

Epidemiology of *Elizabethkingia* spp. Infections in Southeast Asia

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Elizabethkingia spp. is a ubiquitous pathogenic bacterium that has been identified as the causal agent for a variety of conditions such as meningitis, pneumonia, necrotizing fasciitis, endophthalmitis, and sepsis and is emerging as a global threat including in Southeast Asia. *Elizabethkingia* infections tend to be associated with high mortality rates (18.2–41%) and are mostly observed in neonates and immunocompromised patients. Difficulties in precisely identifying *Elizabethkingia* at the species level by traditional methods have hampered the understanding of this genus in human infections. In Southeast Asian countries, hospital outbreaks have usually been ascribed to *E. meningoseptica*, whereas in Singapore, *E. anophelis* was reported as the main *Elizabethkingia* spp. associated with hospital settings. Misidentification of *Elizabethkingia* spp. could, however, underestimate the number of cases attributed to the bacterium, as precise identification requires tools such as MALDI-TOF MS, and particularly whole-genome sequencing, which are not available in most hospital laboratories. *Elizabethkingia* spp. has an unusual antibiotic resistance pattern for a Gram-negative bacterium with a limited number of horizontal gene transfers, which suggests an intrinsic origin for its multidrug resistance.

Keywords: *Elizabethkingia* spp. ; antibiotic resistance ; multidrug resistance ; meningitis ; bacteremia ; outbreak ; Southeast Asia

1. Introduction

The Gram-negative bacteria of the genus *Elizabethkingia* have recently emerged as an important pathogen in hospital-acquired infections and are generally associated with high mortality ^[1]. Recent literature has reported several cases of severe infection in humans owing to this organism, with neonatal meningitis most commonly presented in children ^[2], accompanied by a range of other clinical manifestations such as septicemia and bacteremia ^{[3][4]}, osteomyelitis ^[5], urinary tract infections ^{[6][7]}, endogenous endophthalmitis ^[8], endocarditis ^[9], epididymo-orchitis ^[10], pulmonary abscess ^[11], necrotizing fasciitis ^{[12][13]}, cystic fibrosis ^[14], hydrocephalus ^[15], and secondary infections with a high mortality rate, particularly in immunocompromised patients ^[16]. *Elizabethkingia meningoseptica* infections have also been associated with COVID-19 patients ^[17]. *Elizabethkingia* spp. infects not only immunocompromised patients but also immunocompetent ones ^{[18][19][20]}.

Historically, the first report of human infection due to *Elizabethkingia* was that of 19 cases of meningitis in infants in the United States of America ^[21]. Even in its earliest description, the isolates were demonstrated to be multidrug-resistant. Not long after King's (1959) report, an outbreak of meningitis infection with *E. meningoseptica* was reported among neonates in the Congo ^[22] with varying sensitivities to chloramphenicol, carbomycin, magnamycin, and erythromycin.

Worldwide infections caused by *E. meningoseptica* were reportedly high amongst immunocompetent neonates as well as hospitalized patients with existing underlying infections, and in a comprehensive review, Dzuiban et al. ^[2] showed that from 283 cases reported from 28 countries from 1944 to 2017, 76% of them were neonates aged 0–1 month. From the 283 cases that were reviewed, 209 of the patients were diagnosed with meningitis ^[2]. Infections by this pathogen have been reported in many parts of the world, including in Southeast Asian countries such as Malaysia ^[2], Singapore ^[23], Thailand ^[24], Indonesia ^[25], and Cambodia ^[26]. However, until now, there have been no published reports from other Southeast Asian countries such as the Philippines, Brunei, Myanmar, Laos, and Timor-Leste.

2. Identification

When first discovered in 1959, the suggested name for the bacterium was *Flavobacterium meningosepticum*, which was later recommended to be changed to *Chryseobacterium meningosepticum* (in 1994) ^[27]. In 2005, it was assigned to the genus *Elizabethkingia* (named after the first scientist to report its' discovery, Elizabeth King) under the Flavobacteriaceae family based on 16S rRNA phylogenetic studies ^[28]. Recently, whole-genome sequence analysis along with optical

mapping and MALDI-TOF mass spectrometry led to the revision of the genus *Elizabethkingia* into eight species, namely *E. meningoseptica*, *E. miricola*, *E. anophelis*, *E. bruuniana*, *E. ursingii*, *E. occulta* [29], *E. argenteiflava* sp. nov. [30], and the latest *E. umeracha* [31].

Since correct identification of *Elizabethkingia* is difficult using traditional microbiological methods and misidentification of *E. anophelis* with *E. meningoseptica* has been found to be common (Lau et al., 2016), it is therefore highly likely for this pathogen to be underreported. Correct identification of the organism is crucial for the diagnosis and management strategies, as *E. anophelis* is a nosocomial pathogen [32]. Hence, differentiation between *E. anophelis* and *E. meningoseptica* requires accurate microbial identification, but the phenotypic similarities between *E. anophelis* and *E. meningoseptica* present a challenge to accurate identification, particularly for clinically derived isolates; 16S rRNA gene analysis had identified a 98.6% similarity between *E. meningoseptica* and *E. anophelis*, which has often led to the misidentification of these bacteria [32].

The four automated bacterial identification systems that are commonly used in diagnostic laboratories are: (1) API/ID32 Phenotyping Kits (bioMérieux, Marcy l'Etoile, France); (2) Phoenix 100 ID/AST Automated Microbiology System (Becton Dickinson Co., Sparks, MD, USA); (3) VITEK 2 Automated Identification System [33]; and (4) MALDI-TOF MS System (bioMérieux, Marcy l'Etoile, France) [34]. At the time of writing this research, the four microbial identification systems that are listed above do not, however, contain all eight species of *Elizabethkingia* in their reference spectra database. Studies have also shown that misidentification of *Elizabethkingia* was rife using these automated identification systems, with *E. anophelis* commonly misidentified as *E. meningoseptica* [1][33][35]. When the accuracy of the API/ID32, Phoenix 100 ID/AST, Vitek 2, and Vitek MS *Elizabethkingia*, clinical isolate identifications were compared with 16S rRNA gene sequencing; it was reported that species identification concordance between these identification systems and 16S rRNA gene sequencing was low at only 24.5–26.5% [33]. Nevertheless, MALDI-TOF MS systems with amended databases (labeled as “research-use only” system) either in the Vitek MS Knowledge Base v3.2 and Bruker MALDI Biotyper Library (Bruker Daltonics GmbH, Bremen, Germany) are now able to reliably differentiate *E. meningoseptica* from *E. anophelis*, but not the remaining species of the genus *Elizabethkingia* [33]. In a recent report of 22 clinical and 6 environmental hospital isolates from Queensland, Australia, Burnard et al. (2020) showed that the VITEK MS Knowledge Base v3.2 had a 96.2% accuracy in identifying *Elizabethkingia*, with a solitary isolate of *E. bruuniana* being the only species that was misidentified. Whole-genome sequencing confirmed that the majority of the isolates were *E. anophelis* ($n = 22$), with the rest being *E. miricola* ($n = 3$), *E. meningoseptica* ($n = 2$), and *E. bruuniana* ($n = 1$) [36].

In the near future, the inclusion of novel *Elizabethkingia* species spectra into the databases should ensure highly accurate identification using MALDI-TOF MS systems, making it a reliable identification tool in lieu of whole-genome sequencing.

3. Antibiotic Resistance

Elizabethkingia are intrinsically resistant to most β -lactams, β -lactam/lactamase inhibitors, and carbapenems due to the presence of two unique class B metallo- β -lactamases (MBLs), namely *bla*_{BlaB} and *bla*_{GOB}, along with a class A extended-spectrum β -lactamase (ESBL), *bla*_{CME} [37][38][39]. *Elizabethkingia* are the only known bacteria thus far with multiple chromosomally encoded MBLs [40]. Reports of subclasses of MBL genes such as *bla*_{BlaB-1} [38], *bla*_{BlaB}, and *bla*_{GOB} in both *E. meningoseptica* and *E. anophelis* [39], as well as *bla*_{BlaB-16} and *bla*_{GOB-19} in *E. miricola* isolated from a black-spotted frog in China [41], make *Elizabethkingia* spp. a possible environmental reservoir for β -lactam resistance.

Elizabethkingia isolates are frequently resistant to aminoglycosides, macrolides, tetracycline, and vancomycin but show variable susceptibility to piperacillin, piperacillin-tazobactam, fluoroquinolones, minocycline, tigecycline, and trimethoprim-sulfamethoxazole [3][36][39][42][43][44]; cephalosporins, monobactams, and moderate susceptibilities to piperacillin [45][46][47], ceftazidime, colistin, and meropenem [48]; and levofloxacin [49]. There are currently no established MIC breakpoints for *Elizabethkingia*, and susceptibilities are largely reported based on *Enterobacteriaceae* breakpoints of the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines and/or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) pharmacokinetic–pharmacodynamic (PK–PD) “non-species” breakpoints [35][36]. It has been pointed out that susceptibilities, especially for vancomycin and piperacillin-tazobactam as determined by disk diffusion and E-test, are deemed unreliable and inaccurate for *Elizabethkingia*, and broth microdilution is instead recommended for susceptibility determination [43][50]. Although successful therapy has been attributed to rifampicin, there has been a report of bacterial resistance after three days of starting treatment [51]. A similar case was reported for an *E. meningoseptica* isolate in the Kuala Lumpur General Hospital, which developed resistance during treatment to cefepime, a cephalosporin antibiotic that is normally highly active against both Gram-positive and Gram-negative organisms [52].

Using disk diffusion, Lau, Chow ^[41] reported 21 *Elizabethkingia* isolates from Hong Kong as susceptible to vancomycin. However, studies using broth microdilution tests on isolates from Taiwan ^{[43][53]} and Australia ^[36] indicated that the isolates are likely non-susceptible based on the high MICs obtained (that ranged between 8 and 256 µg/mL). Similar ranges of vancomycin MICs were obtained by Han et al. ^[35], who investigated *Elizabethkingia* isolates from South Korea using the agar dilution method and concluded that all isolates were non-susceptible based on the interpretive criteria used for *Staphylococcus* spp. The vancomycin resistance gene, *vanW*, was reported in the majority of *Elizabethkingia* genomes, although the exact function of *vanW* is currently unknown ^{[36][44]}. However, mutations in *vanW* have been identified in microorganisms with VanB-type glycopeptide resistance ^{[44][54]}. In view of these facts and despite some anecdotal reports of success in using intravenous vancomycin alone to treat *Elizabethkingia* infections ^{[55][56]}, it was recommended that even if intravenous vancomycin is the favored therapy for *Elizabethkingia* meningitis, ciprofloxacin, linezolid, or rifampicin should also be included until future clinical studies could be carried out to conclusively determine the clinical efficacy of these vancomycin-combination regimens for treatment ^[50].

One of the earliest reports of the whole-genome sequences of *Elizabethkingia* spp. strains from Southeast Asia was from Singapore, whereby sputum isolates obtained from three patients (NUHP1, NUHP2, and NUHP3) and four from the hospital's sink (NUH1, NUH4, NUH6, and NUH11) at the National University Hospital, Singapore, were compared against five previously sequenced *E. anophelis* strains Ag1 (PRJNA80705) and R26 (PRJNA178189), *E. meningoseptica* ATCC 12535 (NITE) (PRJNA199489), *E. meningoseptica* ATCC 12535 (OSU) (PRJNA198814), and *E. meningoseptica* 502 (PRJNA176121). This led to the discovery of 16 antibiotic resistance genes from the core genomes and 19 antibiotic resistance genes from the accessory genomes of *Elizabethkingia* spp., and this included genes that confer resistance to aminoglycosides, β-lactams, fluoroquinolones, glycopeptides, macrolide-lincosamide-streptogramins, tetracyclines, trimethoprim, and rifampicin ^[38]. A later study on two African isolates, E27017 and E18064, that compared their genomes with the genomes of 18 strains belonging to the genus *Elizabethkingia* from many different regions, including Malaysian and Singaporean genomic sequences that were available at that time, identified that all *Elizabethkingia* genomes contained at least 17 antimicrobial resistance genes ^[39].

A whole-genome sequencing study on three isolates of *E. meningoseptica* collected from an outbreak from three separate patients living in different counties in the Midwest regions of Michigan led to the identification of 22 resistance genes and 18 multidrug resistance efflux pump-encoded genes in all samples ^[57]. While *Elizabethkingia* spp. genomes shared many antibiotic-resistance genes with each other, minor differences have been reported ^{[3][58]}. Hence, genomic investigations of *Elizabethkingia* spp. offers invaluable novel information on the species, but unfortunately, there have not been any reports of the whole-genome sequence of *Elizabethkingia* spp. isolates from Southeast Asia besides those from Singapore.

4. Virulence Factors

The mechanisms of pathogenesis of *Elizabethkingia* spp. are still being studied ^[57]. When the virulence factor database (VFDB, <http://www.mgc.ac.cn/VFs/>, accessed on 12 December 2021) was used to predict their presence from the genome sequences of various *Elizabethkingia* spp., this led to the prediction of a total of 270 putative virulence factor genes. More than fourteen virulence factor classes for *Elizabethkingia* spp. were identified with the following defined virulence-associated functions: adherence, antimicrobial activity, biofilm, cellular metabolism, effector delivery system, exoenzyme, exotoxin, immune modulation, invasion, motility, nutritional/metabolic factor, post-translational modification, regulation, stress survival, and others. Different species of *Elizabethkingia* shared the same virulence factors (**Figure 1**).

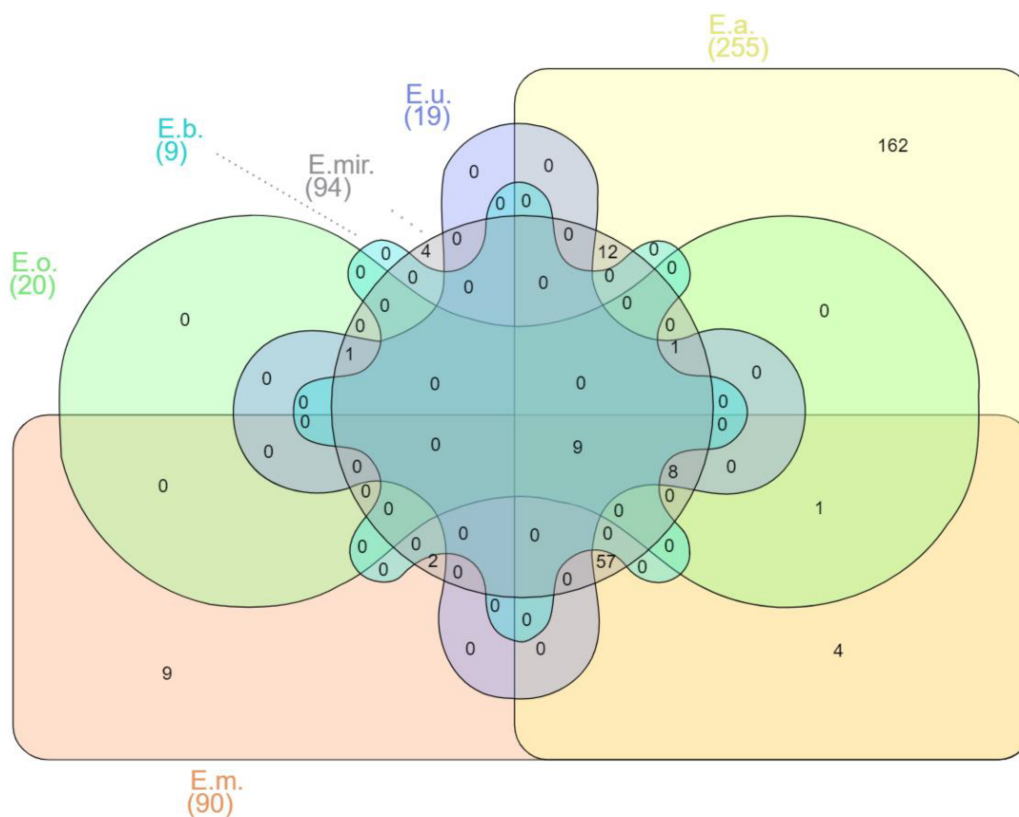


Figure 1. Venn diagram of shared virulence factor genes of *Elizabethkingia* spp. E.m.—*E. meningoseptica*; E.a.—*E. anophelis*; E.mir.—*E. miricola*; E.o.—*E. occulta*; E.u.—*E. ursingii*; E.b.—*E. bruuniana*. Edwards mode was used to process virulence factor gene outputs for Venn diagram visualization with InteractiVenn [59].

Among the 270 predicted genes for virulence factors, 162 have been reported as unique in *E. anophelis*. *E. meningoseptica* carried six unique genes involved in adherence that encode curli nucleator protein (*csgB*), curli assembly proteins (*curEm1*, *curEm2*, *curEm3*, *curEm4*), a curli production assembly protein (*csgG*), and two genes involved in immune modulation encoding a capsular polysaccharide synthesis enzyme (*cap8O*), a gene encoding Rab2-interacting conserved protein A (*ricA*) and a putative carbonic anhydrase-encoded gene (*mig-5*). Four of the *E. miricola* unique virulence genes were predicted to be involved with urease accessory protein (*ureE*), urease alpha subunit (*ureA*), twitching motility protein (*pilG*), and sphingomyelinase-c (*smcL*).

Identification of 6880 gene families in *E. anophelis* highlighted the genomic heterogeneity of *Elizabethkingia* species [39]. Genes homologous to heme iron acquisition, oxidative stress resistance proteins, and hemolysins were reported in earlier studies [32][60][61]. Extensive variations of capsular polysaccharide synthesis genes in *E. anopheles* were first reported by Breurec, Criscuolo [39], with variable *cps* clusters observed amongst the different lineages suggesting virulence heterogeneity among *Elizabethkingia* strains [39]. Identification of the capsule biosynthesis gene, *capD* [57], and the *adeG* gene for the AdeFGH efflux pump [20] in all *Elizabethkingia* species leads to possible biofilm formation [42][62], which empowers the bacteria with the ability to persist on various surfaces [57][63]. Thirty clinical isolates from Malaysia, which comprised *E. anophelis*, *E. meningoseptica*, and *E. miricola*, were recently shown to produce biofilms on polystyrene microtiter plates [64].

Nine virulence factor genes were shared between six of the *Elizabethkingia* spp., including the *E. argenteiflava*-encoded *adeFGH* efflux pump, isocitrate lyase (*icl*), catalase/(hydro)peroxidase (*katA*), 60K heat shock protein (*htpB*), phospholipase C (*plc*), phosphopyruvate hydratase (*eno*), translation elongation factor (*tuf*), catalase/peroxidase HPI (*katG*), and aspartate 1-decarboxylase precursor (*panD*), which is involved with adherence, biofilm formation, cellular metabolism, exotoxin production, and stress survival. Isocitrate lyase (*icl*) plays an important role in the glyoxylate cycle [65], and its presence in *Elizabethkingia* spp. can predict its essential role in stationary-phase survival. An early report had shown that the presence of *icl* in *Mycobacterium tuberculosis* promoted the tenacity of infection by helping the pathogen to survive inside macrophages [66].

However, the specific role of bacterial enzymes in pathogenesis varies with infection. The presence of phospholipases C (*plc*) in all *Elizabethkingia* spp. [44] suggest its crucial role in downregulating host immunity [67]. In *L. monocytogenes*, *plc* aided bacterial escape toward the cytosol and cell-to-cell propagation, whereas, in *C. perfringens*, it helped bacteria induce endothelial damage and platelet aggregation, and in *P. aeruginosa*, it led to the triggering of signaling pathways that lead to inflammation [68].

The catalase-peroxidase genes, *katA* and *katG* (encoding hydroperoxidase I), are crucial against oxidative stress [69]. An earlier report showed that strains with *katA* were resistant to dodecyl sulfate, proteinase K, pepsin, trypsin, chymotrypsin, and the neutrophil protease cathepsin G, and they could survive for a long period once released from lysed cells [70]. Presence of *katA* [38][39][42][44][58][60][71][72] and *katG* [42][44][71][72] could also support *Elizabethkingia* species' resistance to aminoglycosides.

5. Sources of Isolation and Transmission

The genera *Elizabethkingia* are aerobic, non-fermenting, non-motile, catalase-positive, oxidase-positive, indole-positive, and Gram-negative bacilli widely distributed in soil, mosquitoes, plants, fresh and marine fish [28][73], food products [74], hospital settings [75], stagnant water, inland wetlands, and rivers [31]. Due to their biofilm-forming ability [62], they have been isolated from sinks and taps where they colonize the most, leading to nosocomial and community infections [76] (Table 1).

Table 1. Various sources of isolation of *Elizabethkingia* spp. in Southeast Asia.

Source of Isolation	Country of Origin	Citation
Blood	Malaysia, Singapore, Thailand, Indonesia, Cambodia	[8][13][15][23][24][25][26] [34][64][77][78][79][80][81] [82]
Peritoneal fluid	Malaysia	[77]
Cerebrospinal fluid (CSF)	Malaysia, Singapore	[15][23][77][83][84][85][86]
Contact lens	Malaysia	[87]
Hospital environment (aerators, sink drains and traps at ICUs, pediatric wards, surgical wards, orthopedic wards)	Singapore	[8][88][89][90]
Catheter tips	Singapore	[8]
Respiratory specimens	Singapore Malaysia	[8][64]
Rectal swabs	Singapore	[91]
Urine	Malaysia	[64]
Wound swabs	Malaysia	[64]
Nasal swabs	Malaysia	[64]

Source of Isolation	Country of Origin	Citation
Vitreous culture	Singapore	[8]
Frogs (<i>Rana catesbeiana</i> (American bullfrogs) and <i>Theloderma bicolor</i> Chapa bug-eyed frogs, Warty toads (<i>Bombina microdeladigitora</i>), and Northern leopard frogs (<i>Lithobates pipiens</i>)	Malaysia Vietnam	[92][93][94]
Mosquitoes (<i>Anopheles minimus</i> , <i>Anopheles dirus</i> , <i>Anopheles maculatus</i> , <i>Anopheles sawadwongporni</i> , and <i>Anopheles dravidicus</i>)	Thailand	[95][96]
Fish (<i>Clarias gariepinus</i> (African sharptooth catfish) and <i>Pangasius hypophthalmus</i> (Tra catfish)	Malaysia, Vietnam	[73][97][98]
Retail sausages	Malaysia	[74]
<i>Gnetum gnemon</i> (Tree)	Malaysia	[99][100]

Vector-borne transmission of the bacterial pathogen via mosquito bites has been suggested ever since the discovery of *E. anophelis* in the midgut of the *Anopheles gambiae* mosquito [101][102] and, more recently, in the salivary glands and saliva of *Aedes albopictus* [103]. The microbiome of *Anopheles* mosquitoes has evidently revealed the strong symbiotic nature of *E. meningoseptica* [104], which has been isolated from various independent sources, including *Anopheles stephensi*, the vector for the malarial parasite *Plasmodium vivax* [76][105][106], semi-field *Anopheles gambiae* females [104][106][107][108], field sampled mosquitoes in Cameroon [109][110], and laboratory-reared mosquitoes where *Anopheles* were the predominant species [109][111]. Another comparative study on bacterial microbiota isolated from the midgut of various *Anopheles* spp., which were obtained in the same region of Mae Sot District and Sop Moei District in Thailand, reported on the findings of *Elizabethkingia* spp. from *Anopheles minimus*, *Anopheles dirus*, *Anopheles maculatus*, *Anopheles sawadwongporni*, and *Anopheles dravidicus* mosquitoes [95]. However, sequences associated with the genus *Elizabethkingia* could not be definitively assigned to either *E. anophelis* or *E. meningoseptica* as the V3–V4 region of the 16S rRNA gene used for microbiome profiling could not differentiate between the two species [95]. Despite these multiple discoveries of *Elizabethkingia* spp. in the midgut and salivary glands of various mosquito species, there is currently a lack of strong direct evidence that supports *Elizabethkingia* infection, particularly *E. anophelis*, as a mosquito-borne disease [43], although this should not be ruled out with the current level of knowledge. A comparative genomics study of three cases of *E. anophelis* also provided evidence of vertical transmission from mother to her baby [61].

Zainuri et al. (2013) reported on the isolation of *E. meningoseptica* from American bullfrogs (*Lithobates catesbeianus* or *Rana catesbeiana*) suffering from red leg syndrome and cataract in Sabah, Malaysia [93]. Isolation of *E. meningoseptica* from bullfrogs was also described in an earlier study, in which the isolates obtained were found to be resistant to multiple antibiotics [94]. *E. miricola*, which had been implicated in acute infections in humans, caused a disease outbreak associated involving the internal organs of different anuran species, including northern leopard frogs (*Lithobates pipiens*), Chapa bug-eyed frogs (*Theloderma bicolor*), and Vietnamese warty toads (*Bombina microdeladigitora*) captured in Vietnam. The presence of β -lactamases and putative virulence genes in the *E. miricola* isolates were detected in silico [92].

E. miricola was also reportedly isolated from Tra catfish (*Pangasius hypophthalmus*) fillets in the industrial processing lines in Vietnam [98]. Tra catfish is a type of freshwater fish, which is one of the major fish species in the Mekong River, and its processed fillets are exported to more than 80 different countries worldwide [97]. Other scientists have also reported the isolation of *E. meningoseptica* from retail sausages in Kampar, Malaysia, although the identification was performed by traditional biochemical methods and identified as *Chryseobacterium meningosepticum* [74].

Furthermore, 454 pyrosequencing of the 16S rRNA gene from the bacterial community of the root of the gnetalean gymnosperm *Gnetum gnemon* and nearby bulk soils of a tropical forest arboretum at the Forest Research Institute of Malaysia (FRIM) at Kepong, near Kuala Lumpur, identified the mutualistic presence of *E. meningoseptica* and *E. miricola* [99]. *Elizabethkingia* spp. was surprisingly found in relative abundance (4.9%) on the leaves of *Gnetum gnemon* in

comparison with rhizoplane (1.4%) [100]. These reports indicate the ubiquity of *Elizabethkingia* spp. in the environment and, thus, the difficulty in tracing an outbreak should one occur in the community and outside of hospital settings.

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