



HAL
open science

Genomic features of an isolate of *Empedobacter falsenii* harbouring a novel variant of metallo- β -lactamase, blaEBR-4 gene

Ahmed Olowo-Okere, Yakubu Kokori Enevene Ibrahim, Busayo Olalekan Olayinka, Yahaya Mohammed, Larbi Zakaria Nabti, David Lupande-Mwenebitu, Jean-Marc Rolain, Seydina Diene

► To cite this version:

Ahmed Olowo-Okere, Yakubu Kokori Enevene Ibrahim, Busayo Olalekan Olayinka, Yahaya Mohammed, Larbi Zakaria Nabti, et al.. Genomic features of an isolate of *Empedobacter falsenii* harbouring a novel variant of metallo- β -lactamase, blaEBR-4 gene. *Infection, Genetics and Evolution*, 2022, 98, pp.105234. 10.1016/j.meegid.2022.105234. hal-03684605

HAL Id: hal-03684605

<https://amu.hal.science/hal-03684605>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Genomic features of an isolate of *Empedobacter falsenii* harbouring a novel variant of**
2 **metallo- β -lactamase, *bla*_{EBR-4} gene**

3 Ahmed Olowo-okere^{1,5*}, Yakubu Kokori Enevene Ibrahim², Busayo Olalekan Olayinka²,
4 Yahaya Mohammed³, Larbi Zakaria Nabti⁴, David Lupande-Mwenebitu⁵, Jean-Marc Rolain⁵,
5 Seydina M. Diene^{5*}

6

7 **1.** Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo
8 University Sokoto, Nigeria.

9 **2.** Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello
10 University, Zaria, Nigeria.

11 **3.** Department of Medical Microbiology, College of Health Sciences, Usmanu Danfodiyo
12 University, Sokoto, Nigeria.

13 **4.** Université de Sétif 1, Laboratoire de Microbiologie (CHU de Sétif), Sétif, Algérie.

14 **5.** MEPHI, IRD, APHM, IHU-Mediterranee Infection, Faculté de Pharmacie, Aix-Marseille
15 University, Marseille, France

16 ***Corresponding authors:**

17 1. Ahmed Olowo-okere, Department of Pharmaceutics and Pharmaceutical Microbiology,
18 Usmanu Danfodiyo University Sokoto, Nigeria.

19 Email: olowookere.ahmed@udusok.edu.ng

20 2. Seydina M. Diene, Aix Marseille Univ, IRD, APHM, MEPHI, IHU Méditerranée
21 Infection, Faculté de Pharmacie, Marseille CEDEX 05, France. Email:

22 seydneya.m.ddiene@gmail.com

23 **Abstract**

24 *Empedobacter falsenii* is an emerging opportunistic pathogen that has been
25 occasionally implicated in various human infections. In this study, we described the genomic
26 features of a multidrug resistant *E. falsenii* Q1655 obtained from a patient attending a public
27 hospital in Sokoto, northwest Nigeria. The isolate, *E. falsenii* Q1655, was isolated from the
28 stool sample of a patient in Sokoto, Nigeria. The identity of the isolate was confirmed by
29 MALDITOF-MS. The disc diffusion test and modified Carba-NP test were used for
30 phenotypic antibiotic susceptibility test and carbapenemase enzyme production test,
31 respectively. The whole genome of the strain was sequenced using the Illumina MiSeq
32 technique. Resistome analysis was done by annotation of the WGS against the ARG-ANNOT
33 database. The isolate was resistant to all β -lactam antibiotics with the exception of cefepime.
34 The MICs of imipenem and ertapenem as determined by E-test were 12 μ g/ml and 2 μ g/ml,
35 respectively. Modified Carba NP test showed that the strain was carbapenemase producing.
36 Resistome analysis revealed the presence of a novel metallo- β -lactamase, a chromosomal
37 *bla*_{E_{BR}-4} (Accession No: MN997121), which exhibited 94.92 % and 97.02 % nucleotide and
38 protein sequence identities respectively with *bla*_{E_{BR}-3} gene of *E. falsenii* 174820. Seven and
39 eight amino-acid substitutions were observed with the *bla*_{E_{BR}-1} and *bla*_{E_{BR}-2}, respectively. We
40 reported the first isolation and genomic description of an extensively drug resistant isolate of
41 *Empedobacter falsenii* in Nigeria. This report broadens our knowledge of carbapenem
42 resistance in *E. falsenii* and it will serve as a useful guide in the development of antibiotic use
43 policy.

44 **Keywords:** *Empedobacter falsenii*, metallo- β -lactamase, *bla*_{E_{BR}-4}, *tetX*

45 **Introduction**

46 *Empedobacter falsenii* (formally *Wautersiella falsenii*) is a member of the family
47 Flavobacteriaceae [1,2]. The bacteria species was first isolated and described in 2006 in
48 Belgium by Kampfe et al. [3]. The bacterium is an emerging opportunistic, non-fermentative
49 Gram-negative pathogen that has been occasionally implicated in various human infections
50 [4]. The strain has been previously isolated from the respiratory tract of an
51 immunocompromised patient and also from the urine of an infant with pyelonephritis, among
52 others [5,6]. The increasing identification of this strain from varying human clinical samples
53 is facilitated by improvement in the clinical microbiology laboratory capacity including the
54 use of quick and reliable matrix-assisted laser desorption ionization time-of-flight mass
55 spectrometry [7,8].

56 Metallo- β -lactamases (MBLs) are bacterial enzymes which catalyse the formation of a
57 noncovalent reactive complex with β -lactam rings through a hydrolytic reaction facilitated by
58 one or two essential divalent cations, typically zinc, at their active site [9,10]. The reaction
59 results in the formation of microbiologically inactive molecule that is incapable of forming an
60 enzymatically productive complex with a penicillin binding protein (PBP), ultimately leading
61 to loss of activity of β -lactam antibiotics [10]. Several MBLs have been described but the
62 most commonly encountered are the Verona integron encoded metallo- β -lactamase (VIM),
63 Imipenemase (IMP) and New-Delhi Metallo- β -lactamase (NDM) (Bonomo & Szabo, 2006).
64 Other MBL are confined to a specific bacteria species such as *bla*_{EBR} identified in
65 *Empedobacter* spp. or to a particular geographical location such as SPM-1 (Sao Paulo MBL),
66 GIM-1 (German imipenemase); SIM-1 (Seoul imipenemase); TMB-1 (Tripoli MBL) [11].
67 This is likely due to the localisation of the corresponding genes on narrow-host- range
68 plasmids or chromosomes, which makes wide diffusion unlikely [12].

69 Although *E. falsenii* is not a common cause of infection in hospitalized patients, its
70 genome is replete with genes mediating resistance to several antibiotics classes particularly
71 cephalosporin and carbapenems [7,13]. Strain of *E. falsenii* resistant to colistin has also been
72 reported [14]. This makes this bacterium a pathogen of concern as it may serve as reservoir
73 for dissemination of antibiotic resistant to genes to clinically important bacteria. In this
74 study, we described the genomic features of a multidrug resistant isolate of *E. falsenii*
75 obtained from a patient attending a public hospital in Sokoto, northwest Nigeria.

76

77 **Methods**

78 The approval to conduct this study was granted by the Sokoto State Ministry of
79 Health (Approval number: SMH/1580/VIV) and the Research and Ethics Committee of the
80 hospital (Approval number: UDUTH/HREC/2019/770. In April 2019, a strain of *E. falsenii*
81 Q1655 was isolated from the stool sample of a 54 years old male patient admitted into the
82 medical ward of a tertiary healthcare facility in Sokoto, Nigeria as part of antibiotics
83 resistance surveillance. The identity of the isolates was confirmed by the matrix assisted laser
84 desorption ionisation time of flight mass spectrometry (MALDITOF) following a previously
85 described protocol [8]. The disc diffusion test and modified Carba-NP test were used for
86 phenotypic detection of antibiotic susceptibility and production of carbapenemase enzyme,
87 respectively [15,16]. The result of antibiotic susceptibility test was interpreted using the
88 EUCAST breakpoints for non-Enterobacteriaceae. Detection of genes encoding ESBLs
89 (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}) and carbapenemases (*bla*_{OXA-48}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM} and *bla*_{KPC})
90 was done using the real-time PCR (qPCR) as previously described [17]. The whole genome
91 of the strain was thereafter sequenced using the MiSeq technique (Illumina Inc., San Diego,
92 CA, USA). The reads obtained were assembled using Spades software and annotated by the

93 Prokka bacterial genome annotation pipeline [18,19]. The Plasmid incompatibility (*Inc*)
94 groups and resistome analysis were determined *in-silico* using the PlasmidFinder 2.0 platform
95 and annotation of the whole genome sequence (WGS) against antibiotic resistance gene
96 annotation (ARG-ANNOT) database, respectively [20,21]. Alignment of the nucleotide
97 sequences and amino acid sequences of the β -lactamase gene and its variants was done using
98 the Network Protein Sequence Analysis server [22].

99

100 **Results and Discussion**

101 The antibiotic susceptibility assay revealed that the strain was resistant to several
102 antibiotics including penicillins (amoxicillin, amoxicillin-clavulanate), second generation
103 cephalosporin (cephalothin), third-generation cephalosporin (ceftriaxone) and carbapenems
104 (imipenem and ertapenem) with the exception of cefepime (Table 1). The MIC of imipenem
105 and ertapenem were respectively 12 μ g/ml and 2 μ g/ml. Phenotypic modified Carba NP test
106 conducted according to the previously published protocol showed that the strain was
107 carbapenemase producing. The PCR investigation of resistance mechanism revealed the
108 absence of common acquire β -lactamase genes (i.e. *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}, *bla*_{NDM}, *bla*_{OXA-}
109 ₄₈, *bla*_{VIM}, *bla*_{KPC}, *bla*_{IMP}). This was what prompted the genomic investigation of the strain.

110 The genome assembly results to a chromosome size of 3,482,659-bp with a G+C
111 content of 34.4 % (Table 1). Resistome analysis of the WGS of *E. falsenii* Q1655 against
112 ARG-ANNOT database revealed the presence of a novel metallo- β -lactamase type 2, a
113 chromosomal *bla*_{EBR-4} which exhibited 94.92 % and 97.02 % nucleotide and protein identity
114 with *bla*_{EBR-3} gene of *E. falsenii* 174820 (Accession No: NG_066507). Pairwise alignment of
115 the amino acid sequence revealed that the *bla*_{EBR-4} differs from *bla*_{EBR-3} by only 6 amino-acids
116 (fig. 1). Seven and eight amino-acid substitutions were observed with the *bla*_{EBR-1} of

117 *Empedobacter brevis* (Accession No: WP_063860216) and *bla*<sub>E_{BR}-2 of *E. falsenii* (Accession
118 No: WP_038333643), respectively. The various mutations were expected to affect the
119 biological activity of the enzyme. Surprisingly, the enzyme retains its carbapenem
120 hydrolysing activity as demonstrated by its resistance to carbapenem and positive Carba NP
121 test result. The absence of plasmid in the genome of the strain indicates that the *bla*<sub>E_{BR}-4 is
122 chromosomal. This is in line with a previous description of the gene [23]. The *bla*_{E_{BR}} family
123 of metallo-β-lactamase enzyme, efficiently catalyzes the hydrolysis of all penicillin,
124 carbapenems and cephalosporin with a notable exception of cefepime [24]. So far, all
125 detected *bla*_{E_{BR}} genes have been detected in the genus *Empedobacter*. With the exception of
126 *bla*_{E_{BR}-1} which was detected in *E. brevis*, the rest were all detected in *E. falsenii* [23,24]. The
127 phylogenetic tree showing the relationship of the *bla*_{E_{BR}-4} with other β-lactamase genes
128 downloaded from the gene bank is presented in Figure 2. In addition, the genome also
129 contains *tet(X2)* gene, a tetracycline-modifying enzyme involved in enzymatic inactivation of
130 all generations of tetracycline including tigecycline [25].</sub></sub>

131 The resistance of the strain to other antibiotics such as fosfomycin, nitrofurantoin,
132 trimethoprim/sulphamethoxazole may be due to non-permeability of its outer membrane
133 similar to other non-fermentative bacteria [26,27]. Similar to a previously published report,
134 the strain was resistant to colistin [14]. *Empedobacter falsenii* has been suggested to be
135 naturally resistant to colistin [13].

136 **Conclusion**

137 We reported the first isolation and genomic description of an extensively drug
138 resistant isolate of *Empedobacter falsenii* in Nigeria. The isolate was resistant to several
139 medically important antibiotics including third generation cephalosporin, carbapenems,
140 fosfomycin and colistin and harbours a novel variant of metallo-β-lactamase, *bla*_{E_{BR}-4}. This

141 report broadens our knowledge of carbapenem resistance in *E. falsenii* and it will serve as a
142 useful baseline for understanding resistance mechanism in this strain in the future.

143 **Sequences accession number:**

144 The Whole Genome Shotgun project of *E. falsenii* Q1655 has been deposited at
145 DDBJ/ENA/GenBank under the accession JAAGKM000000000. The isolate information,
146 sequencing metrics, and genomic data of the strain are listed in Table 1.

147 **Funding information:**

148 This work was supported by the French Government under the Investments for the
149 Future program managed by the National Agency for Research, Méditerranée Infection, 10-
150 IAHU-03.

151 **Acknowledgement**

152 We sincerely thank Dr Linda Hadjadj for the technical support given to us during the
153 course of this study and for deposition of the WGS in NCBI database.

154 **Conflict of interest:**

155 We declare no conflicts of interest.

- 157 [1] Parte AC. LPSN–List of Prokaryotic names with Standing in Nomenclature (bacterio . net),
158 20 years on. *Int J Syst Evol Microbiol* 2018;68:1825–1829.
- 159 [2] Zhang R-G, Tan X, Liang Y, Meng T-Y, Liang H-Z, Jie Lv. Description of *Chishuiella*
160 *changwenlii* gen . nov ., sp . nov ., isolated from freshwater , and transfer of *Wautersiella*
161 *falsenii* to the genus *Empedobacter* as. *Int J Syst Evol Microbiol* 2014;64:2723–8.
- 162 [3] Kampf P, Avesani V, Michele Janssens, Charlier J, Baere T De, Vaneechoutte M.
163 Description of *Wautersiella falsenii* gen . nov ., sp . nov ., to accommodate clinical isolates
164 phenotypically resembling members of the genera *Chryseobacterium* and *Empedobacter*. *Int J*
165 *Syst Evol Microbiol* 2006;56:2323–9.
- 166 [4] Zaman K, Kaur V, Mohan B, Taneja N. *Empedobacter falsenii*: a rare non-fermenter causing
167 urinary tract infection in a child with bladder cancer. *SOA Clin Med Cases, Reports Rev*
168 2017;1:1–4.
- 169 [5] Giordano C, Falleni M, Capria AL, Caracciolo F, Petrini M, Barnini S. First report of
170 *Wautersiella falsenii* genomovar 2 isolated from the respiratory tract of an immunosuppressed
171 man. *IDCases* 2016;4:27–9.
- 172 [6] Zaman K, Gupta P, Kaur V, Mohan B, Taneja N. *Empedobacter Falsenii*: a Rare Non-
173 Fermenter Causing Urinary Tract Infection in a Child with Carcinoma Bladder. *SOA Clin Med*
174 *Cases, Reports Rev* 2017;1:2–4.
- 175 [7] Dixon C. Identification of two novel β -lactamases from an extensively antibiotic resistant
176 strain of *Empedobacter falsenii*, wf282. California State University, 2017.
- 177 [8] Seng P, Drancourt M, Scola B La, Fournier P, Rolain JM, Raoult D. Ongoing Revolution in
178 Bacteriology : Routine Identification of Bacteria by Matrix-Assisted Laser Desorption
179 Ionization Time-of-Flight Mass Spectrometry. *Clin Infect Dis* 2009;49:543–51.
- 180 [9] Alby K, Miller MB. Mechanisms and Detection of Antimicrobial Resistance. *Princ. Pract.*
181 *Pediatr. Infect. Dis. Fifth Edit*, Elsevier Inc.; 2018, p. 1467-1478.e4.
- 182 [10] Bush K. Epidemiology of Beta-Lactamase-Producing Pathogens. *Clin Microbiol Rev*
183 2020;33:1–37.
- 184 [11] Patel G, Bonomo RA. “Stormy waters ahead”: global emergence of carbapenemases. *Front*
185 *Microbiol* 2013;4:1–17.
- 186 [12] Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, et al. Carbapenemase-
187 Producing Organisms: A Global Scourge. *Clin Infect Dis* 2018;66:1290–7.
- 188 [13] Zeng Y, Dong N, Zhang R, Liu C, Sun Q, Lu J, et al. Emergence of an *Empedobacter falsenii*
189 strain harbouring a tet(X)-variant-bearing novel plasmid conferring resistance to tigecycline. *J*
190 *Antimicrob Chemother* 2019:1–6.
- 191 [14] Traglia GM, Dixon C, Chiem K, Almuzara M, Barberis C, Montaña S, et al. Draft Genome
192 Sequence of *Empedobacter* (Formerly *Wautersiella*) *falsenii* comb . nov . Wf282, a strain
193 isolated from a cervical neck. *Genome Announc* 2015;3:2–3.
- 194 [15] EUCAST. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for
195 interpretation of MICs and zone diameters European Committee on Antimicrobial
196 Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. 2019.
- 197 [16] Bakour S, Garcia V, Loucif L, Brunel JM, Gharout-Sait A, Touati A, et al. Rapid identification
198 of carbapenemase-producing *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter*
199 *baumannii* using a modified Carba NP test. *New Microbes New Infect* 2015;7:89–93.
- 200 [17] Lalaoui R, Bakour S, Livnat K, Assous MV, Diene SM, Rolain J-M. Spread of Carbapenem
201 and Colistin-Resistant *Klebsiella pneumoniae* ST512 Clinical Isolates in Israel: A Cause for
202 Vigilance. *Microb Drug Resist* 2018;00:1–9.
- 203 [18] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: A
204 new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*
205 2012;19:455–77.
- 206 [19] Seemann T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–9.
- 207 [20] Carattoli A, Zankari E, Garcíá-Fernández A, Larsen MV, Lund O, Villa L, et al. In Silico
208 detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing.

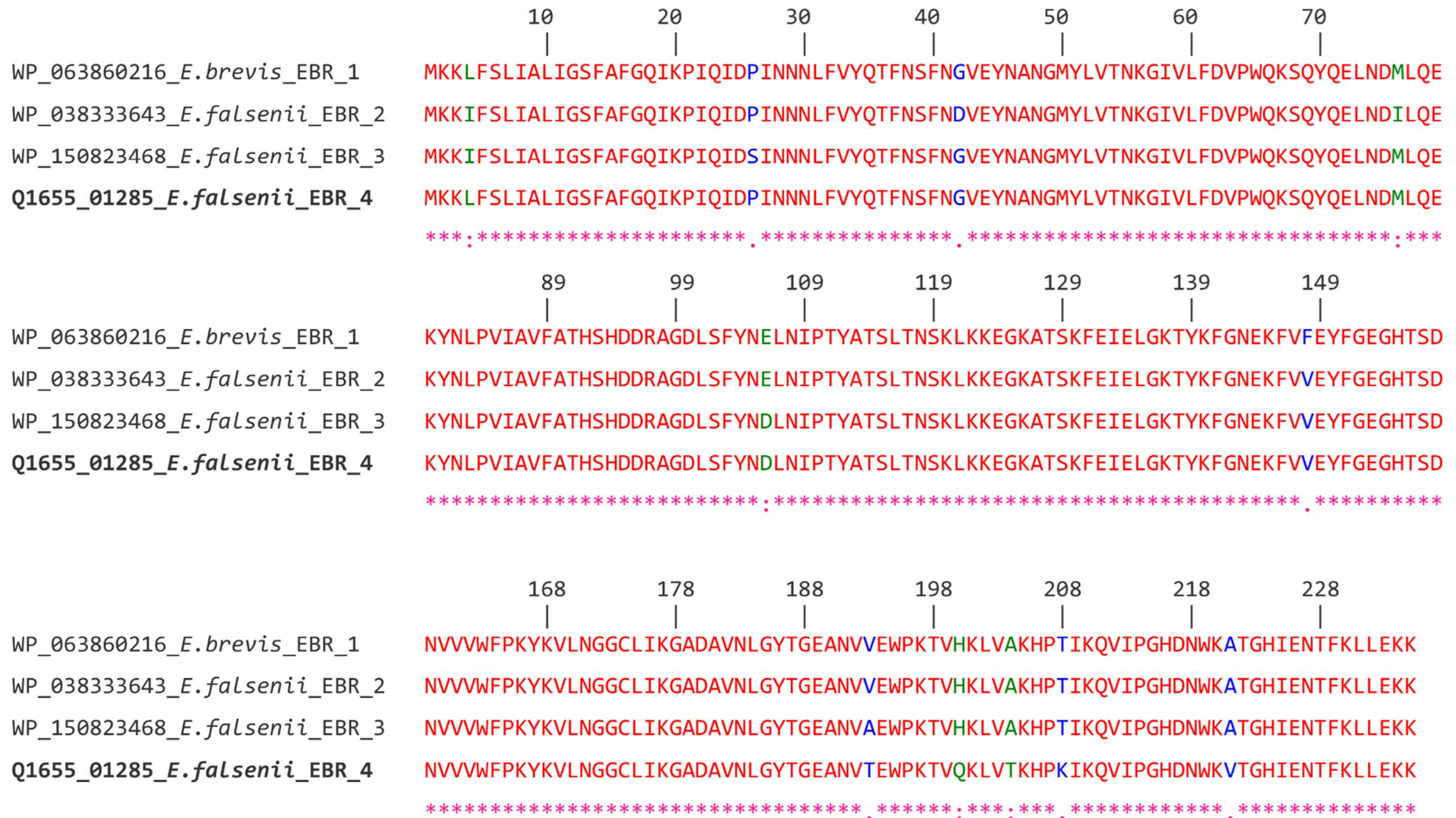
- 209 Antimicrob Agents Chemother 2014;58:3895–903.
- 210 [21] Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-
211 annot, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes.
212 Antimicrob Agents Chemother 2014;58:212–20.
- 213 [22] Combet C, Blanchet C, Geourjon C, Deléage G. NPS@: Network protein sequence analysis.
214 Trends Biochem Sci 2000;25:147–50.
- 215 [23] Bellais S, Girlich D, Karim A, Nordmann P. EBR-1, a novel Ambler subclass B1 Beta-
216 Lactamase from *Empedobacter brevis*. Antimicrob Agents Chemother 2002;46:3223–7.
- 217 [24] Collins C, Almuzara M, Saigo M, Montaña S, Chiem K, Traglia G, et al. Whole-Genome
218 Analysis of an Extensively Drug-Resistance *Empedobacter falsenii* Strain Reveals Distinct
219 Features and the Presence of a Novel Metallo- β -Lactamase (EBR-2). Curr Microbiol
220 2018;75:1084–9.
- 221 [25] Linkevicius M, Sandegren L. Potential of tetracycline resistance proteins to evolve tigecycline
222 resistance. Antimicrob Agents Chemother 2015;10:1–6.
- 223 [26] Vila J, Martinez J. Clinical Impact of the Over-Expression of Efflux Pump in Nonfermentative
224 Gram-Negative Bacilli, Development of Efflux Pump Inhibitors. Curr Drug Targets
225 2008;9:797–807.
- 226 [27] Andersen JL, He GX, Kakarla P, Kc R, Kumar S. Multidrug efflux pumps from
227 *Enterobacteriaceae*, *Vibrio cholerae* and *Staphylococcus aureus* bacterial food Pathogens. Int
228 J Environ Res Public Health 2015;12:1487–547.

229 **Table 1:** Genetic and phenotypic features of *Empedobacter falsenii* Q1655 strain

230

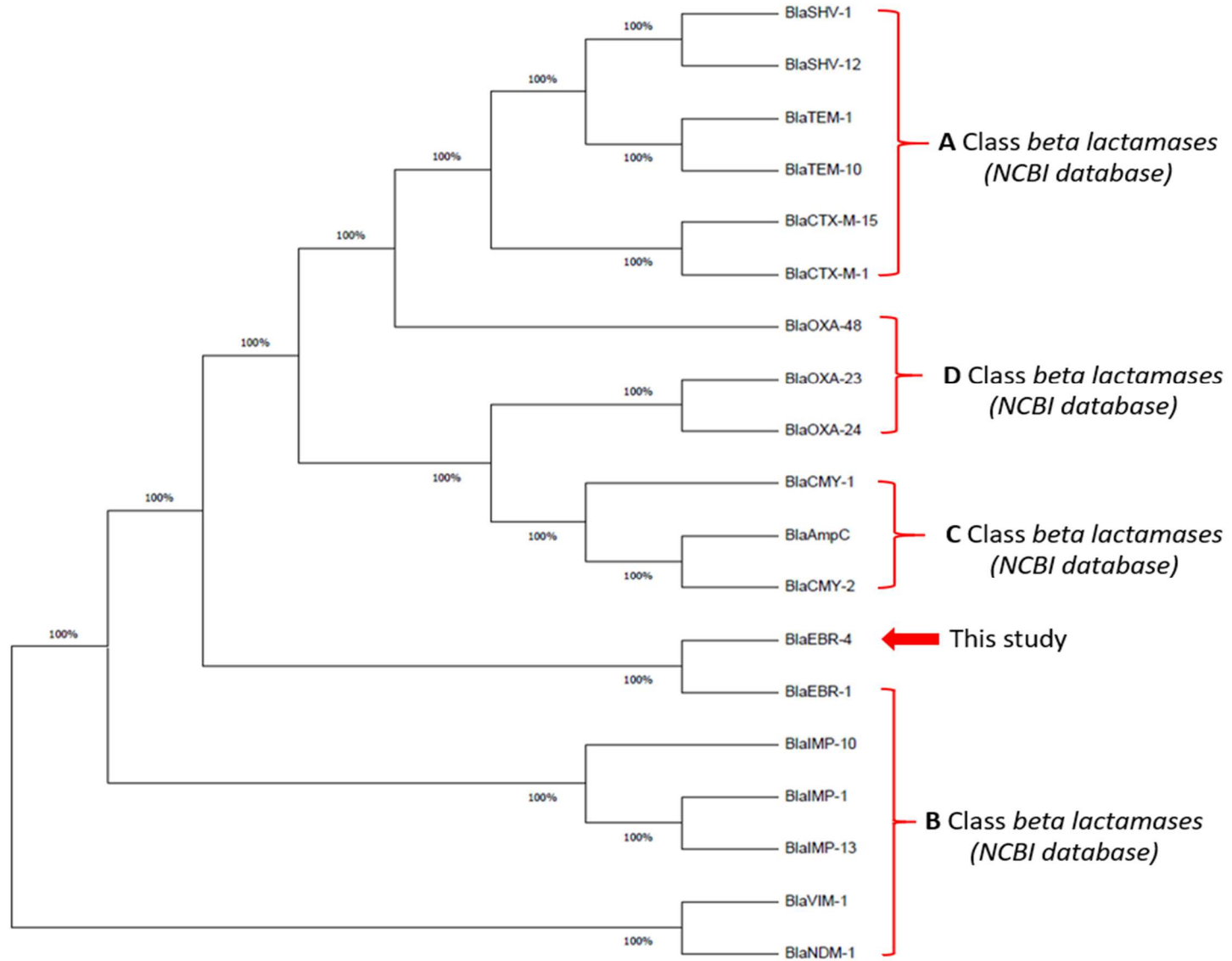
Strain name	Genome size (bp)	% GC content	Antibiotic resistance genes	Resistance phenotype	Susceptible phenotype
<i>E. falsenii</i> Q1655	3,482,659	34.4	<i>bla</i> _{EBR-4} and <i>tet</i> (X)	Amoxicillin, amoxicillin/clavulanate, cefalotin, ceftriaxone, ertapenem, imipenem, fosfomycin, nitrofurantoin, trimethoprim/sulphamethoxazole, colistin	Ciprofloxacin, gentamicin, doxycycline, amikacin and cefepime

231



232

233 **Figure 1:** Protein sequence alignment of the novel metallo-β-lactamase EBR-4 protein and others ERB sequences.



234 **Figure 2:** Phylogenetic tree of the novel *bla*_{EBR-4} gene with other β -lactamase genes downloaded from the NCBI Genbank database.