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1 **Genomic features of an isolate of *Empedobacter falsenii* harbouring a novel variant of**  
2 **metallo- $\beta$ -lactamase, *bla*<sub>EBR-4</sub> gene**

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6

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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  $\beta$ -lactam antibiotics with the exception of cefepime.  
34 The MICs of imipenem and ertapenem as determined by E-test were 12  $\mu$ g/ml and 2  $\mu$ 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- $\beta$ -lactamase, a chromosomal  
37 *bla*<sub>E<sub>BR</sub>-4</sub> (Accession No: MN997121), which exhibited 94.92 % and 97.02 % nucleotide and  
38 protein sequence identities respectively with *bla*<sub>E<sub>BR</sub>-3</sub> gene of *E. falsenii* 174820. Seven and  
39 eight amino-acid substitutions were observed with the *bla*<sub>E<sub>BR</sub>-1</sub> and *bla*<sub>E<sub>BR</sub>-2</sub>, 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- $\beta$ -lactamase, *bla*<sub>E<sub>BR</sub>-4</sub>, *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- $\beta$ -lactamases (MBLs) are bacterial enzymes which catalyse the formation of a  
57 noncovalent reactive complex with  $\beta$ -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  $\beta$ -lactam antibiotics [10]. Several MBLs have been described but the  
62 most commonly encountered are the Verona integron encoded metallo- $\beta$ -lactamase (VIM),  
63 Imipenemase (IMP) and New-Delhi Metallo- $\beta$ -lactamase (NDM) (Bonomo & Szabo, 2006).  
64 Other MBL are confined to a specific bacteria species such as *bla*<sub>EBR</sub> 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*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>) and carbapenemases (*bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>NDM</sub> and *bla*<sub>KPC</sub>)  
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  $\beta$ -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  $\mu$ g/ml and 2  $\mu$ 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  $\beta$ -lactamase genes (i.e. *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA-</sub>  
109 <sub>48</sub>, *bla*<sub>VIM</sub>, *bla*<sub>KPC</sub>, *bla*<sub>IMP</sub>). 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- $\beta$ -lactamase type 2, a  
113 chromosomal *bla*<sub>EBR-4</sub> which exhibited 94.92 % and 97.02 % nucleotide and protein identity  
114 with *bla*<sub>EBR-3</sub> gene of *E. falsenii* 174820 (Accession No: NG\_066507). Pairwise alignment of  
115 the amino acid sequence revealed that the *bla*<sub>EBR-4</sub> differs from *bla*<sub>EBR-3</sub> by only 6 amino-acids  
116 (fig. 1). Seven and eight amino-acid substitutions were observed with the *bla*<sub>EBR-1</sub> of

117 *Empedobacter brevis* (Accession No: WP\_063860216) and *bla*<sub>E<sub>BR</sub>-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<sub>BR</sub>-4 is  
122 chromosomal. This is in line with a previous description of the gene [23]. The *bla*<sub>E<sub>BR</sub></sub> 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*<sub>E<sub>BR</sub></sub> genes have been detected in the genus *Empedobacter*. With the exception of  
126 *bla*<sub>E<sub>BR</sub>-1</sub> 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*<sub>E<sub>BR</sub>-4</sub> 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*<sub>E<sub>BR</sub>-4</sub>. 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.

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150 IAHU-03.

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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.

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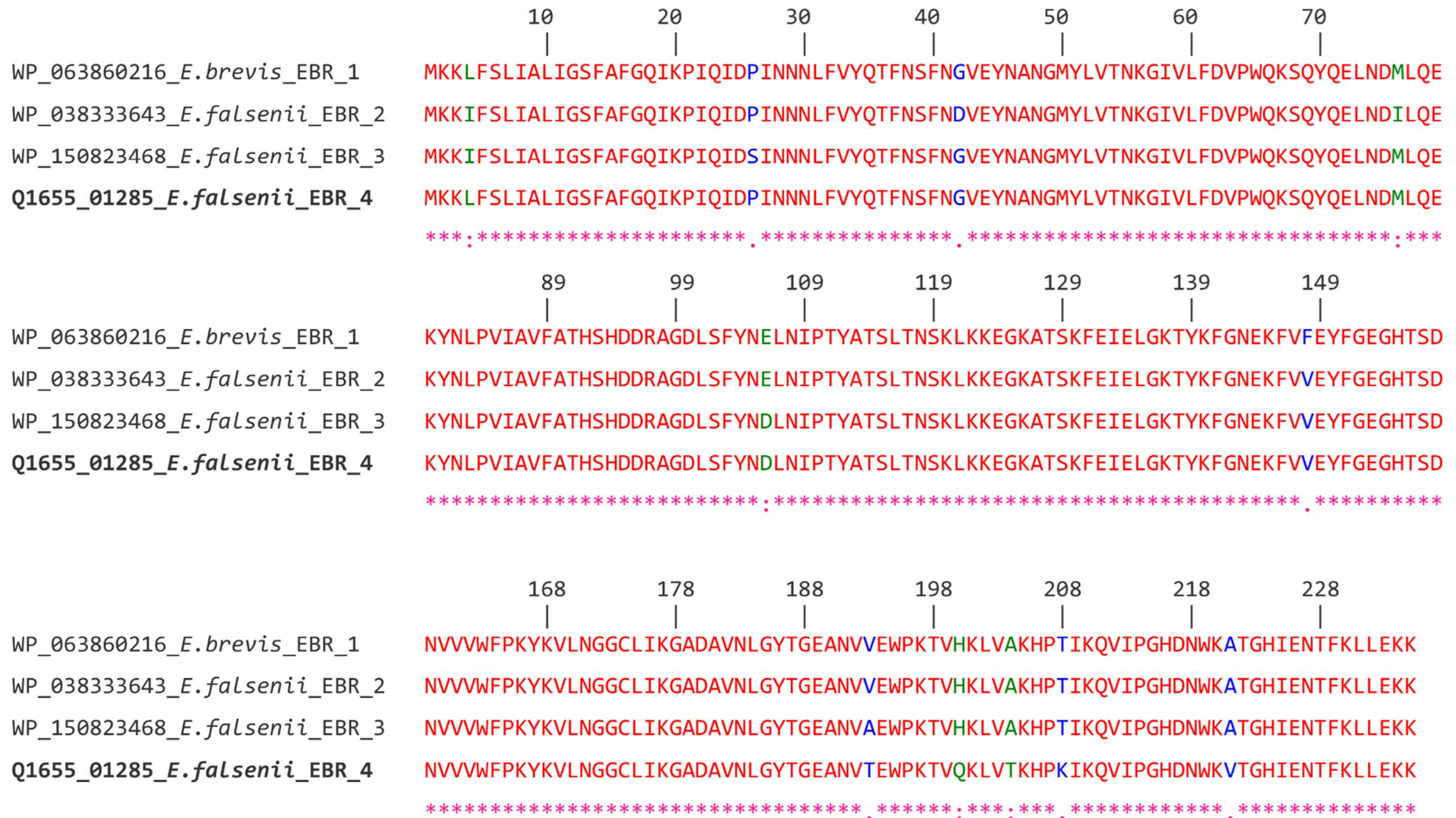
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229 **Table 1:** Genetic and phenotypic features of *Empedobacter falsenii* Q1655 strain

230

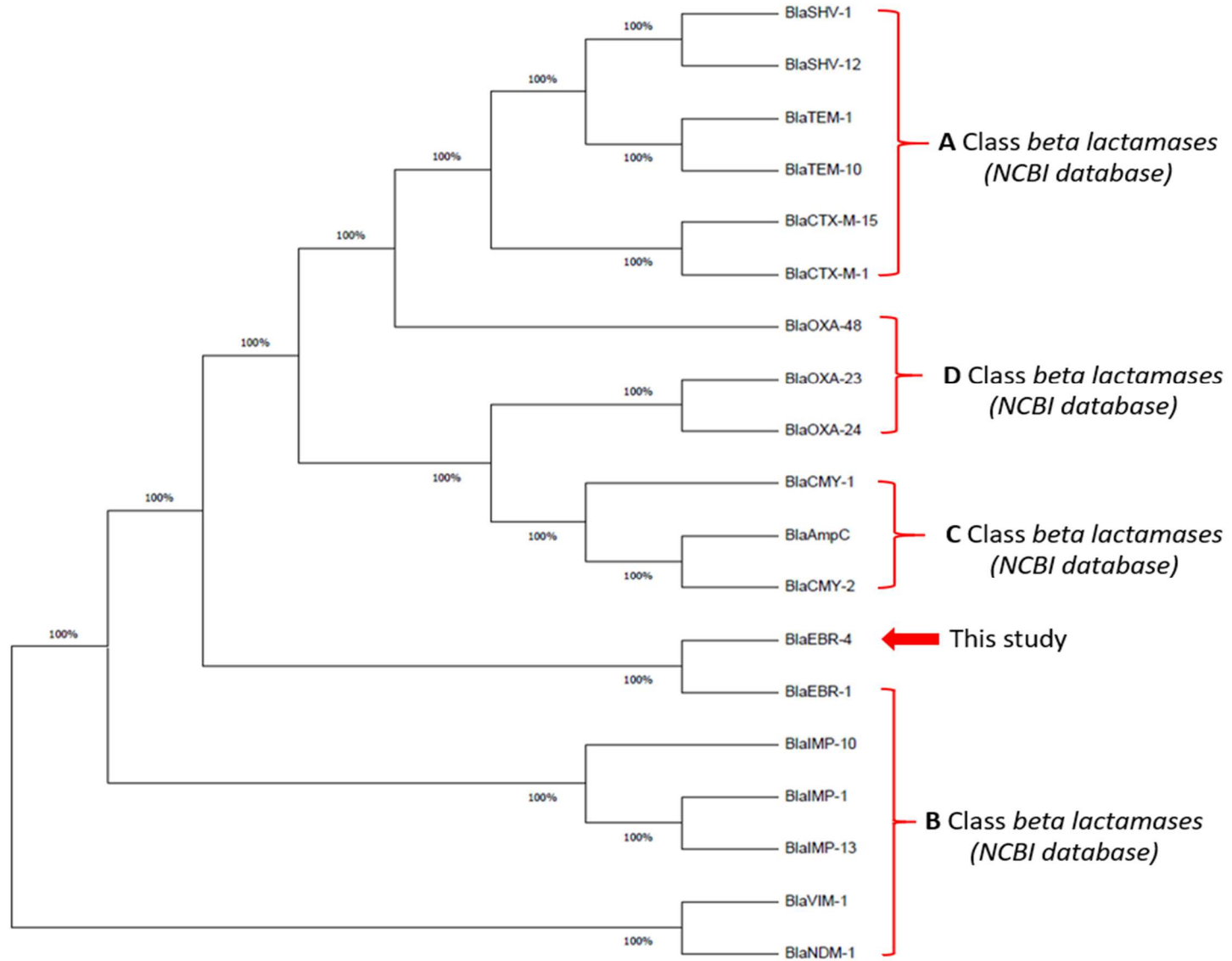
Strain name	Genome size (bp)	% GC content	Antibiotic resistance genes	Resistance phenotype	Susceptible phenotype
<i>E. falsenii</i> Q1655	3,482,659	34.4	<i>bla</i> <sub>EBR-4</sub> and <i>tet</i> (X)	Amoxicillin, amoxicillin/clavulanate, cefalotin, ceftriaxone, ertapenem, imipenem, fosfomycin, nitrofurantoin, 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*<sub>EBR-4</sub> gene with other  $\beta$ -lactamase genes downloaded from the NCBI Genbank database.