	>16	>16
Imipenem	>8	>8	>8	>8	>8	>8
Meropenem	>8	>8	>8	>8	>8	>8
Gentamicin	8	8	4	8	4	8
Tobramycin	>8	>8	>8	>8	>8	>8
Amikacin	<8	32	16	>32	16	32
Tetracycline	>8	>8	>8	>8	>8	>8
Minocycline	<1	<1	<1	4	<1	<1
Tigecycline	<1	8	2	4	<1	<1
Ciprofloxacin	1	2	2	>2	2	1
Levofloxacin	<1	8	<1	>8	<1	<1
TMP/SMX	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76

*BAL, bronchoalveolar lavage; CHF, congestive heart failure; CVC, central venous catheter; TMP/SMX, trimethoprim/sulfamethoxazole; TZP, piperacillin/tazobactam.

†Light gray shading indicates intermediate susceptibility; dark gray, susceptible isolates; no shading, resistant isolates.

in previous studies (7–10). For example, reports from the United States, Hong Kong, and South Korea have revealed that *E. anophelis* and *E. meningoseptica* were frequently resistant to most β -lactams, including ceftazidime, ceftriaxone, and imipenem, but showed variable susceptibility to piperacillin/tazobactam, cefepime, ciprofloxacin, and levofloxacin (7–10).

To investigate the association between target gene mutations and fluoroquinolone resistance, we examined the mutations present in quinolone resistance–determining regions in these 6 isolates. We did not find nonsynonymous substitutions in the quinolone resistance–determining regions of *gyrA*, *gyrB*, *parC*, and *parE*, which suggests that mutations in these genes are not the cause of fluoroquinolone resistance.

In summary, our study demonstrates the clinical manifestations of *E. bruniana* infection and the molecular characteristics of the pathogen. Because cases in our study were limited in number, further large-scale studies are necessary to investigate the antimicrobial susceptibility patterns of *E. bruniana* and elucidate the clinical characteristics and treatment of *E. bruniana* infection.

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About the Author

Dr. Jiun-Nong Lin is an associate professor at the School of Medicine, College of Medicine, I-Shou University, Kaohsiung, Taiwan. His research interests include infectious diseases and clinical microbiology.

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Address for correspondence: Jiun-Nong Lin, E-Da Hospital, Division of Infectious Diseases, Department of Internal Medicine, No. 1, Yida Road, Jiaosu Village, Yanchao District, Kaohsiung 824, Taiwan; email: jinoli@kmu.edu.tw

Human Enterovirus C105, China, 2017

Maozhong Li,¹ Tiegang Zhang,¹ Cheng Gong, Aihua Li, Ming Luo, Mei Dong, Fang Huang

Author affiliation: Beijing Center for Disease Prevention and Control, Beijing, China

¹These authors contributed equally to this article.

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We report a case of enterovirus C105 infection in an 11-year-old girl with lower respiratory tract symptoms that was identified through the Respiratory Virus Surveillance System, which covers 30 sentinel hospitals in all 16 districts of Beijing, China. The presence of this virus strain in China confirmed its geographically wide distribution.

Enteroviruses are small, nonenveloped RNA viruses that cause illnesses in humans ranging from mild to severe (1). Fifteen species of enterovirus are known, 7 of which are known to infect humans. These species include enterovirus A–D and rhinovirus A–C (1,2). The newly emerging genotype C105 (EV-C105) represents a novel monophyletic clade of enterovirus C; this strain was identified in 2010 in the Democratic Republic of the Congo (strain 34S) (3,4). EV-C105 cases from Italy (Pavia/8376, Pavia/9095), Romania (ROM31), the United States (USA/OK/2014-19362), New Zealand (strains not available), and Burundi (BU77, BU5) have been identified and characterized, suggesting that the spread of EV-C105 could be wider than previously hypothesized (5). Here, we report a detected case of EV-C105 in an 11-year-old girl with lower respiratory tract symptoms in Beijing, China.

The Beijing Center for Disease Prevention and Control established the Respiratory Virus Surveillance System (RVSS) in 2014. The RVSS tracks patients with respiratory tract infections (RTIs) and pneumonia in 30 sentinel hospitals throughout Beijing. The RVSS is an active system, designed to alert for future outbreaks of respiratory infections. To study enterovirus infections, we tested 24,093 clinical specimens (nasopharyngeal swab, sputum, and alveolar lavage fluid) from patients with RTIs that were reported through RVSS during June 2014–December 2017. RVSS classifies persons <14 years of age as children and those ≥14 years of age as adults. The ages of the reported patients ranged from 8 months to 93 years (median 33.5 years, mean 37.9 years).

We screened all samples using real-time PCR for influenza virus, parainfluenza virus types 1–4, respiratory syncytial virus, coronaviruses (229E, NL63, HKU1, and OC43), metapneumovirus, adenovirus, bocavirus, and enteroviruses (6). Overall, 445 (445/7,122; 6.2%) children and 276 (276/16,971; 1.6%) adults were positive for enterovirus or other respiratory viruses.

We further genotyped enterovirus-positive samples with primers sequentially targeting the viral protein (VP) 1 region (7,8). We obtained a 699-nt amplicon of EV-C105 from a nasopharyngeal swab sample collected at the time of a hospital visit (GenBank accession no. KX910099). The patient was an 11-year-old girl with no underlying disease who was brought to the outpatient clinic of the Beijing Children's

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Appendix

Antimicrobial Susceptibility Testing

The MICs of antimicrobial drugs were evaluated by using the broth microdilution method with Sensititer 96-well panels per the manufacturer's instructions (Thermo Fisher Scientific/Trek Diagnostics Systems, Oakwood Village, OH, USA). The susceptibilities were interpreted according to the criteria of other non-*Enterobacteriaceae* in the Clinical and Laboratory Standards Institute (CLSI) guidelines (1). There are no established CLSI interpretive criteria of other non-*Enterobacteriaceae* to tigecycline. Therefore, the susceptibility of tigecycline was interpreted according to the *Enterobacteriaceae* susceptibility breakpoints of the US Food and Drug Administration (susceptible MIC ≤ 2 mg/L, intermediate MIC 4 mg/L, resistant MIC ≥ 8 mg/L) (2).

Conditions of PCR Amplification for Quinolone-Resistance Determining Regions

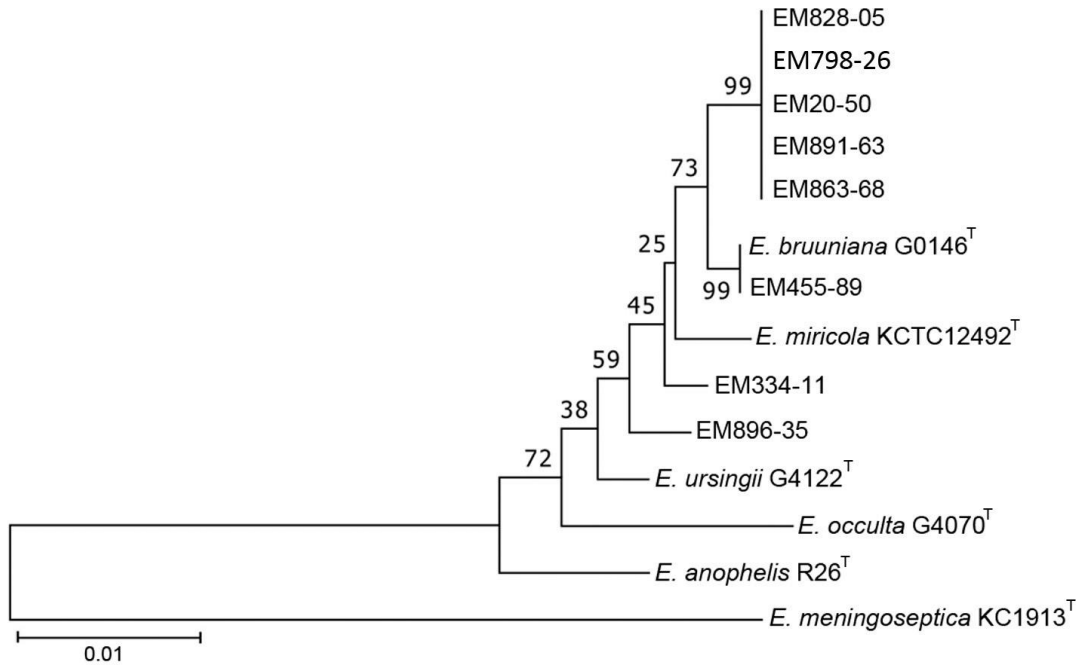
The conditions for amplification of quinolone resistance–determining regions were an initial extended denaturation step of 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C; a final extension at 5 min at 72°C; and then 5 min at 4°C.

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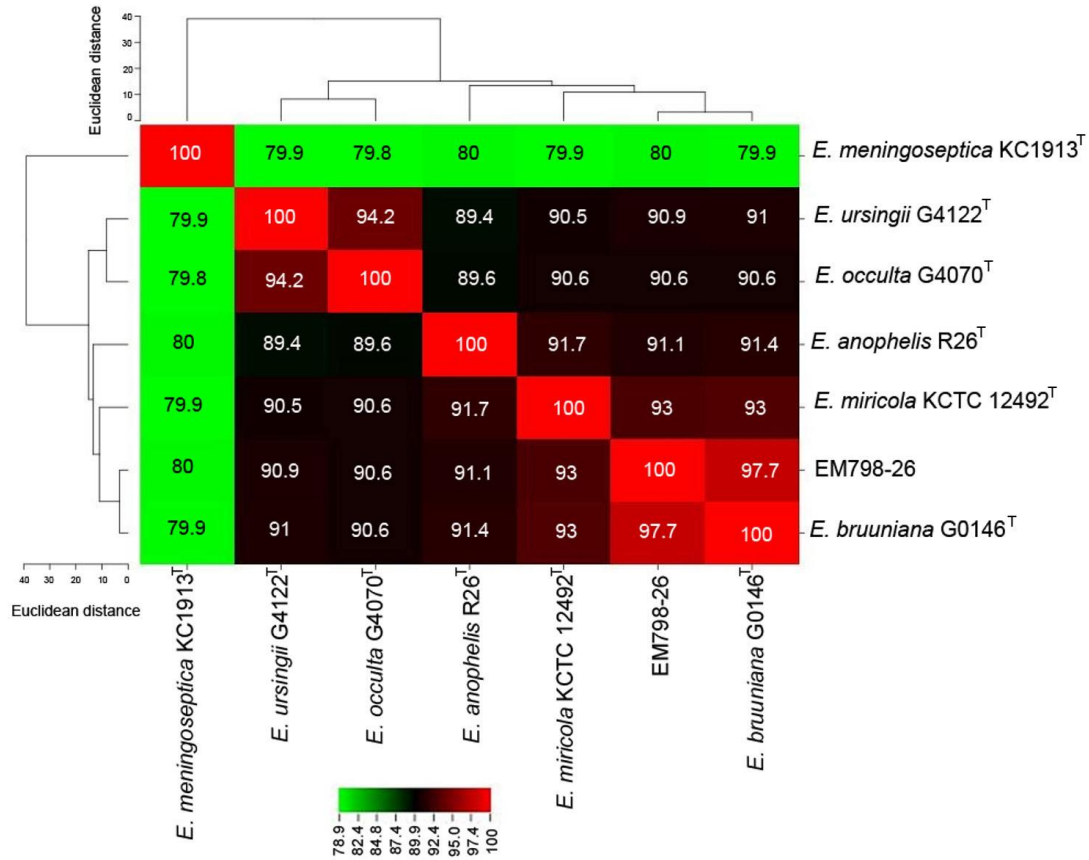
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Appendix Table. Primers for amplification and sequencing of quinolone resistance–determining regions in this study

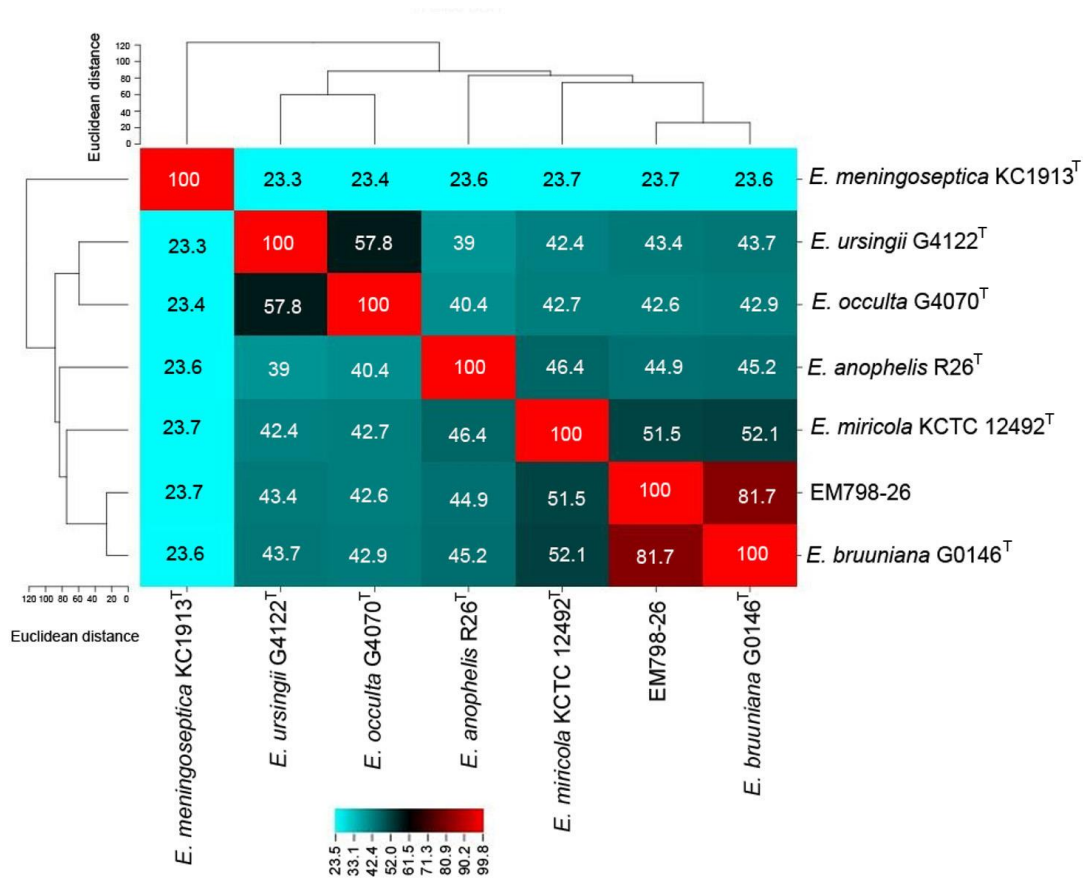
Primer	Sequence, 5'→3'	Amplicon size, bp	Strain, GenBank accession no.
gyrA-f	GGTTATCGTGTCAGAGCG	446	<i>E. bruniana</i> G0146 ^T , CP014337
gyrA-r	CCGCAATACCGGAAGTACCA		
gyrB-f	ATACGCACGAAGGAGGTACG	847	
gyrB-r	CGCTCTTTCTCGTTCCATGC		
parC-f	TGGTTTCTGGATTATGCCTCTT	446	
parC-r	CCTACACCAATCCCTTCTACTCC		
parE-f	GAGCTTCCGGTATTCAG	571	
parE-r	CACCAAGTCCCTTGAATC		



Appendix Figure 1. Phylogenetic analysis of the *rpoB* gene (positions 1,939–3,629) of *Elizabethkingia* genus type strains and isolates in this study. The phylogenetic tree was constructed by the maximum-likelihood method based on the JC69 model using MEGA 7 (3). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test of 1,000 replicates are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The GenBank accession numbers of the type strains are shown as follows: *E. meningoseptica* KC1913^T, CP014338; *E. anophelis* R26^T, CP023401; *E. miricola* KCTC 12492^T, NZ_FLSS01000007; *E. bruuniana* G0146^T, CP014337; *E. ursingii* G4122^T, LNOK01000023; and *E. occulta* G4070^T, NZ_MAHX01000006.



Appendix Figure 2. Average nucleotide identity between *Elizabethkingia* EM798-26 and type strains of the *Elizabethkingia* genus. The average nucleotide identity value between EM798-26 and *E. bruuniana* G0146^T is 97.7%.



Appendix Figure 3. The in silico DNA-DNA hybridization between *Elizabethkingia* EM798–26 and type strains of the *Elizabethkingia* genus. The DNA-DNA hybridization value between EM798-26 and *E. bruuniana* G0146^T is 81.7%.