Title: Genome of the carbapenemase-producing clinical isolate Elizabethkingia miricola EM_CHUV and comparative genomics with Elizabethkingia meningoseptica and Elizabethkingia anophelis: evidence for intrinsic multidrug resistance trait of emerging pathogens.

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Highlights

- MDR clinical isolate *E. miricola* EM_CHUV core genome harbours 40 resistance genes.
- Large majority of the resistance genes are found in other *Elizabethkingia* genomes.
- Identified putative plasmid (pEM_CHUV) did not harbour any resistance genes.
- Limited number of horizontal gene transfers suggested an intrinsic origin of the MDR.

Genome of the carbapenemase-producing clinical isolate *Elizabethkingia miricola* EM_CHUV and comparative genomics with *Elizabethkingia meningoseptica* and *Elizabethkingia anophelis*: evidence for intrinsic multidrug resistance trait of emerging pathogens

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ABSTRACT

Elizabethkingia miricola is a Gram-negative non-fermenting rod emerging as a life-threatening human pathogen. The multidrug-resistant (MDR) carbapenemase-producing clinical isolate *E. miricola* EM_CHUV was recovered in the setting of severe nosocomial pneumonia. In this study, the genome of *E. miricola* EM_CHUV was sequenced and a functional analysis was performed, including a comparative genomic study with *Elizabethkingia meningoseptica* and *Elizabethkingia anophelis*. The resistome of EM_CHUV revealed the presence of a high number of resistance genes, including the presence of the *bla*$_{GOB-13}$ and *bla*B-9 carbapenemase-encoding genes. Twelve mobility genes, with only two of them located in the proximity of resistance genes, and four potential genomic islands were identified in the genome of EM_CHUV, but no prophages or CRISPR sequences. Ten restriction–modification system (RMS) genes were also identified. In addition, we report the presence of a putative conjugative plasmid (pEM_CHUV) that does not encode any antibiotic resistance genes. Altogether, these findings point towards a limited number of DNA exchanges with other bacteria and suggest that multidrug resistance is an intrinsic trait of *E. miricola* owing to the presence of a high number of resistance genes within the bacterial core genome.

1. Introduction

Elizabethkingia miricola, originally identified in 2004 in the Mir Space Station, belongs to the genus *Elizabethkingia* (previously *Chryseobacterium*) that contains four species of ubiquitous Gram-negative non-fermenting rods [1,2]. *Elizabethkingia meningoseptica* mainly, and more recently *E. miricola* and *Elizabethkingia anophelis*, have been incriminated in severe infections in humans. *Elizabethkingia endophytica* is the last identified species of this genera; its pathogenic potential towards humans still remains undetermined [3]. *Elizabethkingia meningoseptica* has been involved in a broad range of infections in humans, including bacteraemia and meningitis, in particular in newborns and children where a high rate (84%) of meningitis has been documented. Noteworthy, nosocomial infections such as ventilator-associated pneumonia and haemodialysis catheter-related infections are a significant part of *E. meningoseptica*-related infections. *Elizabethkingia anophelis*, initially isolated from the midgut of the mosquito malaria vector *Anopheles gambiae* [4], has also been associated with similar severe infections (pneumonia, catheter-related infection and central nervous system infections) with high mortality rates [5,6]. *Elizabethkingia miricola* has been recently incriminated in bacteraemia and sepsis both in immunocompetent and immunocompromised patients, supporting the clinical relevance of this strain [7–10]. The strain *E. miricola* EM_CHUV described here was isolated from a lower respiratory tract specimen in the setting of severe nosocomial pneumonia. Antibiotic susceptibility testing of isolate EM_CHUV revealed a multidrug-resistant (MDR) profile, a phenotype encountered in most of the previously documented *E. miricola* strains. To better understand the genetic basis of this multidrug resistance, in this study the genome of EM_CHUV was sequenced and a

genomics analysis was performed, including a comparison with the genomes of *E. meningoseptica* and *E. anophelis.*

2. Materials and methods

2.1. Microbiology procedures

Strain *E. miricola* EM_CHUV was recovered from the endotracheal secretions and bronchoalveolar lavage fluid of a patient. Isolate identification and antibiotic susceptibility testing, including detection of carbapenemase production, were performed as described in the Supplementary materials and methods.

2.2. Genome sequencing and analysis

Genomic DNA of *E. miricola* EM_CHUV was sequenced, assembled and annotated as described in the Supplementary materials and methods. The genome sequences of *E. miricola* ATCC 33958 type strain (JRFN00000000.1) [11], *E. anophelis* NUHP1 (CP007547), *E. anophelis* R26 (ANIW00000000), *E. meningoseptica* ATCC 13253 (BARD01000018) and *E. meningoseptica* 502 (AVCQ00000000) were retrieved from the National Center for Biotechnology Information (NCBI) database and were annotated using RAST server [12]. The phylogenetic relationship among the *Elizabethkingia* spp. and closely related bacteria was based on the 16S rRNA genes. The resistome of EM_CHUV was investigated from the RAST annotation and was confirmed by BLASTP analysis against the ARG-ANNOT database [13] as well as information from the CARD database [14] in light of the antibiotic resistance phenotype. To address the putative

foreign origin of resistance genes, we searched for the presence of genes harbouring domains of mobile elements within the annotation (transposase, integrase, insertion sequence and recombinase), genomic islands predicted with IslandViewer 3 [15] and prophages predicted using PHASTER [16] in their close vicinity. The absence of CRISPR domain sequences was identified using CRISPRFinder [17]. Average genome identity was evaluated using JSpecies v.1.2.1 [18]. Furthermore, the core proteomes were compared using GET_HOMOLOGUES [19].

3. Results

3.1. Isolation of Elizabethkingia miricola EM_CHUV

In April 2014, an 82-year-old man was admitted to the intensive care unit (ICU) of University Hospital of Lausanne (Lausanne, Switzerland). The medical history of the patient started a year before with cervical spine surgery complicated by post-operative tetraparesia, which was followed by several hospitalisations for the management of recurrent pneumonia. The patient was initially treated by amoxicillin/clavulanic acid, followed by a combination of moxifloxacin and ceftazidime, and finally by amikacin and imipenem. At admission to our hospital, two pairs of blood bottles were drawn from an arterial catheter and were processed using a BD BACTEC^{TM} FX automated blood culture system (Becton Dickinson, Heidelberg, Germany), and a urine sample was collected. In addition, endotracheal secretions and bronchoalveolar lavage fluid were sampled. The urine was negative for detection both of *Legionella* and *Streptococcus pneumoniae* antigens and the urine culture remained sterile. After 28 h in the ICU, the

patient died following treatment withdrawal in the context of a severe neurological disability and nosocomial pneumonia. Cultures of the endotracheal secretions of the patient were positive for two distinct Gram-negative bacteria identified as *Stenotrophomonas maltophilia* and *E. miricola* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). The *E. miricola* strain was also recovered by culture from the bronchoalveolar lavage fluid (10^7 bacteria/mL). Blood cultures remained sterile after 5 days of incubation. The recent literature has reported several cases of severe infections in humans owing to this organism, including pulmonary abscess and sepsis [7–10]. These cases as well as the case described here are summarised in Table 1 and support a significant pathogenic potential of this organism.

3.2. Antibiotic susceptibility profile of Elizabethkingia miricola EM_CHUV

Antibiotic susceptibility testing performed on the *S. maltophilia* strain revealed sensitivity to minocycline, trimethoprim/sulfamethoxazole and levofloxacin. *Elizabethkingia miricola* EM_CHUV was found to be resistant to almost all of the antibiotic families tested, including β-lactams (ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem and meropenem), aminoglycosides (netilmicin and tobramycin) and colistin (Table 2). EM_CHUV was sensitive to the aminoglycosides gentamicin and amikacin and to minocycline. Susceptibility to levofloxacin but resistance to ciprofloxacin was also observed. A carbapenemase was detected using the rapid Carba NP test [20] and was characterised as a metallo-β-lactamase (MBL) using MBL Etest strips (MBL IP/IPI; bioMérieux, Lyon, France) by comparison of

differential inhibition with and without ethylene diamine tetra-acetic acid (EDTA) as chelator in the presence of imipenem (Supplementary Fig. S1) [21]. Thus, EM_CHUV exhibited a MDR phenotype, already documented for other *E. miricola* species, which we investigated through a genomic analysis.

3.3. Comparative genomics and phylogeny of the Elizabethkingia spp.

The general features of the genome of EM_CHUV and the other genomes included in this study are given in Supplementary Table S1. The genome size of *E. miricola* EM_CHUV was estimated to be 4 286 503 bp with a GC content of 35.78%. A putative plasmid (pEM_CHUV) of 176 107 bp with a GC content of 40.15% was also identified, which encodes two full copies of the conjugative transposon operon *tra* (11 525 bp and 13 394 bp), likely involved in bacterial plasmid transmission by conjugation [22]. This plasmid showed no similarity to the complete genome of *E. miricola* BM10, but some regions shared similarity with different contigs of *E. miricola* ATCC 33958, suggesting that a similar plasmid could be found in various strains as an epitope or integrated within the genome. Interestingly, no resistance genes were detected on pEM_CHUV. The phylogenetic tree of the 16S rRNA gene revealed that the three *E. miricola* strains constitute a monophyletic cluster distinct from the other *Elizabethkingia* spp. (Fig. 1A). As expected, *E. miricola* ATCC 33958 was the most closely related strain to EM_CHUV when considering whole-genome nucleotide identity and core proteome identity (Fig. 1A,B; Supplementary Fig. S2). In contrast, *E. anophelis*, *E. meningoseptica* and *Chryseobacterium meningoseptica* strains grouped together, suggesting that the current taxonomy does not appropriately reflect their phylogenetic relationships. The very close

genetic proximity of *E. anophelis* NUPH1, *E. anophelis* R26 and *E. meningoseptica* 502 was confirmed by pairwise genome comparison (>97% identity) as well as by analysis of the core proteomes (>96% identity) (Supplementary Fig. S2). Moreover, the group is paraphyletic due to the deeper branching of *E. meningoseptica* ATCC 13253, which suggests that the latter strain might belong to a different species. This finding was also supported by the low average genome nucleotide identity (78%) and average core proteome identity (84%) of *E. meningoseptica* ATCC 13253 with the other genomes (Fig. 1B; Supplementary Fig. 2). Finally, over three-fold more proteins are shared between *E. miricola* EM_CHUV and *E. anophelis* NUHP1 (448 proteins) than *E. miricola* EM_CHUV and *E. meningoseptica* ATCC 13253 (134 proteins) (see Fig. 1C). In congruence with our findings, the classification of *E. meningoseptica* 502 has been changed to *E. anophelis* 502 in NCBI taxonomy during the revision process of this article.

As depicted in Fig. 1C, the core proteome of *E. miricola* EM_CHUV, *E. anophelis* NUPH1 and *E. meningoseptica* ATCC 13253 consists of 2842 proteins. EM_CHUV encodes 254 strain-specific proteins mainly with an unknown function (214/254; 84.25%). Interestingly, annotated proteins include a type I restriction–modification system (RMS), a DNA mismatch repair (*mutT*) gene, a heavy metal transporter and a gene encoding for a colicin-like protein, a cytotoxin with bactericidal activity.

3.4. Resistome of Elizabethkingia miricola EM_CHUV

The resistome of EM_CHUV revealed a total of 40 antibiotic resistance genes (Table 2; Supplementary Table S2). A high number of genes (*n* = 20) are involved in resistance to β-lactams, including the *bla*$_{GOB-13}$ and *bla*B-9 genes encoding for class B carbapenemases [23]. The genome of EM_CHUV also contains the aminoglycoside 6-adenylyltransferase gene (*ant-6*) associated with tobramycin resistance. Furthermore, four genes associated with resistance to sulfonamides and six genes associated with resistance to macrolides could be identified. Four tetracycline resistance genes were also present. Finally, the resistome of the EM_CHUV isolate contained a chloramphenicol resistance gene. Analysis of the genes associated with resistance to quinolones revealed the mutation T83S of GyrA, M437L of GyrB and M437F/A473L of ParE; no mutation was found in ParC. None of the resistance genes were located on the putative plasmid pEM_CHUV. Noteworthy, the genome of *E. miricola* ATCC 33958 and the other *Elizabethkingia* genomes analysed in this study also contained all these genes, except for four β-lactamase genes (Supplementary Table S2) [11].

We investigated whether the resistance determinants could have been acquired horizontally as part of genomic islands, but very few mobile elements located close to the resistance genes were identified. Indeed, among the 12 mobile elements (transposases, integrases, insertion sequences and recombinases) found in the genome of EM_CHUV, only 2 were located in the proximity of a class C β-lactamase-encoding gene and a chloramphenicol acetyltransferase-encoding gene (at 5.6 kb and

1.1 kb, respectively). The four genomic islands predicted along the chromosome did not contain any resistance genes, and no prophages or CRISPR sequences were found.

Despite the presence of two *tra* operons likely encoding for a conjugative DNA transfer system on the plasmid that could favour genetic exchange, this bacterium could partially limit the integration of exogenous DNA owing to RMSs. Indeed, ten genes belonging to RMSs were identified, including four copies of the type I DNA methyltransferase gene, four copies of the type I specificity domain and two copies of the type I restriction endonuclease gene.

4. Discussion

In this study, the genome of the clinical isolate *E. miricola* EM_CHUV was sequenced and analysed with the aim of identifying the genetic basis of the MDR phenotype of this emerging pathogen. Such a MDR phenotype, including resistance to carbapenems, has also recently been documented for several other *E. miricola* strains incriminated in human infections [8–10]. Genome analysis of *E. miricola* EM_CHUV revealed the presence of a high number of genes involved in resistance to antibiotics (β-lactamases, aminoglycoside 6-adenylyltransferase, and sulfonamide, tetracycline and chloramphenicol resistance genes) that were not associated with mobile elements. Moreover, all except four resistance genes were found to be conserved and most similar to orthologues in other *E. miricola* strains and other *Elizabethkingia* spp. Altogether, these data suggested that the observed MDR phenotype of *E. miricola*

EM_CHUV might be an intrinsic characteristic of this species and might be common within the *Elizabethkingia* genera.

Isolation of *E. miricola* EM_CHUV in the setting of nosocomial pneumonia in this study, together with two other documentations of *E. miricola* occurring in the setting of severe infections (bacteraemia and sepsis) involving respiratory tract infections [8,9], raises the question of a pulmonary tropism of this micro-organism. Similar to what is being described for *E. meningoseptica* and *E. anophelis*, such a tropism might be associated with increased risk of nosocomial pneumonia and more generally of nosocomial infection, which is further supported by the fact that in most reported cases the patient becomes colonised by *E. miricola* following broad-spectrum antibiotic treatment.

Overall, this study supports that *E. miricola* be considered as a potential pathogen with significant nosocomial risk based on frequent MDR phenotypes. Novel accurate identification methods such as MALDI-TOF/MS might reveal the true incidence of this bacterium. As a consequence, particular attention should be paid to this pathogen when isolated from clinical samples, and antibiotic susceptibility testing should be systematically performed.

Sequence accession no.

The Whole Genome Shotgun project of *E. miricola* EM_CHUV has been deposited at DDBJ/EMBL/GenBank under the accession no. **LIQC00000000**.

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References

[1] Kim KK, Kim MK, Lim JH, Park HY, Lee ST. Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. Int J Syst Evol Microbiol 2005;55:1287–93.

[2] Li Y, Kawamura Y, Fujiwara N, Naka T, Liu H, Huang X, et al. *Chryseobacterium miricola* sp. nov., a novel species isolated from condensation water of space station Mir. Syst Appl Microbiol 2003;26:523–8.

[3] Kämpfer P, Busse HJ, McInroy JA, Glaeser SP. *Elizabethkingia endophytica* sp. nov., isolated from *Zea mays* and emended description of *Elizabethkingia anophelis* Kämpfer et al. 2011. Int J Syst Evol Microbiol 2015;65:2187–93.

[4] Kämpfer P, Matthews H, Glaeser SP, Martin K, Lodders N, Faye I. *Elizabethkingia anophelis* sp. nov., isolated from the midgut of the mosquito *Anopheles gambiae*. Int J Syst Evol Microbiol 2011;61:2670–5.

[5] Frank T, Gody JC, Nguyen LB, Berthet N, Le Fleche-Mateos A, Bata P, et al. First case of *Elizabethkingia anophelis* meningitis in the Central African Republic. Lancet 2013;381:1876.

[6] Lau SK, Chow WN, Foo CH, Curreem SO, Lo GC, Teng JL, et al. *Elizabethkingia anophelis* bacteremia is associated with clinically significant infections and high mortality. Sci Rep 2016;6:26045.

[7] Rossati A, Kroumova V, Bargiacchi O, Brustia D, Luigi Garavelli P. *Elizabethkingia miricola* bacteriemia in a young woman with acute alcoholic pancreatitis. Presse Med 2015;44:1071–2.

[8] Green O, Murray P, Gea-Banacloche JC. Sepsis caused by *Elizabethkingia miricola* successfully treated with tigecycline and levofloxacin. Diagn Microbiol Infect Dis 2008;62:430–2.

[9] Gonzalez C, Coolen-Allou N, Allyn J, Esteve JB, Belmonte O, Allou N. Severe sepsis and pulmonary abscess with bacteremia due to *Elizabethkingia miricola* [in French]. Med Mal Infect 2016;46:49–51.

[10] Colapietro M, Endimiani A, Sabatini A, Marcoccia F, Celenza G, Segatore B, et al. *bla*B-15, a new BlaB metallo-β-lactamase variant found in an *Elizabethkingia miricola* clinical isolate. Diagn Microbiol Infect Dis 2016;85:195–7.

[11] Matyi SA, Hoyt PR, Ayoubi-Canaan P, Hasan NA, Gustafson JE. Draft genome sequence of strain ATCC 33958, reported to be *Elizabethkingia miricola*. Genome Announc 2015;3:pii: e00828-15.

[12] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75.

[13] Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 2014;58:212–20.

[14] McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, et al. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 2013;57:3348–57.

[15] Dhillon BK, Laird MR, Shay JA, Winsor GL, Lo R, Nizam F, et al. IslandViewer 3: more flexible, interactive genomic island discovery, visualization and analysis. Nucleic Acids Res 2015;43:W104–8.

[16] Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, et al. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 2016;44:W16–21.

[17] Grissa I, Vergnaud G, Pourcel C. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 2007;35:W52–7.

[18] Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 2009;106:19126–31.

[19] Contreras-Moreira B, Vinuesa P. GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis. Appl Environ Microbiol 2013;79:7696–701.

[20] Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing *Pseudomonas* spp. J Clin Microbiol 2012;50:3773–6.

[21] Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a new Etest for detecting metallo-β-lactamases in routine clinical testing. J Clin Microbiol 2002;40:2755–9.

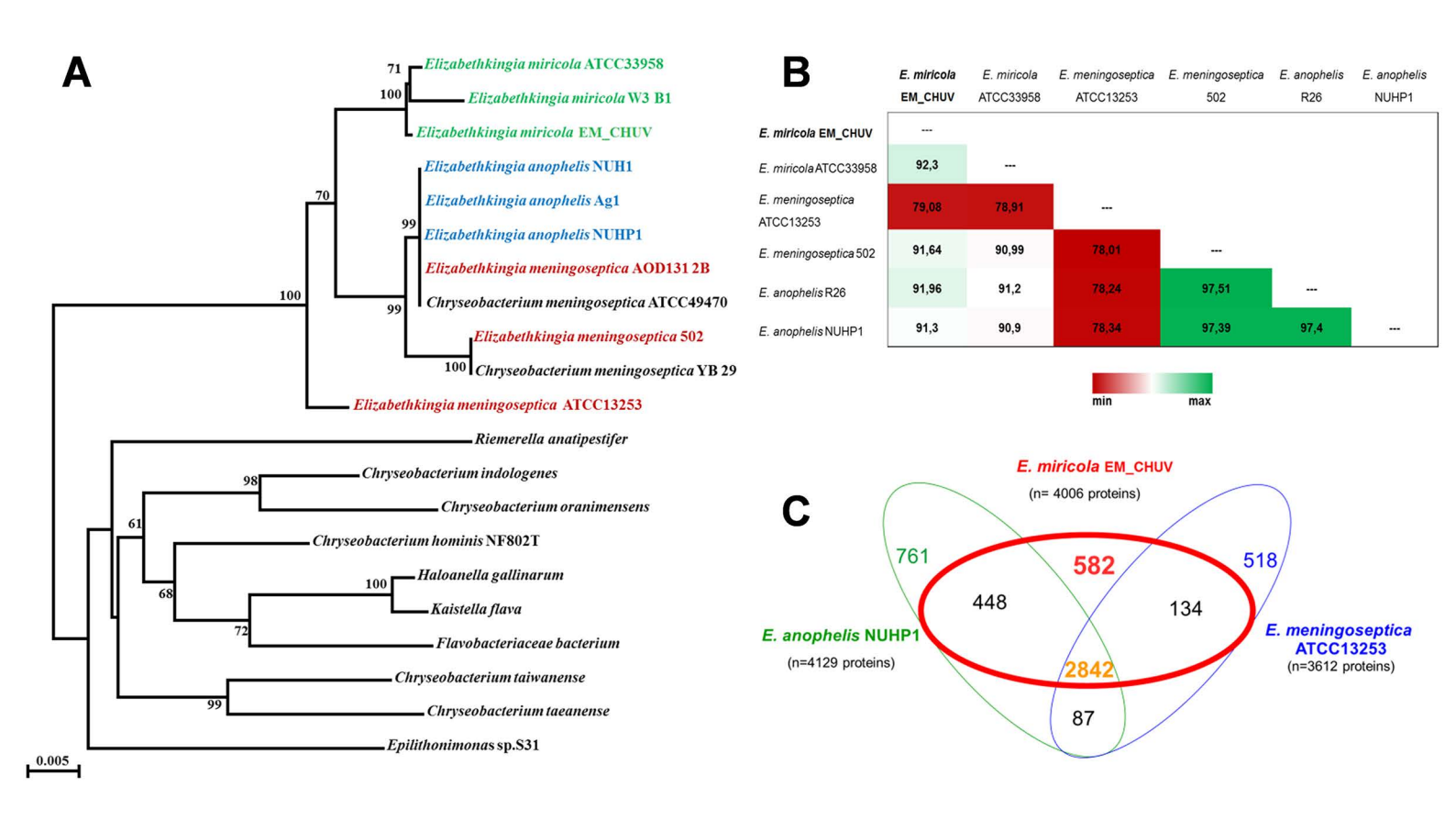
[22] Lawley TD, Gilmour MW, Gunton JE, Tracz DM, Taylor DE. Functional and mutational analysis of conjugative transfer region 2 (Tra2) from the IncHI1 plasmid R27. J Bacteriol 2003;185:581–91.

[23] Yum JH, Lee EY, Hur SH, Jeong SH, Lee H, Yong D, et al. Genetic diversity of chromosomal metallo-β-lactamase genes in clinical isolates of *Elizabethkingia meningoseptica* from Korea. J Microbiol 2010;48:358–64.

[24] Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23:2947-8.

[25] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular biology and evolution. 2013;30:2725-9.

Fig. 1. Phylogenetic relationship of *Elizabethkingia* spp. and comparative genomics of *Elizabethkingia miricola* EM_CHUV with other *Elizabethkingia* spp. (A) Phylogenetic relationship of *Elizabethkingia* spp. and closely related bacteria based on 16S rRNA sequences. The tree was constructed using the free Clustal X software [24] v.2.0 and MEGA software v.6.06 [25] using the neighbour-joining method with the model Kimura 2-parameter and 1000 bootstrap replicates. Bootstrap values are expressed by percentage of the 1000 replicates, and only those up to 60% are shown at branch points. (B) Pairwise comparison of the average nucleotide identity of the genomes performed using the JSpecies v.1.2.1 program [18]. (C) Proteome comparison between *E. miricola* EM_CHUV, *Elizabethkingia anophelis* NUPH1 and *Elizabethkingia meningoseptica* ATCC 13253.


A
Elizabethkingia miricola ATCC33958
Elizabethkingia miricola W3 B1
Elizabethkingia miricola EM_CHUV
Elizabethkingia anophelis NUH1
Elizabethkingia anophelis Ag1
Elizabethkingia anophelis NUHP1
Elizabethkingia meningoseptica AOD131 2B
Chryseobacterium meningoseptica ATCC49470
Elizabethkingia meningoseptica 502
Chryseobacterium meningoseptica YB 29
Elizabethkingia meningoseptica ATCC13253
Riemerella anatipestifer
Chryseobacterium indologenes
Chryseobacterium oranimensens
Chryseobacterium hominis NF802T
Haloanella gallinarum
Kaistella flava
Flavobacteriaceae bacterium
Chryseobacterium taiwanense
Chryseobacterium taeanense
Epilithonimonas sp.S31
71
100
70
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E. miricola EM_CHUV
E. miricola ATCC33958
E. meningoseptica ATCC13253
E. meningoseptica 502
E. anophelis R26
E. anophelis NUHP1
92,3
79,08 78,91
91,64 90,99 78,01
91,96 91,2 78,24 97,51
91,3 90,9 78,34 97,39 97,4
min
max
C
E. miricola EM_CHUV
(n= 4006 proteins)
761
582
518
448
134
E. anophelis NUHP1
(n=4129 proteins)
2842
E. meningoseptica ATCC13253
(n=3612 proteins)
87

Table 1

Summary of *Elizabethkingia miricola* infections reported to date

Year	Age (years)/ sex	Underlying condition(s)	Clinical manifestation(s)	Clinical sample	Treatments	Outcome	Reference
2008	55/M	Stage IV mantle cell lymphoma, salvage chemotherapy	Febrile neutropenia, pulmonary nodule, sepsis	Tracheal aspirate, blood cultures	Tigecycline, levofloxacin (consecutive to ceftazidime, vancomycin, meropenem)	Persistent pulmonary colonisation, sepsis	[8]
2015	34/F	Alcohol consumption	Acute alcoholic pancreatitis, respiratory distress requiring non-invasive ventilation, lung atelectatic areas	Blood cultures	Ciprofloxacin, imipenem, (consecutive to imipenem alone)	Favourable	[7]
2016	31/F	Arterial hypertension	Worsening of general state, dry cough, fever dyspnoea, lung abscess and pleural effusion, dilated cardiomyopathy with left ventricular thrombus	Blood cultures	TZP, gentamicin (consecutive to TZP alone)	Favourable	[9]

2016	2/M	Spina bifida, bladder exstrophy, atelectasis, tracheostomy, chronic kidney insufficiency, Mitrofanoff stoma fistula	Fever and severe clinical conditions	Urine collected from an intermittent catheter	Unknown	Unknown	[10]
2016	82/M	Status post bone marrow surgery, recurrent pneumonia	Severe nosocomial pneumonia	Bronchoalveolar lavage fluid and tracheal aspirate	Amikacin, imipenem (consecutive to moxifloxacin, ceftazidime)	Death [a]	This study

TZP, piperacillin/tazobactam;

[a] Treatment withdrawal in the context of tetraparesia and nosocomial pneumonia.

Table 2

Antibiotic resistance pattern and resistance genes of *Elizabethkingia miricola* EM_CHUV

Antibiotic class	Tested antibiotics	Inhibition diameter (mm)	Etest MIC (mg/L)	Interpretation	Resistance genes identified in the genome [a]
β-Lactams	Ticarcillin/clavulanic acid	6	>256	R	β-Lactamase (*n* = 16)
	Piperacillin/tazobactam	6	32	R	MBL fold metallo-hydrolase (*n* = 2)
	Ceftazidime	6	>256	R	
	Cefepime	11	24	R	Class B carbapenemase *bla*$_{GOB-13}$
	Imipenem	6	>32	R	
	Meropenem	6	>32	R	Class B carbapenemase *bla*$_{B-9}$
	Aztreonam	6	>256	R	
Aminoglycosides	Amikacin	21	8	S	Aminoglycoside 6-adenylyltransferase
	Gentamicin	22	4	S	
	Netilmicin	15	24	R	
	Tobramycin	6	32	R	
Tetracyclines	Minocycline	ND	1	S	Tetracycline resistance protein TetX
	Tigecycline	ND	1	NA	Tetracycline efflux protein TetA
					Transmembrane efflux protein
					Antibiotic transporter
Polymyxins	Colistin	6	>256	R	

Sulfonamides	Trimethoprim/sulfamethoxazole	13	3	I	Dihydrofolate reductase DHFR Dihydrofolate reductase FolA Bifunctional deaminase-reductase protein Dihydropteroate synthase FolP (EC 2.5.1.15)
Quinolones	Ciprofloxacin	32	2	R	DNA gyrase GyrA subunit A mutation T83S DNA gyrase GyrB subunit A mutation M437L
	Levofloxacin	31	1	S	
Rifampicin	Rifampicin	ND	0.75	NA	
Resistance genes for untested classes of antibiotics					
Macrolides	–	–	–	–	Macrolide resistance, ABC transporter Macrolide efflux protein, MFS transporter DHA3 Erythromycin resistance, EmrB/QacA (*n* = 3) Erythromycin esterase
Chloramphenicol	–	–	–	–	Chloramphenicol acetyltransferase CatB Bcr/CflA family drug resistance efflux pump

MIC, minimum inhibitory concentration; ND, not done; R, resistant; S, sensitive; NA, not applicable (no interpretation criteria); I, intermediate; MBL, metallo-β-lactamase.

[a] Details of the resistance genes as well as their locus tag and their presence/absence in *E. miricola* ATCC 33958 are presented in Supplementary Table S2.

Supplementary materials and methods

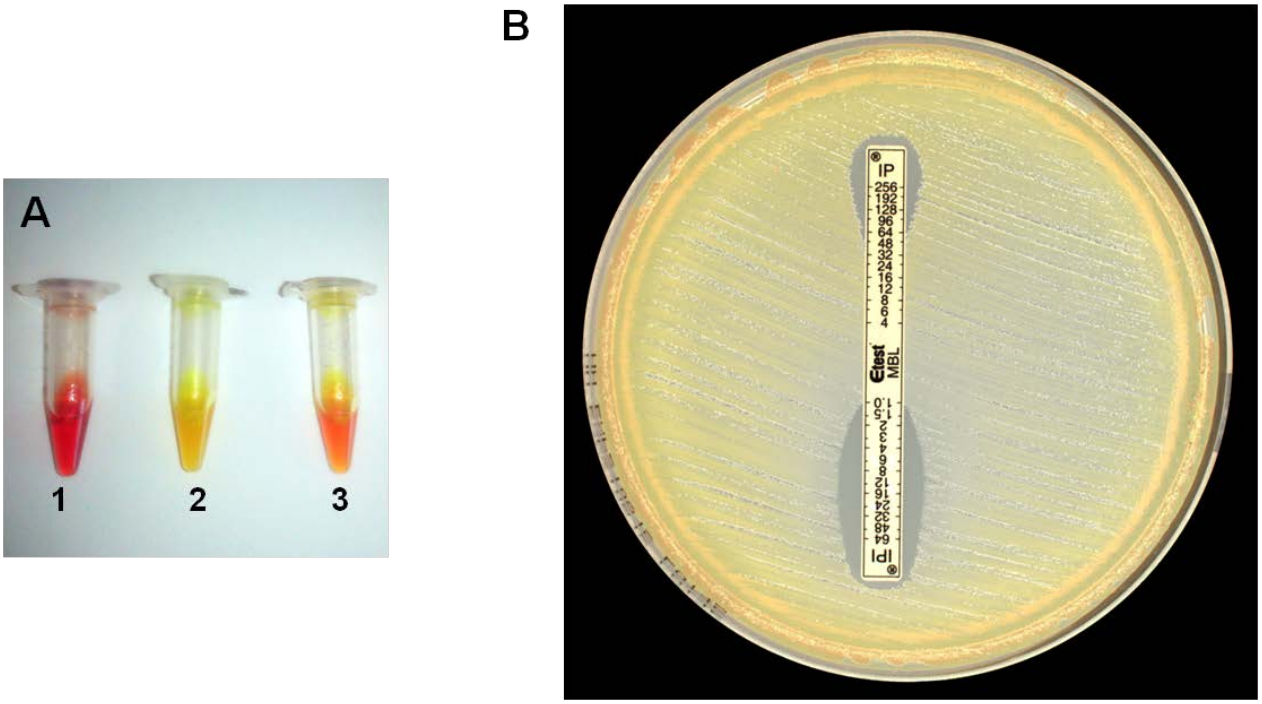
Microbiology procedures

The *Elizabethkingia miricola* strain EM_CHUV and the *Stenotrophomonas maltophilia* isolate grew on blood agar plates and MacConkey plates and were lactose-negative and oxidase-positive. Identification to species level was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonics, Bremen, Germany) with a score above 2. Minimum inhibitory concentrations (MICs) were determined by Etest (bioMérieux, Lyon, France) and their interpretations were achieved using the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://eucast.org/). For *E. miricola* EM_CHUV, the antibiotics commonly analysed for non-fermenting bacteria were tested; however, in the absence of specific criteria, the EUCAST criteria for *Pseudomonas* spp. were used for categorical interpretation as susceptible or resistant. Production of carbapenem-degrading enzymes was determined using the Carba NP test [1] and by metallo-β-lactamase (MBL) Etest strips (MBL IP/IPI; bioMérieux).

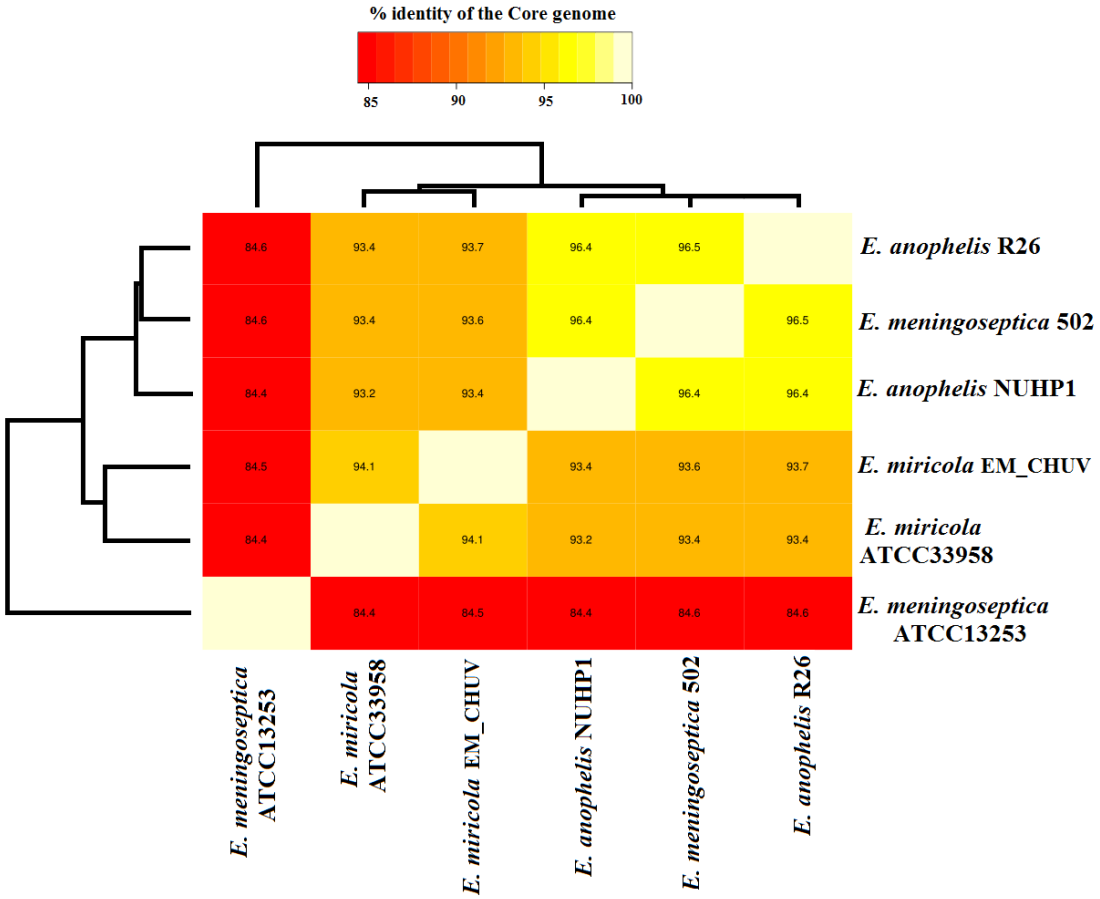
Genomic sequencing, assembly and annotation

Genomic DNA of *E. miricola* EM_CHUV was extracted and purified using a Wizard Genomic DNA Purification Kit (Promega, Dübendorf, Switzerland). Purified genomic DNA was subjected to whole-genome shotgun sequencing using 2 × 150 bp paired-end sequencing on a MiSeq sequencer (Illumina, San Diego CA) from a single library. The quality of the raw sequence data was checked using the FastQC program (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The paired-end reads were filtered

according to quality values and sizes using the Fastq-Mcf program (Ea-utils: command-line tools for processing biological sequencing data; https://expressionanalysis.github.io/ea-utils/). Genome assembly of the 1 707 522 filtered reads was performed using the SPAdes v.3.5.0 program [2] with different Kmer values (from 51 to 109). The assembly with lowest number of contigs (*n* = 24) and highest N50 (374 739 bp) was kept for subsequent genome analysis. The 24 contigs ranged in size from 1038 bp to 671 192 bp with a mean coverage of 25-fold. The genome of *E. miricola* EM_CHUV contained 4 286 503 bp with a GC content of 35.78%, and a plasmid of 176 107 bp with a GC content of 40.15%. All of the assembled contigs were submitted to the RAST server [3] for automatic annotation that predicted 4006 coding sequences (CDS), 46 tRNA genes and 6 ribosomal RNAs (rRNAs).



Supplementary Fig. S1. Carbapenemase detection in *Elizabethkingia miricola* EM_CHUV. (A) Carbapenemase detection using the rapid Carba NP test based on hydrolysis of the β-lactam ring of the carbapenem imipenem associated with a colour change induced by a pH indicator in the presence of a carbapenemase [1]. Strain 1, *Klebsiella pneumoniae* ATCC BAA-1705, which served as a carbapenemase-positive control; strain 2, *K. pneumoniae* ATCC BAA-1706, which served as a negative control strain; and strain 3, *E. miricola* EM_CHUV. (B) Characterisation as metallo-β-lactamase (MBL) using MBL Etest strips (MBL IP/IPI; bioMérieux, Lyon, France) with comparison of differential inhibition with (IP = 0.1 mg/L) and without ethylene diamine tetra-acetic acid (EDTA) as chelator (IPI = 64 mg/L) in the presence of imipenem.



Supplementary Fig. S2. Pairwise comparison of the core proteomes of *Elizabethkingia* spp. This comparison was performed using a published code 'get_homologues.pl' [4]. Values shown in the boxes refer to the average percentage identity of each pair of proteomes.

Supplementary Table S1

General features the *Elizabethkingia miricola* EM_CHUV genome and all of the other *Elizabethkingia* genomes studied

Name	Sources	Number of contigs	Genome size (Mbp)	%GC content	No. of genes	No. of proteins	tRNAs	Genome identity (%)
***E. miricola* EM_CHUV**	Human	**24**	**4.29**	**35.78**	**4058**	**4006**	**46**	**–**
		1 (plasmid)	**0.176**	**40.15**	**171**	**171**	**0**	**–**
E. miricola ATCC 33958	Collection	75	4.58	35.35	4501	4454	47	**92.30**
E. anophelis NUHP1	Human	64	4.34	35.6	4178	4130	48	91.30
E. anophelis R26	Gut of mosquitoes	66	4.03	35.4	3879	3839	40	91.96
E. meningoseptica ATCC 13253	Human	34	3.84	36.4	3612	3571	41	79.08
E. meningoseptica 502	Human	21	3’96	35.5	3765	3714	51	91.64

Supplementary Table S2

Antibiotic resistance genes identified in the genome of *Elizabethkingia miricola* EM_CHUV, their best BLAST hit in the non-redundant (nr) database and their presence (+) or absence (–) in the genomes of other *Elizabethkingia* spp.

Locus number	Gene in *E. miricola* EM_CHUV	Putative function	Gene size (bp)	Identity (%)	E-value	Organism with the best hit in NCBI database	Gene in *Elizabethkingia* spp. [a]
	β-LACTAM RESISTANCE GENES						
AMC91_07925	–	β-Lactamase	1047	90	0	*Chryseobacterium* sp. OV715	–
AMC91_08300	bla_{GOB-13}	Class B carbapenemase Bla_{GOB-13}	879	99	0	*E. meningoseptica*	+
AMC91_03610	–	β-Lactamase	1134	95	0	*E. miricola*	+
AMC91_08990	–	β-Lactamase (EC 3.5.2.6)	1083	93	0	*E. meningoseptica*	+
AMC91_09070	bla_{ACME}	β-Lactamase (Bla_{ACME}) VEB-1-like	891	94	0	*E. meningoseptica*	+
AMC91_09240	–	β-Lactamase (EC 3.5.2.6)	1089	42	2e-144	*Colwellia psychrerythraea*	–
AMC91_09510	–	β-Lactamase (EC 3.5.2.6)	1428	93	0	*E. anophelis*	+
AMC91_09825	bla_{B}	BJP β-Lactamase	723	95	2e-166	*E. anophelis*	+
AMC91_05755	bla_{A}	Class A β-lactamase (EC 3.5.2.6)	891	98	0	*E. anophelis*	+
AMC91_11605	*ampC*	Class C AmpC β-lactamase	348	47	1e-19	*Mariniradius saccharolyticus*	–
AMC91_11615	–	β-Lactamase (EC 3.5.2.6)	873	83	1e-180	*E. miricola* BM10	+
AMC91_00570	–	MBL fold metallo-hydrolase	999	92	0	*E. miricola*	+
AMC91_04315	–	β-Lactamase (EC 3.5.2.6)	1491	93	0	*E. anophelis*	+
AMC91_07385		β-Lactamase (EC 3.5.2.6)	1293	44	5e-103	*Chryseobacterium* sp. YR561	–
AMC91_04980	–	β-Lactamase	684	94	2e-159	*E. meningoseptica*	+

AMC91_05565	–	β-Lactamase (EC 3.5.2.6)	1062	93	0	*E. anophelis*	+
AMC91_17770	–	β-Lactamase (EC 3.5.2.6)	1131	97	0	*E. miricola* BM10	+
AMC91_18185	–	MBL fold metallo-hydrolase	867	100	0	*E. miricola* BM10	+
AMC91_06520	*bla*B-9	Class B carbapenemase BlaB-9	747	100	2e-180	*E. meningoseptica*	+
AMC91_02425	–	β-Lactamase (EC 3.5.2.6)	1545	98	0	*E. meningoseptica*	+
	AMINOGLYCOSIDE RESISTANCE GENES						
AMC91_13030	*ant-6*	Aminoglycoside 6-adenylyltransferase	867	91	0	*E. miricola* BM10	+
	CHLORAMPHENICOL RESISTANCE GENES						
AMC91_05240	*catB*	Chloramphenicol acetyltransferase CatB	861	95	9e-135	*E. miricola*	+
AMC91_14995	*bcr*/*cflA*	Bcr/CflA family drug resistance efflux pump	1146	92	0	*E. anophelis*	+
	SULFONAMIDE RESISTANCE GENES						
AMC91_17570	*dhfR*	Dihydrofolate reductase DHFR	525	100	6e-123	*E. miricola*	+
AMC91_06105	*folA*	Dihydrofolate reductase FolA	558	95	9e-122	*E. miricola*	+
AMC91_07670	–	Bifunctional deaminase-reductase protein	570	96	1e-130	*E. meningoseptica*	+
AMC91_05240	*folP*	Dihydropteroate synthase FolP (EC 2.5.1.15)	861	99	2e-180	*E. miricola* BM10	+
	TETRACYCLINE RESISTANCE GENES						
AMC91_04870	*tetX*	Tetracycline resistance protein TetX	1131	91	0	*E. meningoseptica*	+
AMC91_01490	*tetA*	Tetracycline efflux protein TetA	1212	98	0	*E. anophelis*	+
AMC91_18395	–	Transmembrane efflux protein	1409	97	0	*E. miricola* BM10	+
AMC91_07415	–	Antibiotic transporter	1386	99	0	*E. anophelis*	+
	MACROLIDE RESISTANCE GENES						

AMC91_17220	*lolD*	Macrolide resistance, ABC transporter	693	100	8e-163	*E. anophelis*	+
AMC91_00700	*dhA3*	Macrolide efflux protein, MFS transporter DHA3	1248	97	0	*E. miricola*	+
AMC91_05800	*emrB*	Erythromycin resistance, EmrB/QacA	1551	96	0	*E. meningoseptica*	+
AMC91_08945	*emrB*	Erythromycin resistance, EmrB/QacA	1410	99	0	*E. anophelis*	+
AMC91_13070	*emrB*	Erythromycin resistance, EmrB/QacA	1578	99	0	*E. anophelis*	+
AMC91_07245	–	Erythromycin esterase	1269	96	0	*E. miricola* BM11	+
	QUINOLONE RESISTANCE GENES [b]						
AMC91_00350	*gyrA*	DNA gyrase GyrA subunit A (T83S)	2573	100	0	*E. miricola*	+
AMC91_00615	*gyrB*	DNA gyrase GyrB subunit A (M437L)	1934	99	0	*E. miricola*	+
AMC91_00940	*parE*	DNA topoisomerase IV subunit B (M437F/A473L)	1896	100	0	*E. miricola*	+

NCBI, National Center for Biotechnology Information.

[a] Presence in the genomes of the *E. miricola* ATCC 33958, *Elizabethkingia anophelis* NUHP1, *E. anophelis* R26, *Elizabethkingia meningoseptica* ATCC 13253 and *E. meningoseptica* 502: +, present in all of the genomes; –, absent in all of the genomes.

[b] These mutations were determined with respect to those from *Pseudomonas aeruginosa* PAO1.

References

[1] Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing Pseudomonas spp. Journal of clinical microbiology. 2012;50:3773-6.
[2] Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. Journal of computational biology : a journal of computational molecular cell biology. 2013;20:714-37.
[3] Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC genomics. 2008;9:75.
[4] Contreras-Moreira B, Vinuesa P. GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis. Applied and environmental microbiology. 2013;79:7696-701.