



## Genome Note

# Complete genome sequence of an IMP-8, CTX-M-14, CTX-M-3 and QnrS1 co-producing *Enterobacter asburiae* isolate from a patient with wound infection

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## ABSTRACT

**Objectives:** The aim of this study was to investigate the characteristics and complete genome sequence of an IMP-8, CTX-M-14, CTX-M-3 and QnrS1 co-producing multidrug-resistant *Enterobacter asburiae* isolate (EN3600) from a patient with wound infection.

**Methods:** Species identification was confirmed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). Carbapenemase genes were identified by PCR and Sanger sequencing. The complete genome sequence of *E. asburiae* EN3600 was obtained using a PacBio RS II platform. Genome annotation was done by Rapid Annotation using Subsystem Technology (RAST) server. Acquired antimicrobial resistance genes (ARGs) and plasmid replicons were detected using ResFinder 2.1 and PlasmidFinder 1.3, respectively.

**Results:** The genome of *E. asburiae* EN3600 consists of a 4.8-Mbp chromosome and five plasmids. The annotated genome contains various ARGs conferring resistance to aminoglycosides, β-lactams, fluoroquinolones, fosfomycin, macrolides, phenicols, rifampicin and sulfonamides. In addition, plasmids of incompatibility (Inc) groups IncHI2A, IncFIB(pECLA), IncFIB(pQil) and IncP1 were identified. The genes *bla*<sub>IMP-8</sub>, *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-3</sub> were located on different plasmids. The *bla*<sub>IMP-8</sub> gene was carried by an 86-kb IncFIB(pQil) plasmid. The *bla*<sub>CTX-M-3</sub> and *qnrS1* genes were co-harboured by an IncP1 plasmid. In addition, *bla*<sub>CTX-M-14</sub> was associated with *bla*<sub>TEM-1B</sub>, *bla*<sub>OXA-1</sub>, *catB3* and *sul1* genes in a 116-kb non-typeable plasmid.

**Conclusion:** To our knowledge, this is the first complete genome sequence of an *E. asburiae* isolate co-producing IMP-8, CTX-M-14, CTX-M-3 and QnrS1. This genome may facilitate the understanding of the resistome, pathogenesis and genomic features of *Enterobacter cloacae* complex (ECC) and will provide valuable information for accurate identification of ECC.

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Species of the *Enterobacter cloacae* complex (ECC) are widely encountered in nature, however they can also act as pathogens in clinical settings. Biochemical and molecular studies on ECC have shown its genomic heterogeneity, which comprises six species, namely *E. cloacae*, *Enterobacter asburiae*, *Enterobacter hormaechei*, *Enterobacter kobei*, *Enterobacter ludwigii* and *Enterobacter nimipressuralis* [1,2]. *Enterobacter cloacae* and *E. hormaechei* are the most frequently isolated species in human clinical specimens. Of note, *E. cloacae* and *E. asburiae* are difficult to differentiate by

matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS), with a low discrimination result [3]. Girlich et al. suggested that *E. cloacae* of sequence types ST25, ST229, ST249 and ST250 should be identified as *E. asburiae* by sequencing of the chromosomal *ampC* gene [4].

ECC has become the third most important drug-resistant pathogen among the Enterobacteriaceae owing to dissemination of respective antimicrobial resistance genes (ARGs) via horizontal gene transfer. Carbapenemase-producing Enterobacteriaceae are recognised as the most worrying threat to public health owing to the limited availability of drugs to treat such pathogens. Members of the ECC commonly produce IMP carbapenemases [5].

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IMP-type metallo- $\beta$ -lactamases (MBLs) have been reported globally since the first report of IMP-1 from *Pseudomonas aeruginosa* in Japan [6]. IMP-type MBLs are typically carried by class 1 integrons and there are more than 20 variants. IMP-8 was first discovered in a clinical *Klebsiella pneumoniae* isolate in Taiwan harboured by a plasmid [7]. Since then, *bla*<sub>IMP-8</sub> has been successively identified in *E. cloacae*, *Klebsiella oxytoca*, *Escherichia coli* and *Raoultella planticola* isolates. Here we describe the complete sequence and resistome of an IMP-8-producing *E. asburiae* isolate from a wound infection.

In May 2015, a carbapenem-resistant ECC isolate was recovered from a blood sample of a patient with wound infection and was identified as *E. asburiae* EN3600 using MALDI-TOF/MS (Bruker Daltonik, Bremen, Germany). Genomic DNA of *E. asburiae* EN3600 was extracted from 2 mL of pure cell culture using a Genomic DNA Isolation Kit (QIAGEN, Hilden, Germany). Carbapenemase genes was identified by PCR and Sanger sequencing. Whole-genome sequencing was performed at Novogen Tech. Co. Ltd. (Beijing, China) using two single-molecule real-time (SMRT) cells on a PacBio RS II platform (Pacific Biosciences, Menlo Park, CA). Raw reads were filtered using the RS Filter Only protocol in the SMRT portal (Pacific Biosciences) with default settings. Short contigs of <500 nucleotides in length were discarded. Genome annotation was done by Rapid Annotation using Subsystem Technology (RAST) server (<http://rast.nmpdr.org/>), and specific genes were manually curated. Plasmid sequences and acquired ARGs were identified using PlasmidFinder 1.3 (<https://cge.cbs.dtu.dk/services/Plasmid-Finder/>) and ResFinder 2.1 (<https://cge.cbs.dtu.dk/services/Res-Finder/>), respectively.

The complete genome of *E. asburiae* EN3600 has a G + C content of 55% and consists of one circular DNA chromosome of 4 824 514 bp. Five extrachromosomal elements of 289 962, 116 221, 103 522, 86 605 and 63 601 bp, respectively, were identified (Supplementary Table S1). The RAST server predicted a total of 5288 protein-coding sequences, 204 pseudogenes and 85 tRNAs in the whole genome.

The acquired ARGs detected in the genome of *E. asburiae* EN3600 are summarised in Table 1. The following genes were identified: aminoglycoside resistance genes *aac*(6′)-*Ib*-cr, *aac*(6′)-*Ib*3 and *aadA1*;  $\beta$ -lactam resistance genes *bla*<sub>TEM-1B</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>IMP-8</sub>, *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-3</sub>; fluoroquinolone resistance genes *aac*(6′)-*Ib*-cr and *qnrS1*; fosfomycin resistance gene *fosA*; macrolide

resistance gene *mph*(A); phenicol resistance gene *catB3*; rifampicin resistance gene *arr-3*; and sulfonamide resistance genes *sul1* and *sul2*.

Plasmids of incompatibility (Inc) groups HI2A, FIB(pECLA), FIB(pQil) and P1 were identified (Supplementary Table S1). The genes *bla*<sub>IMP-8</sub>, *bla*<sub>CTX-M-14</sub> and *bla*<sub>CTX-M-3</sub> were identified on different plasmids. The plasmid harbouring *bla*<sub>IMP-8</sub> (pIMP-8-EN3600) was identified as an IncFIB(pQil) plasmid with the length of 86 605 bp. IncFIB(pQil) variants are one of the plasmid types most represented in clinical samples [7]. The IncFIB plasmids in Enterobacteriaceae are of particular interest since they contribute to the carriage and spread of ARGs [8]. Plasmid pIMP-8-EN3600 was further compared with the *bla*<sub>IMP-8</sub>-encoding plasmid pEKP0787-1 (*K. pneumoniae*, China; GenBank accession no. **AF322577**) and pFP10-2 (*K. oxytoca*, China; GenBank accession no. **HQ651093**). As shown in Supplementary Fig. S1, the backbone sequences of the three plasmids are almost identical. The *bla*<sub>IMP-8</sub> gene was situated within a class 1 integron with the following cassette combination: *intI1*-*bla*<sub>IMP-8</sub>-*aac*(6′)-*Ib*-*tinR*-*hp*- $\Delta$ ISArsp14\_aa3- $\Delta$ ISArsp14\_aa2- $\Delta$ ISArsp14\_aa14, which was inserted into an IS26 insertion sequence backbone. The *bla*<sub>CTX-M-3</sub> and *qnrS1* genes were co-harboured by an IncP1 plasmid. In addition, *bla*<sub>CTX-M-14</sub> was associated with *bla*<sub>TEM-1B</sub>, *bla*<sub>OXA-1</sub>, *catB3* and *sul1* genes in a non-typeable plasmid with a size of 116 221 bp.

In the past decade, the co-occurrence of *bla*<sub>IMP-8</sub> and *qnrS1* in *E. asburiae* was rarely detected in the world, except occasionally from Taiwan and Mainland China [9]. However, the co-existence of *bla*<sub>CTX-M-3</sub> and *qnrS1* in *E. coli* ST131 isolates has been described [10]. Co-occurrence of *bla*<sub>CTX-M-3</sub> and *qnrS1* genes in an IncP1 plasmid from an *Enterobacter* strain has not been reported. In this study, plasmid-mediated ARGs conferring resistance to aminoglycosides,  $\beta$ -lactams and fluoroquinolones were observed in the same clinical isolate, highlighting the horizontal mobility of these resistance genes. The spread of such plasmid-encoded ARGs in clinical ECC isolates is of serious concern.

To our knowledge, this is the first complete genome sequence of an *E. asburiae* isolate co-producing IMP-8, CTX-M-14, CTX-M-3 and QnrS1. This genome may facilitate the understanding of the resistome, pathogenesis and genomic features of ECC.

Sequence and annotation data of *E. asburiae* strain EN3600 have been deposited in GenBank under accession nos. **CP035633–CP035638**. The version described here is the first version.

**Table 1**

Acquired antimicrobial resistance genes (ARGs) identified in IMP-8-producing *Enterobacter cloacae* EN3600.

Antimicrobial class	ARG	Identity	Query/HSP	Contig	Position in contig	Accession no.
Aminoglycosides	<i>aac</i> (6′)- <i>Ib</i> -cr	100.00	600/600	3	11593–12192	<b>DQ303918</b>
	<i>aac</i> (6′)- <i>Ib</i> 3	99.64	555/555	5	23426–23980	<b>X60321</b>
	<i>aadA1</i>	100.00	792/792	1	3258–4049	<b>JQ414041</b>
$\beta$ -Lactams	<i>bla</i> <sub>TEM-1B</sub>	100.00	861/861	1	10373–11233	<b>AY458016</b>
	<i>bla</i> <sub>OXA-1</sub>	100.00	831/831	3	12323–13153	<b>HQ170510</b>
	<i>bla</i> <sub>IMP-8</sub>	100.00	741/741	5	22587–23327	<b>DQ845788</b>
	<i>bla</i> <sub>CTX-M-14</sub>	100.00	876/876	3	23171–24046	<b>AF252622</b>
	<i>bla</i> <sub>CTX-M-3</sub>	100.00	876/876	6	41714–42589	<b>Y10278</b>
	<i>bla</i> <sub>TEM-1B</sub>	100.00	861/861	6	43371–44231	<b>AY458016</b>
	<i>bla</i> <sub>TEM-1B</sub>	100.00	861/861	3	98945–99805	<b>AY458016</b>
Fluoroquinolones	<i>aac</i> (6′)- <i>Ib</i> -cr	100.00	600/600	3	11593–12192	<b>DQ303918</b>
	<i>aac</i> (6′)- <i>Ib</i> -cr	99.42	519/519	5	23462–23980	<b>EF636461</b>
	<i>qnrS1</i>	100.00	657/657	6	38655–39311	<b>AB187515</b>
Fosfomycin	<i>fosA</i>	97.65	426/426	1	38839–39264	<b>AEXB01000013</b>
Macrolides	<i>mph</i> (A)	100.00	906/906	3	4263–5168	<b>D16251</b>
Phenicol	<i>catB3</i>	100.00	633/633	3	13291–13923	<b>U13880</b>
Rifampicin	<i>arr-3</i>	100.00	453/453	3	14008–14460	<b>JF806499</b>
Sulfonamides	<i>sul1</i>	100.00	840/840	3	15024–15863	<b>U12338</b>
	<i>sul2</i>	99.88	816/816	5	17098–17913	<b>AY034138</b>
	<i>sul1</i>	100.00	840/840	1	1914–2753	<b>U12338</b>

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## Competing interests

None declared.

## Ethical approval

Not required.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jgar.2019.05.029>.

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