Elizabethkingia anophelis bacteremia is associated with clinically significant infections and high mortality

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Unlike Elizabethkingia meningoseptica, the clinical importance of E. anophelis is poorly understood. We determined the clinical and molecular epidemiology of bacteremia caused by Elizabethkingia-like species from five regional hospitals in Hong Kong. Among 45 episodes of Elizabethkingia-like bacteremia, 21 were caused by Elizabethkingia, including 17 E. anophelis, three E. meningoseptica and one E. miricola; while 24 were caused by other diverse genera/species, as determined by 16S rRNA gene sequencing. Of the 17 cases of E. anophelis bacteremia, 15 (88%) were clinically significant. The most common diagnosis was pneumonia (n = 5), followed by catheter-related bacteremia (n = 4), neonatal meningitis (n = 3), nosocomial bacteremia (n = 2) and neutropenic fever (n = 1). E. anophelis bacteremia was commonly associated with complications and carried 23.5% mortality. In contrast, of the 24 episodes of bacteremia due to non-Elizabethkingia species, 16 (67%) were clinically insignificant. Compared to non-Elizabethkingia bacteremia, Elizabethkingia bacteremia was associated with more clinically significant infections (P < 0.01) and positive cultures from other sites (P < 0.01), less polymicrobial bacteremia (P < 0.01), and higher complication (P < 0.05) and mortality (P < 0.05) rates. Elizabethkingia bacteremia is predominantly caused by E. anophelis instead of E. meningoseptica.

Elizabethkingia bacteremia, especially due to E. anophelis, carries significant morbidity and mortality, and should be considered clinically significant unless proven otherwise.

The genus Elizabethkingia comprises aerobic, non-fermenting, non-motile and non-spore-forming gram-negative rods that were previously named Flavobacterium or belonged to CDC group IIa and later reclassified as Chryseobacterium in 1994. In 2005, Chryseobacterium meningosepticum and C. miricola were transferred to a new genus, Elizabethkingia, on the basis of combined phenotypic and phylogenetic characteristics. The genus comprises three medically important species, Elizabethkingia anophelis, E. meningoseptica and E. miricola. A novel species, E. endophytica, isolated from sweet corn, was also recently proposed. E. meningoseptica, previously named Flavobacterium meningosepticum or C. meningosepticum, is the best known species among the genus. E. meningoseptica is a causative agent of nosocomial infections especially in immunocompromised patients, as well as neonatal meningitis and sepsis. Besides soil, fresh water and plants, the bacterium can be found in hospital environments and may contaminate flushing solutions and medical devices. Infections caused by E. meningoseptica can be difficult to treat and carry high mortalities, which may be partly explained by their intrinsic multidrug resistance towards commonly used antibiotics such as β-lactams and aminoglycosides. Therefore,
accurate diagnosis is important to guide appropriate antibiotic regimens which often consist of a combination of ciprofloxacin or rifampicin with piperacillin-tazobactam or vancomycin.

In contrast to *E. meningoseptica*, the epidemiology and pathogenicity of *E. anophelis* and *E. miricola* were less well understood. *E. miricola*, originally named *C. miricola* when first isolated from condensed water obtained from the Russian space station, Mir, only rarely causes nosocomial infections in human7,8. On the other hand, *E. anophelis* was first isolated from midgut of the mosquito *Anopheles gambiae* in 20119. Soon after its discovery, it was reported to cause neonatal meningitis in the Central African Republic and a nosocomial outbreak in an intensive-care unit in Singapore10,11. The first discovery of *E. anophelis* from mosquito gut has raised suspicion on mosquitoes as the source of neonatal meningitis cases in Africa10. However, our recent report on *E. anophelis* meningitis in two neonates and chorioamnionitis in a neonate’s mother in Hong Kong suggested that mosquitoes were unlikely the vehicles of transmission12. Since the transmission route was initially obscure, draft genome sequencing was performed and showed evidence for perinatal vertical transmission from a mother to her neonate12. The ultimate resolution power of genome sequencing also enabled species confirmation and discrimination from the phenotypically similar species, *E. meningoseptica*12.

Since *E. anophelis* was commonly misidentified as *E. meningoseptica* in previous reports10–13, we hypothesize that many previously described *E. meningoseptica* isolates were actually *E. anophelis* and that *E. anophelis* may account for a significant proportion of *Elizabethkingia* infections. To better understand the epidemiology and clinical disease spectrum of *E. anophelis* and *Elizabethkingia* as a whole, we determined the clinical and molecular epidemiology of bacteremia caused by *Elizabethkingia*-like species from five regional hospitals in Hong Kong. All bacteremia episodes caused by *Elizabethkingia*-like species identified by conventional phenotypic tests from 2004 to 2013 during the study period were included. For the 45 episodes of *Elizabethkingia*-like bacteremia identified, 16S rRNA gene sequencing was performed for species identification and clinical characteristics and outcomes were analyzed.

### Results

**Identification of *Elizabethkingia*-like bacteremia by 16S rRNA gene sequencing.** Twenty-one of the 45 episodes of *Elizabethkingia*-like bacteremia were caused by *Elizabethkingia*, while 24 episodes were caused by diverse genera/species including *Chryseobacterium* (n = 15), *Flavobacterium* (n = 1), *Planobacterium* (n = 6), *Sphingobacterium* (n = 1) and *Weeksella* species (n = 1) according to 16S rRNA gene analysis (Fig. 1 and see Supplementary Table 1). Of the 21 episodes of *Elizabethkingia* bacteremia, 17 were caused by *E. anophelis* (99.0–99.9% nucleotide identity to *E. anophelis* type strain R265), three by *E. meningoseptica* (99.4–99.8% nucleotide identity to *E. meningoseptica* type strain ATCC 13253) and one by *E. miricola* (99.5% nucleotide identity to *E. miricola* type strain LMG 22470). Among the 24 episodes of non-*Elizabethkingia* bacteremia, 15 were caused by *Chryseobacterium* species, among which two (C13 and C13) represented two potentially novel *Chryseobacterium* species (≤98.4% nucleotide identity to existing *Chryseobacterium* species). One episode was caused by another potentially novel species most closely related to *Weeksella* (91.9% nucleotide identity to *Weeksella virosa* DSM 16922). The other eight

| Characteristics                        | Number of patients (%) | P-value |
|----------------------------------------|------------------------|---------|
|                                       | Elizabethkingia bacteremia (n = 21) | Non-Elizabethkingia bacteremia (n = 24) | |
| Sex (male:female)                      | 11:10                  | 13:11   | 0.90   |
| Underlying diseases                    | 19 (90.5)              | 24 (100) | 0.12   |
| Hospital vs community-acquireda        | 15:4                   | 7:1     | 0.60   |
| Diagnosisb                            |                        |         |        |
| Biliary tract infection                | 2 (9.5)                | 0 (0)   | 0.12   |
| Catheter-related bacteremia            | 5 (23.8)               | 1 (4.2) | 0.05   |
| Community-acquired pneumonia           | 3 (14.3)               | 0 (0)   | 0.06   |
| Neonatal meningitis                    | 3 (14.3)               | 0 (0)   | 0.06   |
| Neutropenic fever                      | 1 (4.8)                | 2 (8.3) | 0.63   |
| Nosocomial bacteremia                  | 3 (14.3)               | 5 (20.8)| 0.57   |
| Nosocomial pneumonia                   | 2 (9.5)                | 0 (0)   | 0.12   |
| Primary bacteremia                     | 0 (0)                  | 1 (4.2) | 0.34   |
| Pseudobacteremia                       | 2 (9.5)                | 16 (76.1)| 0.00009|
| >1 positive blood cultures             | 3 (14.3)               | 1 (4.2) | 0.23   |
| Polymicrobial bacteremia               | 0 (0)                  | 10 (41.7)| 0.00008|
| Positive cultures from other sites     | 8 (38.1)               | 1 (4.2) | 0.005  |
| Complications                          | 7 (33.3)               | 1 (4.2) | 0.01   |
| Attributable mortality                 | 4 (19.0)               | 0 (0)   | 0.025  |

Table 1. Characteristics of the 45 patients with bacteremia caused *Elizabethkingia*-like organisms

*a*excluding pseudobacteremia. *b*The percentages add up to more than 100% because some patients have more than one diagnosis. *c*by Chi-square test.
Episodes were caused by *Planobacterium* (n = 6), *Flavobacterium* (n = 1) and *Sphingobacterium* (n = 1) species respectively.

**Clinical characteristics of patients with Elizabethkingia-like bacteremia.** The clinical characteristics of the 45 episodes of *Elizabethkingia*-like bacteremia were summarized in Tables 1–3. Of the 17 episodes of *E. anophelis* bacteremia, the male:female ratio was 11:6, with a median age of 58 years (range, 1 day to 104 years) (Table 2). Most patients had underlying diseases. Except two patients with pseudobacteremia, most cases were associated with clinically significant bacteremia, with 12 cases being hospital-acquired and three community-acquired. The most common diagnosis was pneumonia (n = 5), followed by catheter-related bacteremia (n = 4), neonatal meningitis (n = 3), nosocomial bacteremia (n = 2) and neutropenic fever (n = 1). Among the five cases of pneumonia, three were community-acquired, including one in a 51-year-old previously healthy man (case EA10). All three cases of neonatal meningitis were hospital-acquired. Details of two (HKU36 and HKU38) of the three neonatal meningitis cases have been reported previously 

![Phylogenetic tree showing the relationship of the 45 Elizabethkingia-like blood culture isolates to related bacterial species using 16S rRNA gene sequence analysis.](image)

The tree was constructed by maximum likelihood method using General Time Reversible model and *Escherichia coli* (CP010304) as the root. A total of 1325 nucleotide positions were included in the analysis. Bootstrap values were calculated from 1000 replicates. The scale bar indicates the number of substitutions per site. Names and accession numbers are given as cited in GenBank database.
| Case/strain no. | Sex | Age | Underlying diseases | Diagnosis | Community- or hospital-acquired | No. of positive blood cultures (concomitant isolates) | Other culture-positive specimens | Antibiotic treatment | Complications/outcome |
|----------------|-----|-----|---------------------|-----------|-------------------------------|---------------------------------------------------|----------------------------------|---------------------|----------------------|
| EA1            | M   | 54  | DM, hyperlipidemia, ischemic cardiomyopathy | Nosocomial pneumonia | HA | 1 | None | Piperacillin-tazobactam | Acute pulmonary edema, CHF, survived |
| EA2            | M   | 65  | HT, DM, CAD, PVD, RAS, hyperlipidemia, carotid stenosis | Pseudobacteremia | NA | 1 | None | Cloxacillin and levofloxacin, then amoxicillin-clavulanate | Survived |
| EA3            | F   | 1m  | Prematurity, twin, RDS, PDA | Catheter-related bacteremia | HA | 3 | None | Vancomycin, cefoperazone-sulbactam | Multi-organ failure, died |
| EA4            | F   | 8d  | Imperforated anus, RDS, PDA | Neonatal meningitis | HA | 2 | CSF | Vancomycin and rifampicin | Survived |
| EA5            | M   | 65  | COPD, CAD, CHF, AF, CRHD, right hip AVN | Community-acquired pneumonia | CA | 1 | Sputum | Ciprofloxacin | Survived |
| EA6            | M   | 37  | Dilated cardiomyopathy, CAD | Nosocomial bacteremia | HA | 1 | None | Ciprofloxacin | DIC, survived |
| EA7            | F   | 64  | HT, hyperlipidemia, stage 4 DLBCL lymphoma | Neutropenic fever, severe mucositis | HA | 2 | None | Meropenem | Septic shock, haematomasis, died |
| EA8            | M   | 68  | HT, CRF, AAA | Nosocomial bacteremia | HA | 1 | Tracheal aspirate | Levofloxacin and vancomycin | Acute renal failure, died |
| EA9            | M   | 73  | CA hypopharynx, CA ampulla of Vater | Pseudobacteremia | NA | 1 | None | Cefuroxime | Survived |
| EA10           | M   | 51  | None | Community-acquired pneumonia | CA | 1 | Sputum | Amoxicillin-sulbactam, then ciprofloxacin | Survived |
| EA11           | F   | 104 | HT, CAD, CVA, nephrotic syndrome | Community-acquired pneumonia | CA | 1 | None | Amoxicillin-clavulanate | Died |
| EA12           | M   | 59  | obesity, DM, CAD | Catheter-related bacteremia, drip site cellulitis | HA | 1 | None | Levofloxacin | Survived |
| EA13           | F   | 35  | Epilepsy, HT, ESRF on HD | Catheter-related bacteremia | HA | 1 | None | Levofloxacin | Survived |
| EA14           | M   | 58  | CRHD with AVR, ESRF on HD | Catheter-related bacteremia | HA | 1 | Catheter tip | Piperacillin-tazobactam, then levofloxacin | Septic shock, survived |
| EA15           | M   | 88  | COPD, old PTB | Nosocomial (aspiration) pneumonia | HA | 1 | None | Levofloxacin | Survived |
| HKU36          | M   | 21d | None | Neonatal meningitis | HA | 1 | CSF | Vancomycin, piperacillin and rifampicin | Survived |
| HKU38          | F   | 1d  | Apnea of prematurity | Neonatal meningitis | HA | 1 | CSF | Vancomycin, piperacillin-tazobactam and rifampicin | Metabolic acidosis, IVH, survived |

### Table 2. Clinical characteristics of patients with *Elizabethkingia* bacteremia.

- AAA, abdominal aortic aneurysm; AF, atrial fibrillation; AVN, avascular necrosis; AVR, atrial valve replacement; CA, carcinoma; CA, community-acquired; CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; CRHD, chronic rheumatic heart disease; CSF, cerebrospinal fluid; CVA, cerebrovascular accident; CVP, central venous pressure; DIC, disseminated intravascular coagulation; DLBCL, diffuse large B cell; DM, diabetes mellitus; ESRF, end-stage renal failure; HA, hospital-acquired; HD, hemodialysis; HT, hypertension; IVH, intraventricular hemorrhage; MSSA, methicillin-sensitive...
Staphylococcus aureus: NA, not applicable; PDA, patent ductus arteriosus; PTB, pulmonary tuberculosis; PVD, peripheral vascular disease; RAS, renal artery stenosis; RDS, respiratory distress syndrome; RPC, recurrent pyogenic cholangitis.

carbapenems, vancomycin and rifampicin. Removal of catheter was often required in catheter-related bacteremia cases in addition to antibiotic treatment.

All the four patients with E. meningoseptica or E. miricola bacteremia also had underlying disease and clinically significant infections (Table 2). Two patients with E. meningoseptica bacteremia had biliary tract infections, while one had nosocomial bacteremia. Interestingly, the only episode of E. miricola bacteremia (case EMI1) was detected in the same patient with E. anophelis nosocomial pneumonia (case EA1) which occurred more than one month previously during the same admission and was complicated by heart failure and acute pulmonary edema requiring intubation and inotropes. Five weeks after successful treatment with piperacillin-tazobactam, the patient developed another episode of bacteremia caused by E. miricola which was also isolated from central venous catheter. The patient recovered with levofloxacin and catheter removal.

In contrast, 16 of the 24 patients with bacteremia due to non-Elizabethkingia species had pseudobacteremia (Table 3). The other eight patients were diagnosed to have nosocomial bacteremia (three cases), transfusion-related bacteremia (two cases), catheter-related bacteremia (one case), neutropenic fever (one case) and sepsis (one case). Except for a case of community-acquired sepsis in a 90-year-old man (case P1), all other cases were hospital-acquired. Only one episode of catheter-related bacteremia was complicated by septic shock, where C. arthrosphaerae was isolated from six blood cultures. All the eight patients survived.

Comparison between Elizabethkingia and non-Elizabethkingia cases showed that clinically significant infections were more common in patients with Elizabethkingia bacteremia than those with non-Elizabethkingia bacteremia (i.e. fewer pseudobacteremia cases were observed) (P < 0.01). Moreover, patients with Elizabethkingia bacteremia had more positive cultures from other sites (P < 0.05) and lower incidence of polymicrobial bacteremia (P < 0.01). They also showed higher complication (P < 0.05) and mortality (P < 0.05) rates than those with non-Elizabethkingia bacteremia (Table 1).

Microbiological characteristics of Elizabethkingia isolates. All the 21 Elizabethkingia isolates were non-motile, oxidase-positive, catalase-positive, indole-positive, non-glucose-fermenting, Gram-negative bacilli. Growth on MacConkey agar, citrate utilization, urea hydrolysis and fermentation of cellulose and melibiose fermentation were variable. All 21 isolates were identified as E. meningoseptica by Vitek 2 GNI system with 91–99% confidence. All 21 isolates were susceptible to ciprofloxacin, ceftazidime, piperacillin, rifampicin and cotrimoxazole were variable (see Supplementary Table 2).

MALDI-TOF MS using Reference Library Biotyper v3.1.2.0 (Bruker Daltonik, Germany) failed to identify the 17 E. anophelis isolates (10 misidentified as E. meningoseptica with score 2.073 to 2.403, two identified as Elizabethkingia species with score 1.952–1.971 and five unidentified with score 1.32 to 1.42 to E. meningoseptica). When the database was expanded with inclusion of mass spectra from seven E. anophelis isolates, all the other 10 E. anophelis isolates were correctly identified as E. anophelis with score 2.321 to 2.634. The three E. meningoseptica and one E. miricola strains were correctly identified by the Bruker reference library (see Supplementary Table 2). Hierarchical cluster analysis showed that the protein mass spectra of E. anophelis and E. miricola were clustered together but formed a distinct branch from E. meningoseptica (Fig. 2).

PFGE typing of E. anophelis. To determine the genetic relatedness of the 17 E. anophelis strains isolated from five different hospitals, PFGE was performed using the bacterial genomic and DNA restriction endonuclease XbaI. In general, the PFGE patterns among the 17 E. anophelis strains were distinct from each other and different from that of the type strain R26

Dendrogram constructed using the PFGE images showed that some adjacent clusters were isolated from the same hospital (Fig. 3). For example, strains EA2, EA3 and EA4 were from hospital 5, while strains EA13 and EA15 were from hospital 6. However, no clear epidemiological linkage could be identified in terms of place and time of blood culture isolation. Specifically, strains EA2, EA3 and EA4 were isolated from different years. Strains EA13 and EA15 were isolated four months apart and the two patients were hospitalized in different wards of hospital 6.

Discussion

E. anophelis bacteremia should be considered clinically significant unless proven otherwise and should prompt appropriate antibiotic therapy. In contrast to the traditional belief that E. meningoseptica was the most important Elizabethkingia species associated with bacteremia, the present study showed that the majority of Elizabethkingia bacteremia cases were caused by E. anophelis. In particular, neonatal meningitis was associated exclusively with E. anophelis in this study. In the present series, E. anophelis bacteremia mainly occurred in neonates or adults with underlying medical illnesses, and was most commonly associated with pneumonia and hospital-acquired infections such as catheter-related bacteremia and neonatal meningitis. Notably, community-acquired pneumonia occurred in three patients including a healthy middle-aged adult, where the infective source remained obscure. Apart from the two previous reported cases of neonatal meningitis (HKU36 and HKU38) which were most likely acquired from maternal-to-infant transmission based on comparative genomics

no obvious epidemiological linkage or genetic relatedness was identified among the other cases. Nevertheless, some potentially genetically related strains upon PFGE analysis may have circulated in the same hospital. Further studies should be performed to better understand the disease spectrum and transmission routes of E. anophelis.
| Case/strain no. | Sex | Age | Underlying diseases | Diagnosis | Community- or hospital-acquired | No. of positive blood cultures (concomitant isolates) | Other culture-positive specimens | Treatment + removal of catheter | Complications + outcome |
|----------------|-----|-----|---------------------|-----------|----------------------------------|------------------------------------------------------|----------------------------------|-------------------------------|---------------------------|
| **Potentially novel Chryseobacterium species** | | | | | | | | | |
| C13            | M   | 64  | Metastatic pancreatic carcinoma, sepsis | Postmortem pseudobacteremia | NA | 1 | None | NA | Died (non-attributable) |
| C15            | M   | 68  | Head injury | Pseudobacteremia | NA | 1 (Acinetobacter sp., Enterococcus faecium) | No | Amoxicillin-clavulanate | Survived |
| **Chryseobacterium arthropaeae** | | | | | | | | | |
| C1             | M   | 82  | HT, gout, BPH, bilateral hydronephrosis and hydrourerter | Nosocomial bacteremia | HA | 1 | None | Piperacillin-tazobactam, then levofloxacin | Survived |
| C2             | F   | 48  | Stage IV DLBC lymphoma | Cathe-ter-related bacteremia, neutropenic fever | HA | 6 (Klebsiella pneumoniae, Acinetobacter baumanii) | Central catheter | Imipenem-cilastatin and amikacin | Septic shock, survived |
| C9             | M   | 56  | COPD, pneumothorax | Nosocomial bacteremia | HA | 1 | None | Ticarcillin-clavulanate, levofloxacin | Survived |
| **Chryseobacterium gallinarum** | | | | | | | | | |
| C14            | M   | 1   | Cow milk allergy | Nosocomial bacteremia | HA | 1 | None | Piperacillin-tazobactam | Survived |
| **Chryseobacterium hominis** | | | | | | | | | |
| C8             | F   | 76  | DM, HT, CVA, CHF, AAA, PVD, hyperlipidemia | Pseudobacteremia | NA | 1 | None | None | Survived |
| C12            | F   | 51  | AML | Neutropenic fever | HA | 1 (Moraxella sp.) | None | Piperacillin-tazobactam, vancomycin | Survived |
| **Chryseobacterium indologenes** | | | | | | | | | |
| C3             | F   | 58  | Hyperlipidemia, IgA nephropathy, Multiple myeloma | Pseudobacteremia | NA | 1 (Stenotrophomonas maltophilia, Com- mamonas sp., Balstonia sp.) | None | Levofloxacin, piperacillin-tazobactam | Survived |
| C4             | F   | 49  | DM, CREF, bilateral hydronephrosis, perinephric and psoas abscess | Pseudobacteremia | NA | 1 (Enterococcus faecalis, A. baumanii, Streptococcus mitis) | None | Cefuroxime, ampicillin, levofloxacin | Survived |
| C5             | M   | 78  | HT, gout, CA c ecum with metastases, thyroidectomy | Pseudobacteremia | NA | 1 (A. baumanii) | None | Amoxicillin-clavulanate, ciprofloxacin | Survived |
| C6             | F   | 57  | CA rectum, mania, small bowel obstruction | Pseudobacteremia | NA | 1 (E. faecalis, A. baumanii) | None | Amoxicillin-clavulanate | Survived |
| C7             | M   | 84  | HT, AFCRHD, CVA, gout, BPH COPD | Pseudobacteremia | NA | 1 | None | Amoxicillin-clavulanate | Survived |
| C10            | F   | 56  | Acute encephalopathy, aspiration pneumonia, sepsis | Postmortem pseudobacteremia | NA | 1 (Pseudomonas putida, Bacillus sp., CNS, MSSA, non-hemolytic Streptococcus) | None | NA | Died (non-attributable) |
| **Chryseobacterium taihuense** | | | | | | | | | |
| C11            | M   | 85  | DM, DU, BPH | Pseudobacteremia | NA | 1 | None | Amoxicillin-clavulanate | Survived |
| **Flavobacterium lindanitolerans** | | | | | | | | | |
| F1             | F   | 30  | SLE, lupus nephritis | Nosocomial (post-transfusion) bacteremia | HA | 1(Bacillus sp.) | None | Levofloxacin | Survived |
| **Flavobacterium taklimakanense** | | | | | | | | | |
| P1             | M   | 90  | CAD, COPD, DM, BPH | Primary bacteremia | CA | 1 | None | Ceftriaxone | Survived |
| P2             | M   | 1d  | RDS, congenital pneumonia | Pseudobacteremia | NA | 1 | None | Penicillin and netilmicin | Survived |
| P3             | F   | 99  | COPD, CVA, intertrochanteric fracture | Pseudobacteremia | NA | 1 | None | Amoxicillin-clavulanate | Survived |
| P4             | M   | 56  | COPD, BPH, PTB | Pseudobacteremia | NA | 1 | None | Levofloxacin | Survived |
| P5             | M   | 52  | NPC, thyroidectomy, hypothyroidism | Nosocomial (post-transfusion) bacteremia | HA | 1 | None | Piperacillin-tazobactam | Survived |
| P6             | F   | 1m  | Sepsis, hypoglycemia | Pseudobacteremia | NA | 1 | None | Cefotaxime | Survived |

Continued
Table 3. Clinical characteristics of patients with non-Elizabethkingia bacteremia. *AAA, abdominal aortic aneurysm; AF, atrial fibrillation; AML, acute myeloid leukemia; BPH, benign prostatic hyperplasia; CA, carcinoma; CAD, coronary artery disease; CHF, congestive heart failure; CNS, coagulase-negative Staphylococcus; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; CRHD, chronic rheumatic heart disease; CVA, cerebrovascular accident; DLBC, diffuse large B cell; DM, diabetes mellitus; DU, Duodenal ulcer; HA, hospital-acquired; HT, hypertension; MSSA, methicillin-sensitive Staphylococcus aureus; NA, not applicable; NPC, nasopharyngeal carcinoma; PTD, pulmonary tuberculosis; PVD, peripheral vascular disease; RDS, respiratory distress syndrome; RSV, respiratory syncytial virus; SLE, systemic lupus erythematosus.

| Case/strain no. | Sex | Age | Underlying diseases | Diagnosis | Community- or hospital-acquired | No. of positive blood cultures (concomitant isolates) | Other culture-positive specimens | Treatment + removal of catheter | Complications + outcome |
|----------------|-----|-----|---------------------|-----------|-------------------------------|-----------------------------------------------|---------------------------------|-------------------------------|--------------------------|
| S1             | M   | 94  | COPD, cor pulmonale, BPH, gout, Shy-Drager syndrome, CAD | Pseudobacteremia | NA                           | 1 (K. pneumoniae)                             | None                            | Cotrimoxazole                | Survived                 |
| Potentially novel |     |     |                     |           |                               |                                               |                                 |                               |                          |
| W1             | F   | 18d | RSV pneumonia       | Pseudobacteremia | NA                           | 1                                             | None                            | Ampicillin, netilmicin and erythromycin | Survived                 |

_E. anophelis_ bacteremia carries significant morbidity and mortality. Various complications were observed and four patients died, giving a mortality rate of 23.5%. This is in line with previous reports of _E. anophelis_ neonatal meningitis being associated with poor outcomes. Nevertheless, all the three present cases of neonatal meningitis were cured with early use of appropriate antibiotics. Although _E. anophelis_ usually confers resistance to multiple antibiotics such as ceftazidime, imipenem and aminoglycosides, all the 17 isolates were susceptible to ciprofloxacin, cefoperazone-sulbactam and vancomycin, which should be considered in empirical treatment while awaiting susceptibility results. In cases of catheter-related bacteremia, infected catheters should be removed in addition to antibiotic treatment. Future prospective studies with population-based data should be performed to determine the prevalence or incidence of _E. anophelis_ bacteremia.

_E. meningoseptica_ and _E. miricola_ appeared to be much less prevalent than _E. anophelis_, although similar studies in other countries are required to more accurately assess their relative importance. Similar to _E. anophelis_, _E. meningoseptica_ and _E. miricola_ bacteremia were associated with clinically significant infections. Besides one case of _E. meningoseptica_ nosocomial bacteremia and one case of _E. miricola_ catheter-related bacteremia, biliary tract infections were also noted in two cases of _E. meningoseptica_ bacteremia. It remains to be determined if _E. meningoseptica_ may have the propensity to cause biliary tract infections among the genus. Given their similar antibiotic susceptibility profiles to that of _E. anophelis_, ciprofloxacin, cefoperazone-sulbactam and vancomycin should also be included in treatment regimens for _E. meningoseptica_ and _E. miricola_ bacteremia.

In contrast to _Elizabethkingia_ species, isolation of non- _Elizabethkingia_ species from blood cultures should raise suspicion of their clinical significance. In this study, non- _Elizabethkingia_ bacteremia is associated with higher incidence of pseudobacteremia and polymicrobial bacteremia than _Elizabethkingia_ bacteremia. In particular, all six isolates of _C. indologenes_ and the three potentially novel species were associated with pseudobacteremia. Bacteremia caused by non- _Elizabethkingia_ species is also associated with lower incidence of complications and mortality than _Elizabethkingia_ bacteremia, suggesting that these bacterial species may be less virulent than _Elizabethkingia_. Moreover, these environmental, non- _Elizabethkingia_ bacterial species may contaminate blood cultures when asymptotic techniques during blood taking are breached. Careful clinical assessment is required to determine the clinical significance and the need for antibiotics when these bacteria are isolated from blood cultures.

Although the two _E. anophelis_ strains, HKU36 and EA14, are phylogenetically genetically close to _E. endophytica_ strain JM-87, they should belong to _E. anophelis_ instead of _E. endophytica_ or a novel species. The two strains possessed 99.9% nucleotide identities to both the newly proposed species, _E. endophytica_ strain JM-87 and _E. anophelis_ R26 in their 16S rRNA gene sequences. Although strain JM-87 was reported to possess 51–52% similarities to _E. anophelis_ R26 by DNA-DNA hybridization, our previous study showed that the draft genome of strain HKU36 possessed 78.3% nucleotide identity to the genome of _E. anophelis_ R26 by estimation of intergenomic distance. Given the close relatedness of strains HKU36, EA14 and JM-87 in their 16S rRNA genes (Fig. 1), it is likely that the genome sequences of strain JM-87 and EA14 may also possess >70% identity to that of _E. anophelis_ R26. Since genome-based comparison can offer ultimate resolution for species delineation which is superior to traditional DNA-DNA hybridization methods, genome sequencing of strain JM-87 and related strains such as EA14 should be performed to more accurately define their taxonomic positions.

The present results confirmed our suspicion that _E. anophelis_ was a previously under-reported bacterium which can be easily misidentified as _E. meningoseptica_. Although _E. anophelis_ was first discovered from mosquito gut, we previously showed that maternal chorioamnionitis, instead of mosquitoes, was more likely the source of neonatal meningitis. Similarly, mosquitoes are unlikely the route of transmission in other _E. anophelis_ infections. We speculate that contaminated environments, such as infected catheters, are the source of infection in most cases, as in the case of previously described _E. meningoseptica_ infections. Given their similar phenotypic characteristics, _E. anophelis_ isolates from previous reports were often mistaken as _E. meningoseptica_ initially. Phenotypic tests, such as acid production from cellobiose and citrate utilization, previously reported as potentially useful for species discrimination, were unlikely to be reliable. In our previous study, the three _E.
anophelis strains were also initially misidentified as *E. meningoseptica* even with MALDI-TOF MS, owing to the absence of *E. anophelis* spectra in commercial databases. The 16S rRNA genes of *E. anophelis* possessed >98% nucleotide identity to those of *E. meningoseptica* and *E. miricola*, which should offer sufficient discriminative

**Figure 2.** Results of MALDI-TOF MS identification of the 21 *Elizabethkingia* strains. In panel (A), representative MALDI-TOF MS spectra of the three *Elizabethkingia* species are shown. In panel (B), dendrogram was generated from hierarchical clustering of MALDI-TOF MS spectra of 21 *Elizabethkingia* isolates and reference strains of *E. meningoseptica* and *E. miricola*, using ClinProTools 3.0 (Bruker Daltonics, Germany). Distances are displayed in relative units.

**Figure 3.** Pulsed-field gel electrophoresis (PFGE) analysis of the 17 *E. anophelis* isolates and *E. anophelis* type strain R26. (lane 1 = EA1, lane 2 = EA2, lane 3 = EA3, lane 4 = EA4, lane 5 = EA5, lane 6 = EA6, lane 7 = EA7, lane 8 = EA8, lane 9 = EA9, lane 10 = EA10, lane 11 = EA11, lane 12 = EA12, lane 13 = EA13, lane 14 = EA14, lane 15 = EA15, lane 16 = HKU38, lane 17 = HKU36, lane 18 = R26, M = lambda marker). In Panel (A), PFGE was performed using CHEF Mapper XA system (Bio-Rad) and restriction endonuclease *Xba*I. Results showed that the 17 isolates possessed distinct PFGE patterns. In Panel (B), dendrogram was constructed with PFGE data by similarity and clustering analysis using the Dice coefficient (1% tolerance and 0.5% optimization) and unweighted pair-group method using average linkages with GelCompar II.
power. However, some “E. meningoseptica” strains with 16S rRNA sequences deposited in GenBank, such as strains G3-1-08 and 50215, should belong to E. anophelis based on phylogenetic analysis. These “misidentified” strains in GenBank may confuse the interpretation of 16S rRNA gene sequencing results and should be rectified. While E. anophelis can be distinguished from E. meningoseptica by MALDI-TOF MS when the database is expanded with mass spectra from E. anophelis strains, E. anophelis and E. miricola appear to be indistinguishable from each other. With an expanded database using E. anophelis isolates, MALDI-TOF MS is the method of choice for rapid and accurate diagnosis of E. anophelis infections, which is crucial to better understand its epidemiology and clinical disease spectrum.

**Methods**

**Ethics statement.** The use of blood culture isolates and anonymous clinical data were approved by Institute Review Board, The University of Hong Kong/Hospital Authority, Hong Kong (reference UW 04-278 T/600). The methods and all experimental protocols were carried out in accordance with the approved guidelines. Since this study does not involve experimentation on human subjects or the use of tissue samples from human subjects, written informed consent has been waived by our institutional review board.

**Settings and Patients.** Patients were hospitalized in five regional hospitals located in different areas of Hong Kong from 2004 to 2013. To identify potential cases of Elizabethkingia bacteremia, all bacteremia episodes caused by oxidase-positive, non-glucose fermenters that were identified as Elizabethkingia, Flavobacterium or Chryseobacterium species by conventional phenotypic tests during the study period were included with clinical data analyzed. Bacteremia was categorized as clinically significant or pseudobacteremia (calibration of blood culture) by clinical and laboratory criteria. The criteria included the patient's clinical presentation, physical examination findings, body temperature at the time of the blood culture, leukocyte and differential cell counts, imaging or operative results, histopathological findings, number of positive blood cultures out of the total number performed, and response to treatment.

**Bacterial isolates.** Collection of clinical specimens, bacterial cultures and conventional phenotypic identification were performed according to standard protocols. Two of the 45 Elizabethkingia-like isolates from two neonates (HKU36 and HKU38) have been reported previously. The same isolate recovered from the same patient was counted only once.

**16S rRNA gene sequencing for species identification.** The 45 blood culture isolates were subject to 16S rRNA gene sequencing according to previously published protocols with modifications, using primers LPW57 (5′-AGTTTGATCCTGGCTCAG-3′) and LPW58 (5′-AGGCCCGGGAACGTATTCAC-3′). The sequences of PCR products were compared to known gene sequences in GenBank by multiple sequence alignment using CLUSTAL_W in MEGA version 6. Phylogenetic tree was constructed by maximum likelihood method using MEGA version 6.

**Statistical analysis.** A comparison of characteristics was made between patients with Elizabethkingia and non-Elizabethkingia bacteremia using Chi-square test (IBM SPSS Statistics version 19). P < 0.05 was regarded as statistically significant.

**Phenotypic characterization and matrix-assisted laser-desorption ionization-time-of-flight mass-spectrometry (MALDI-TOF MS) of Elizabethkingia isolates.** The 21 Elizabethkingia isolates were characterized by phenotypic tests, Vitek 2 GNI system (bioMérieux, France) and MALDI-TOF MS. MALDI-TOF MS was performed by ethanol formic acid extraction method as described previously, using Bruker Daltonics microflex LT system with Reference Library Biotype v3.1.2.0 (Bruker Daltonik, Germany). Since E. anophelis is not included in the Bruker reference, mass spectra generated from seven E. anophelis strains with identity confirmed by 16S rRNA gene sequencing were later added to the database. Obtained spectra were subject to hierarchical cluster analysis as described previously. Antibiotic susceptibility testing was performed by Kirby Bauer disk diffusion method with results interpreted according to Clinical and Laboratory Standards Institute for Staphylococcus aureus (vancomycin) and Pseudomonas aeruginosa (other drugs), because of the lack of interpretative criteria for Elizabethkingia.

**Pulsed-field gel electrophoresis (PFGE).** The 17 E. anophelis isolates were characterized by PFGE using CHEF Mapper XA system (Bio-Rad, CA, USA) and restriction endonuclease XhoI as described previously. After PFGE, the gel was stained with ethidium bromide (1 μg/ml) for 30 minutes and the patterns of the genomic DNA digest were visualized with a UV transilluminator. Digital images were stored electronically as TIFF files and analyzed visually and with GelCompar II (version 3.0; Applied Maths, Kortrijk, Belgium), and represented by UPGMA method.

**Nucleotide sequence accession number.** The 16S rRNA gene sequences of the 45 blood culture isolates have been deposited in the GenBank sequence database under accession no. KP875383 to KP875427.

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Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Lau, S. K. P. et al. *Elizabethkingia anophelis* bacteremia is associated with clinically significant infections and high mortality. *Sci. Rep.* 6, 26045; doi: 10.1038/srep26045 (2016).

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