Insights from the draft genome into the pathogenicity of a clinical isolate of Elizabethkingia meningoseptica Em3

Shicheng Chen¹*, Marty Soehnlen², Frances P. Downes³ and Edward D. Walker¹

Abstract

Elizabethkingia meningoseptica is an emerging, healthcare-associated pathogen causing a high mortality rate in immunocompromised patients. We report the draft genome sequence of E. meningoseptica Em3, isolated from sputum from a patient with multiple underlying diseases. The genome has a length of 4,037,922 bp, a GC-content 36.4%, and 3673 predicted protein-coding sequences. Average nucleotide identity analysis (>95%) assigned the bacterium to the species E. meningoseptica. Genome analysis showed presence of the curli formation and assembly operon and a gene encoding hemagglutinins, indicating ability to form biofilm. In vitro biofilm assays demonstrated that E. meningoseptica Em3 formed more biofilm than E. anophelis Ag1 and E. miricola Emi3, both lacking the curli operon. A gene encoding thiol-activated cholesterol-dependent cytolysin in E. meningoseptica Em3 (potentially involved in lysing host immune cells) was also absent in E. anophelis Ag1 and E. miricola Emi3. Strain Em3 showed α-hemolysin activity on blood agar medium, congruent with presence of hemolysin and cytolysin genes. Furthermore, presence of heme uptake and utilization genes demonstrated adaptations for bloodstream infections. Strain Em3 contained 12 genes conferring resistance to β-lactams, including β-lactamases class A, class B, and metallo-β-lactamases. Results of comparative genomic analysis here provide insights into the evolution of E. meningoseptica Em3 as a pathogen.

Keywords: Draft genome, Infections, Elizabethkingia meningoseptica, Human isolate

Introduction

Elizabethkingia meningoseptica, a Gram-negative, aerobic bacillus, belongs to the family Flavobacteriaceae within the phylum Bacteroidaeota [1–3]. Among the three clinically important Elizabethkingia species (including E. meningoseptica, Elizabethkingia anophelis and Elizabethkingia miricola), E. meningoseptica has been intensively investigated for its pathogenicity [4–6]. Most of the E. meningoseptica infections are nosocomial, often transmitted in intensive care units [1, 7]. This bacterium survives in tap water, in disinfection fluid, on wet surfaces of sinks, in ventilators, hemodialysis equipment, catheters, and other medical apparatus. E. meningoseptica infection causes neonatal meningitis, nosocomial pneumonia, bactereemia, osteomyelitis, endocarditis, and skin infections [1, 4, 8].

Moreover, older (age > 65) and immunocompromised patients are more susceptible to infection; case-fatality rates have reached 50% [9].

Infections by E. meningoseptica are difficult to treat with antimicrobial agents due to multiple drug resistance [4]. Tetracycline, chloramphenicol, and β-lactams have been used to treat patients [10], but increasingly clinical isolates lack susceptibility to these antibiotics [11]. Analysis of the resistome in the related bacterium E. miricola revealed multiple drug resistance genes [12]. Some antibiotics effective against Gram-positive bacteria such as vancomycin, quinolones, tigecycline, and rifampin have been used for treating E. meningoseptica-infected patients, though the mechanism of action remains unclear [12, 13]. Also, the effectiveness of these antibiotics varied; many patients resolved infection but isolates showed high MICs in vitro, thus the relationship between MICs and clinical response was obscure [14]. Further genome analyses will elucidate the breadth of antibiotic susceptibility and resistance mechanisms in Elizabethkingia spp.
Differentiation of Elizabethkingia species using routine morphological and biochemical tests is difficult in clinical laboratories [14]. Comparison of 16S rRNA identity does not provide sufficient resolution to identify and separate these closely-related Elizabethkingia species [2, 14]. Characterization of Elizabethkingia species by MALDI-TOF mass spectrometry would facilitate it if species reference spectra were added to the database [14]. A limitation is that MALDI-TOF mass spectrometry is not available in many smaller clinical microbiology laboratories. Whole genome analysis facilitates the development of molecular diagnosis tools (such as single nucleotide polymorphisms) that can be potentially useful for small laboratories. In this study, we sequenced, annotated and analyzed a clinical E. meningoseptica genome, with the aim of providing a better understanding of antibiotic resistance and pathogenesis mechanisms in this pathogen, and of unveiling useful biologically systematic molecular markers.

Organism information
Classification and features
E. meningoseptica Em3 (Fig. 1) was isolated from a sputum sample from a patient with multiple underlying diseases and on life support. E. meningoseptica Em3 is Gram-negative, non-motile and non-spore-forming (Fig. 1 and Table 1). A taxonomic analysis was performed by comparing the 16S rRNA gene sequence to those in the GenBank (Fig. 2). The phylogenetic tree based on the 16S rRNA gene sequences indicated that strain Em3 was clustered within a branch containing other E. meningoseptica and departing from the clusters E. anophelis and E. miricola in the genus Elizabethkingia (Fig. 2). We further calculated the ANI and DDH values among the representative Elizabethkingia (Table 2). Our results showed that strain Em3 belongs to E. meningoseptica because of the high ANI (>95%, cutoff for species differentiation) and DDH (>70%, cutoff for species differentiation) values between strain Em3 and E. meningoseptica ATCC 13253 T [15].

The motility was tested on semi-TSA. The cells of strain Em3 are straight and rods and have a diameter of 0.7 μm and length of 24.0 μm. Strain Em3 grew on TSA, producing yellow pigment (Fig. 1). This bacterium also grew well on SBA with greyish discoloration around the colonies, showing it had the α-hemolytic activity (Fig. 1). E. meningoseptica Em3 did not grow on MacConkey agar, a finding consistent with strain-dependent growth on this medium; e.g., E. meningoseptica CCUG 214 T grew on MacConkey agar whereas other hospital-associated E. meningoseptica strains did not [2]. Of those
strains growing on MacConkey agar, lactose was not utilized [2]. The optimal growth temperature for strain Em3 was 37 °C (Table 1). Carbon source, nitrogen source utilization and osmotic tolerance were assayed by incubating cells in Biolog GEN III microplates at 37 °C overnight (CA, USA). The results showed that E. meningoseptica Em3 did not tolerate 4% NaCl. E. meningoseptica Em3 utilized several carbon sources, including D-maltose, D-trehalose, D-gentibiose, D-melibiose, D-glucose, D-mannose, D-fructose, D-fucose, D-mannitol and D-glycerol. The ability to use D-melibiose can differentiate E. meningoseptica from E. anophelis and E. miricola [16]. The inability to grow on cellobiose or citrate was consistent with previous reports [16]. Moreover, E. meningoseptica Em3 utilized D-serine, L-alanine, L-aspartic acid, L-glutamic acid, L-histidine and L-serine when tested on Biolog GEN III microplates.

Extended feature descriptions
Phylogenetic analysis (Additional file 1: Figure S1) was further conducted by using 19 genomes with 1181 core genes per genome (22,439 in total). As expected, E. meningoseptica Em3 grouped together with the selected E. meningoseptica species and separated from the clusters E. anophelis, E. endophytica and E. miricola, a finding similar to the phylogenetic analysis based on 16 s rRNA sequences. Further, both trees (Fig. 1 and Additional file 1: Figure S1) show that species E. anophelis and E. endophytica are not separated well, which is consistent with previous reports [17].

Genome sequencing information
Genome project history
The genome of E. meningoseptica Em3 was selected for whole genome sequencing because of its association...
with pulmonary disease. Comparison of strain Em3 genome with other Elizabethkingia species may provide insights into the molecular basis of pathogenicity and metabolic features of this strain. The high-quality draft genome sequence was completed on August 1, 2016 and was deposited to GenBank as a Whole Genome Shotgun project under accession number MDTY00000000 and the Genome OnLine Database with ID Gp0172366 (Table 3).

**Growth conditions and genomic DNA preparation**

For genomic DNA isolation, *E. meningoseptica* Em3 (CL16-200185, Bureau of Laboratories, Michigan Department of Health and Human Services) culture was grown overnight and genomic DNA isolation, *E. meningoseptica* Em3 (CL16-200185, Bureau of Laboratories, Michigan Department of Health and Human Services) culture was grown overnight.

**Table 2** Percentage of in silico DNA-DNA hybridization (DDH) and average nucleotide identities (ANI) among the selected Elizabethkingia genomes

|                   | E. meningoseptica EM3 | E. anophelis R26<sup>†</sup> [43] | E. meningoseptica ATCC 13253<sup>†</sup> [44] | E. miricola BM10<sup>†</sup> [45] | E. endophytica JM-87<sup>†</sup> [46] |
|-------------------|-----------------------|-----------------------------------|-----------------------------------------------|----------------------------------|-------------------------------------|
| E. meningoseptica EM3 | 31.90                 | 91.10                             | 31.20                                         | 32.70                            |
|                   | 80.15                 | 98.52                             | 80.44                                         | 80.25                            |
| E. anophelis R26<sup>†</sup> | 91.10                 | 33.60                             | 68.80                                         | 78.60                            |
|                   | 80.15                 | 98.52                             | 91.52                                         | 97.49                            |
| E. meningoseptica ATCC 13253<sup>†</sup> | 31.20                 | 98.52                             | 31.40                                         | 33.30                            |
|                   | 80.15                 | 98.52                             | 68.70                                         | 91.41                            |
| E. miricola BM10<sup>†</sup> | 32.70                 | 80.25                             | 68.70                                         | 91.41                            |
|                   | 78.60                 | 97.49                             | 68.70                                         | 91.41                            |

Nucleotide sequences were downloaded from GenBank. The accession numbers for *E. anophelis* R26<sup>†</sup>, *E. meningoseptica* ATCC 13253<sup>†</sup>, *E. miricola* BM10<sup>†</sup> and *E. endophytica* JM-87<sup>†</sup> are NZ_ANIW01000001.1, NZ_ASAN01000001.1, NZ_CP011059.1 and NZ_CP016372, respectively.

<sup>†</sup>In silico DNA-DNA hybridization was calculated by using Genome-to-Genome Distance Calculator (GGDC) [47]. The percentage of DDH was shown on the top and bolded.

<sup>b</sup>ANI values were computed for pairwise genome comparison with using the OrthoANIu algorithm [48]. The percentage of ANI was shown on the bottom.
in 25 mL LB medium at 37 °C with vigorous shaking. DNA was isolated using a Wizard Genomic DNA Purification Kit (Promega, Madison). The amount of genomic DNA was measured using a Nanodrop2000 UV-Vis Spectrophotometer (Thermo scientific) and Qubit DNA assay kit. DNA integrity was confirmed by agarose gel assay (1.5%, w/v).

Genome sequencing and assembly
NGS libraries were prepared using the Illumina TruSeq Nano DNA Library Preparation Kit. Completed libraries were evaluated using a combination of Qubit dsDNA HS, Caliper LabChipGX HS DNA and Kapa Illumina Library Quantification qPCR assays. Libraries were combined in a single pool for multiplex sequencing and the pool was loaded on one standard MiSeq flow cell (v2) and sequencing performed in a 2x250bp, paired end format using a v2, 500 cycle reagent cartridge. Base calling was done by Illumina Real Time Analysis [18] v1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v1.8.4.

The Illumina data were assembled into contiguous sequences using SPAdes version 3.9.0 [19], then short contigs (<400 bp) were filtered out. The 11 contigs identified in this strain were therefore submitted to the NCBI database as a Whole Genome Shotgun project.

Genome annotation
Annotation of the 11 contigs was first done through the NCBI Prokaryotic Genome Automatic Annotation Pipeline [20]. The predicted CDSs were next translated and analyzed against the NCBI non-redundant database, iPfam, TIGRfam, InterPro, KEGG and COG. The RAST system was used to check the annotated sequences [21, 22]. Additional gene prediction and manual revision was performed by using the IMG/MER platform. E. meningoseptica Em3 genome is available in IMG (genome ID = 2,703,719,242).

Genome properties
The draft genome sequence is 4,037,922 bp long, 36.37% G + C rich and contains 11 scaffolds (Table 4). Of 3729 genes predicted, 3673 encoded proteins and 56 were RNAs. 2585 (69.32%) were assigned a putative function, while the other 1088 (30.68%) were designated as hypothetical proteins. The distribution of coding genes into general COG functional categories analyzed by IMG is listed in Table 5. Collectively, the genome features were similar to those in other sequenced E. meningoseptica (Additional file 2: Table S1).

Insights from the genome sequence
Elizabethkingia bacteria cause sepsis, bacteremia, meningitis or respiratory tract infections in hospitalized patients, indicating that they have the ability to colonize host tissues, suppress the host immune response, and disrupt erythrocytes to obtain nutrients and propagate in the host bloodstream [1, 13, 14]. Genome analysis showed that E. meningoseptica Em3 carried a gene (BFF93_RS1398) encoding a hemagglutinin protein. Hemagglutinins as adhesins are required for virulence in bacterial pathogens [23]. Hemagglutinins facilitate infection via adherence to epithelial cell lines from the human respiratory tract in Bordetella pertussis [24]. Darvish et al. showed that filamentous hemagglutinin adhesins were crucial for bacterial attachment and subsequent cell accumulation on target substrates [25]. An in vitro biofilm assay showed that, compared to the

| Table 3 Project information |
|-----------------------------|
| MIGS ID | Property | Term            |
| MIGS 31 | Finishing quality | High-quality draft |
| MIGS-29 | Libraries used | two paired-end 250 bp library |
| MIGS 29 | Sequencing platforms | MiSeq-Illumina |
| MIGS 31.2 | Fold coverage | 50.0X |
| MIGS 30 | Assemblers | SPAdes 3.9.0 |
| MIGS 32 | Gene calling method | NCBI Prokaryotic Genome, Annotation Pipeline |
| Locus Tag | BFF93_ |
| Genbank ID | MDTY0000000.1 |
| GenBank Date of Release | October 25, 2016 |
| GOLD ID | Gpo0172366 |
| BIOPROJECT | PRJNA338129 |
| MIGS 13 | Source Material Identifier | CL16–200185 |
| Project relevance | Clinical pathogen |

| Table 4 Genome statistics of E. meningoseptica Em3 |
|------------------------|-----------------|------------|
| Attribute          | Value          | % of total |
| Genome size (bp)   | 4,037,922      | 100        |
| DNA coding (bp)    | 3,571,073      | 88.44      |
| DNA G + C (bp)     | 1,468,714      | 36.37      |
| DNA scaffolds       | 11             | NA         |
| Total genes        | 3729           | 100        |
| Protein coding genes | 3673          | 98.50      |
| RNA genes          | 56             | 1.50       |
| Pseudo genes       | 0              | 0          |
| Genes in internal clusters | 752 | 20.17 |
| Genes with function prediction | 2585 | 69.32 |
| Genes assigned to COGs | 1993          | 53.45      |
| Genes with Pfam domains | 2740          | 73.48      |
| Genes with signal peptides | 452 | 12.12 |
| Genes with transmembrane helices | 818 | 21.94 |
| CRISPR repeats      | 0              | 0          |
mosquito isolate *E. anophelis* Ag1, clinical isolates *E. meningoseptica* Em3 and *E. miricola* Emi3 formed a higher amount of biofilm (Fig. 3). Furthermore, *E. meningoseptica* Em3 had better ability to form biofilm than did *E. miricola* Emi3. The capacity for strain Em3 to form biofilm was further exemplified by discovery of an operon involved in curli biosynthesis and assembly (BFF93_RS03755, BFF93_RS03760, BFF93_RS03765, BFF93_RS03725 and BFF93_RS03775). In vitro studies demonstrated that curli fibers participated in bacterial adhesion to target cell surfaces, cell aggregation, as well as biofilm formation [26, 27]. Moreover, some studies showed that curli mediated host cell attachment and invasion in vivo [28]. Curli were involved in inducing the host inflammatory response [29]. It should be noted that this curli synthesis operon is present in *E. meningoseptica* while it is absent in *E. miricola*. Further experiments are warranted to test if the curli gene cluster contributed to biofilm formation in strain Em3 because biofilm formation may involve other genes.

A cytolysin encoding gene (BFF93_RS16990) was found in the strain Em3 genome, whose product belonged to a thiol-activated, CDC family [30]. CDC as a virulence factor is widely distributed among Gram-positive, opportunistic pathogens [31]. For example, *Streptococcus pyogenes* utilized pore-forming CDC to translocate a protein into eukaryotic cells [32]. Disruption of expression of a hemolysin (CDC) gene in the intracellular pathogen *Listeria monocytogenes* reduced virulence in mice, showing that CDC was critical for full virulence [33]. Furthermore, perfringolysin, a CDC toxin, has cytotoxicity and leukostasis activities, allowing the cells to escape from macrophage phagosomes during *Clostridium perfringens* gas gangrene [34]. Only a few CDCs have been found in Gram-negative bacteria [31], and this is the first report of CDC genes in *E. meningoseptica*. It should also be noted that this cytolysin gene is located immediately downstream of *hmuY*, which together comprise part of an iron metabolism gene cluster. Such gene organization was only seen in *E. meningoseptica*. This CDC protein sequence in strain Em3 shared 87%, 83% and 81% identity to that in *E. meningoseptica* ATCC 13253, *E. meningoseptica* B2D and *E. meningoseptica* NBRC 12535, respectively. It is interesting that it did not have close identity to that in *E. meningoseptica* FMS-007 (48%) and *E. meningoseptica* 502 (48%); it was absent in an *E. meningoseptica* strain associated with endophthalmitis [35]. Similarly, it was not conserved in *E. anophelis* (identity ranging from 0 to 50%) and absent in

| Code | Value | %age | Description |
|------|-------|------|-------------|
| J    | 186   | 8.58 | Translation, ribosomal structure and biogenesis |
| A    | 0     | 0    | RNA processing and modification |
| K    | 170   | 7.84 | Transcription |
| L    | 91    | 4.20 | Replication, recombination and repair |
| B    | 0     | 0    | Chromatin structure and dynamics |
| D    | 21    | 0.97 | Cell cycle control, Cell division, chromosome partitioning |
| V    | 81    | 3.74 | Defense mechanisms |
| T    | 82    | 3.78 | Signal transduction mechanisms |
| M    | 184   | 8.49 | Cell wall/membrane biogenesis |
| N    | 10    | 0.46 | Cell motility |
| U    | 17    | 0.78 | Intracellular trafficking and secretion |
| O    | 110   | 5.08 | Posttranslational modification, protein turnover, chaperones |
| C    | 106   | 4.89 | Energy production and conversion |
| G    | 120   | 5.54 | Carbohydrate transport and metabolism |
| E    | 184   | 8.49 | Amino acid transport and metabolism |
| F    | 60    | 2.77 | Nucleotide transport and metabolism |
| H    | 134   | 6.18 | Coenzyme transport and metabolism |
| I    | 96    | 4.43 | Lipid transport and metabolism |
| P    | 153   | 7.06 | Inorganic ion transport and metabolism |
| Q    | 39    | 1.80 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 203   | 9.37 | General function prediction only |
| S    | 105   | 4.85 | Function unknown |
| –    | 1736  | 46.55 | Not in COGs |
all *E. miricola* species. Such observations may stress that a diverse pathogenesis process exists in various *E. meningoseptica* and other *Elizabethkingia* species.

Besides a CDC gene in strain Em3 genome, we found that there was a gene encoding the hemolysin with a CBS domain (BFF93_RS14485). Hemolysin can be possibly secreted and involved in lysis of the erythrocytes [35]. The predicted amino acid sequence was conserved in most *E. meningoseptica* strains (> 90%). Further examination of hemin-degrading/transporter/utilization proteins led to a discovery of the gene cluster including SAM-dependent methyltransferases (BFF93_RS02055), iron ABC transporter (BFF93_RS02045), hemin-degrading protein (hmuS, BFF93_RS02060), hemin importer ATP-binding protein (BFF93_RS02050) and iron-regulated protein (BFF93_RS02065). Furthermore, there was a gene encoding a hemin receptor (BFF93_RS03140).

*Elizabethkingia* infections can be fatal in immune-compromised patients if appropriate antibiotic therapy is delayed or the antimicrobial treatment is not properly provided [9, 14]. However, *Elizabethkingia* spp. are multi-drug resistant [4, 13]. The prediction results by CARD and RAST (Table 6) showed that there are at least 31 genes involved in antibiotic resistance including antibiotic inactivation enzymes and related efflux pumps in *E. meningoseptica* Em3. Many of them are possibly involved in mupirocin, vancomycin, β-lactam, aminocoumarin, elfamycin, isoniazid, tetracycline and fluoroquinolone resistance (Table 6). Several drugs used to treat *Elizabethkingia*-infected patients in the past are not effective anymore [4], which agrees with recent resistome assays in clinical *E. meningoseptica* isolates [12]. Genes associated with resistance to β-lactams, aminoglycosides, tetracycline, vancomycin, and chloramphenicol, reported here in strain Em3, are present in most of the studied *Elizabethkingia* spp. (Table 6). Remarkably, at least 12 β-lactam resistance genes encoding MBL fold metallo-hydrolases, metallo-β-lactamases and β-lactamases (class A and B) were found in *E. meningoseptica* Em3 genome (Table 6). Alternatively, antibiotics such as ciprofloxacin, minocycline, trimethoprim-sulfamethoxazole, rifampin and novobiocin may remain effective due to absence of relevant antibiotic resistance genes in *Elizabethkingia* sp. [36]. Therefore, a combination of antimicrobial tests and resistome analysis, combined with rapid identification of infections, will contribute to efficient management for *E. meningoseptica* infections in the future.

**Conclusions**
The draft genome sequence of *E. meningoseptica* Em3 isolated from a sputum sample in a patient was sequenced, annotated and described. We found that *E. meningoseptica* Em3 had novel genes encoding thiol-activated cholesterol-dependent cytolysin, curli and heme metabolism related proteins, showing that *E. meningoseptica* Em3 may be a causative agent. Our results also indicated that *E. meningoseptica* might be resistant to β-lactam antibiotics due to the production of diverse MBLs and β-lactamases. Furthermore, these β-lactamase encoding genes were also found in other *Elizabethkingia* species, indicating that *Elizabethkingia* species were important reservoirs of novel β-lactamase genes. Comparative genomics is a crucial approach in the discovery of novel virulence determinants in *Elizabethkingia* species. Genome-based approaches contribute to develop novel genetic markers for future molecular diagnosis of *Elizabethkingia* infections.
### Table 6 Antibiotic genes prediction

| Locus number | Gene in Em3 | Putative function | E. meningoseptica | E. anophels | E. miricola |
|--------------|-------------|-------------------|-------------------|-------------|-------------|
|              |             |                   | Em3   | S02 | R26 | NUHP1 | ATCC 33958 | EM_CHUV |
| BFF93_RS01220 | bla\_Gob-13 | Class B carbapenemase Bla\_Gob-13 | +    | +  | +   | +     | +       |         |
| BFF93_RS04805 | –           | β-lactamase       | +    | +  | +   | +     | +       |         |
| BFF93_RS05700 | –           | β-lactamase (EC 3.5.2.6) | +    | +  | +   | +     | +       |         |
| BFF93_RS07625 | bla\_ACME   | β-lactamase (Bla\_ACME) VEB-1-like | +    | +  | +   | +     | +       |         |
| BFF93_RS09600 | bla\_A      | β-lactamase       | +    | +  | +   | +     | +       |         |
| BFF93_RS09265 | –           | MBL fold metallo-hydrolase | +    | +  | +   | +     | +       |         |
| BFF93_RS06995 | –           | β-lactamase (EC 3.5.2.6) | +    | +  | +   | +     | +       |         |
| BFF93_RS14540 | –           | β-lactamase       | +    | +  | +   | +     | +       |         |
| BFF93_RS12085 | –           | β-lactamase (EC 3.5.2.6) | +    | +  | +   | +     | +       |         |
| BFF93_RS12510 | –           | MBL fold metallo-hydrolase | +    | +  | +   | +     | +       |         |
| BFF93_RS14000 | bla\_B      | Class B carbapenemase Bla\_B | +    | +  | +   | +     | +       |         |
| BFF93_RS01365 | –           | β-lactamase (EC 3.5.2.6) | +    | +  | +   | +     | +       |         |
| BFF93_RS00125 | dhfR         | Dihydrofolate reductase DHFR | +    | +  | +   | +     | +       |         |
| BFF93_RS17395 | –           | Bifunctional deaminase-reductase | +    | +  | +   | +     | +       |         |
| BFF93_RS00125 | dhfR         | Dihydrofolate reductase DHFR | +    | +  | +   | +     | +       |         |
| BFF93_RS17395 | –           | Bifunctional deaminase-reductase protein | +    | +  | +   | +     | +       |         |
| BFF93_RS14765 | folP         | Dihydropteroate synthase FolP (EC 2.5.1.15) | +    | +  | +   | +     | +       |         |
| BFF93_RS08380 | tetA         | Tetracycline efflux protein TetA | +    | +  | +   | +     | +       |         |
| BFF93_RS07335 | –           | Transmembrane efflux protein | +    | +  | +   | +     | +       |         |
| BFF93_RS12745 | –           | Antibiotic transporter | +    | +  | +   | +     | +       |         |
| BFF93_RS00370 | lolD         | Macrolide resistance, ABC transporter | +    | +  | +   | +     | +       |         |
| BFF93_RS05670 | emrB         | Erythromycin resistance, EmrB/QacA | +    | +  | -   | -     | +       |         |
| BFF93_RS05670 | emrB         | Erythromycin resistance, EmrB/QacA | +    | +  | +   | +     | +       |         |
| BFF93_RS10830 | emrB         | Erythromycin resistance, EmrB/QacA | +    | +  | +   | +     | +       |         |
| BFF93_RS03320 | –           | Erythromycin esterase | +    | +  | +   | +     | +       |         |
| BFF93_RS04670 | gyrA         | DNA gyrase GyrA subunit A (T83S) | +    | +  | +   | +     | +       |         |
| BFF93_RS09245 | gyrB         | DNA gyrase GyrB subunit A (M437 L) | +    | +  | +   | +     | +       |         |
| BFF93_RS08895 | parE         | DNA topoisomerase IV subunit B (M437F/A473L) | +    | +  | +   | +     | +       |         |
| BFF93_RS10790 | ant-6        | Aminoglycoside 6-adenylytransferase | +    | +  | +   | +     | +       |         |
| BFF93_RS14765 | catB         | Chloramphenicol acetyltransferase CatB | +    | +  | +   | +     | +       |         |
| BFF93_RS04080 | bcr/cflA     | Bcr/CflA efflux pump | +    | +  | +   | +     | +       |         |

*+* or *−* indicates the presence or absence of genes in the selected Elizabethkingia
The authors declare that they have no competing interests.

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Abbreviations

ANL: Average nucleotide identities; CDC: Cholesterol-dependent cytolsin; IMG/MER: Integrated Microbial Genomes and Microbiome Samples Expert

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