



Whole-Genome Characterization of a *Shewanella algae* Strain Coharboring *bla*_{CTX-M-15} and *armA* Genes on a Novel IncC Plasmid

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Multidrug-resistant *Shewanella* species strains are emerging worldwide (1, 2). Here, we describe an unusual *Shewanella algae* (18064-CSB-B-B) isolated from chicken stool collected in July 2018 on the island of Zanzibar (Tanzania) during an ongoing study (<http://p3.snf.ch/Project-170063>).

The sample was screened by implementing selective enrichments (3). Species identification was confirmed by analyzing the whole-genome sequence (WGS) with the tools of the Type (Strain) Genome Server (<https://tygs.dsmz.de/>) (4, 5). The following MICs were obtained by implementing the Sensititre GNX2F/ESB1F panels (Thermo Fisher): ceftaxime, ≤ 4 $\mu\text{g/ml}$; ceftazidime, 4 $\mu\text{g/ml}$; cefotaxime, 64 $\mu\text{g/ml}$; ceftazidime-clavulanate and cefotaxime-clavulanate, ≤ 0.12 $\mu\text{g/ml}$; cefepime, ≤ 2 $\mu\text{g/ml}$; meropenem, ≤ 0.5 $\mu\text{g/ml}$; ciprofloxacin, ≤ 0.25 $\mu\text{g/ml}$; gentamicin, > 16 $\mu\text{g/ml}$; amikacin, > 32 $\mu\text{g/ml}$; trimethoprim-sulfamethoxazole, $> 4/76$ $\mu\text{g/ml}$; doxycycline, ≤ 2 $\mu\text{g/ml}$; and colistin, ≤ 0.25 $\mu\text{g/ml}$.

WGS was achieved by combining MinION (Oxford Nanopore) and NovaSeq-6000 (Illumina) as previously described (6). The output was interpreted by implementing the tools of the Center for Genomic Epidemiology (www.genomic epidemiology.org/) and PubMLST (www.pubmlst.org/plasmid/). A 149,553-bp IncC (formerly A/C₂) type 2 ST1 plasmid (p18064-CSB-B-B) coharboring *bla*_{CTX-M-15}, *armA*, *sul1*, and *dfrA12* antibiotic resistance genes (ARGs), was identified (7). The *bla*_{CTX-M-15} was flanked upstream by insertion sequence *ISEcp1*, whereas *armA* was 56,750 bp apart from *bla*_{CTX-M-15} and within the ARI-A antibiotic resistance island (see Fig. S1 and S2 in the supplemental material) (8). In particular, *armA* was flanked upstream by *ISCR1* followed by a partial *ISEc28* and downstream by two hypothetical proteins trailed by *ISEc29* and *IS26*. Moreover, a class I integron carrying *dfrA12* was located downstream of *ISCR1* and separated by *sul1*. Overall, the integron and *armA* regions were flanked upstream by *IS5075* and *Tn1696* and downstream by *Tn2-IS4321* elements. The transposon appeared nonfunctional, because no target site duplications were identified (Fig. 1).

BLASTn analysis showed that the *ISCR1-ISEc28-armA-ISEc29* element was previously reported in 19 IncC plasmids from *Enterobacteriales* and in 107 *Acinetobacter baumannii* isolates, of which 8 were on plasmids negative for *Enterobacteriales* replicons (data not shown).

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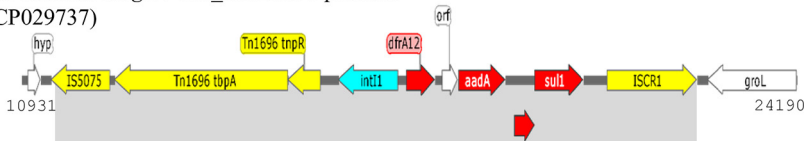
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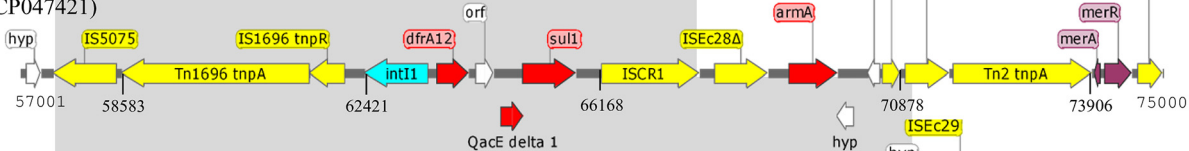
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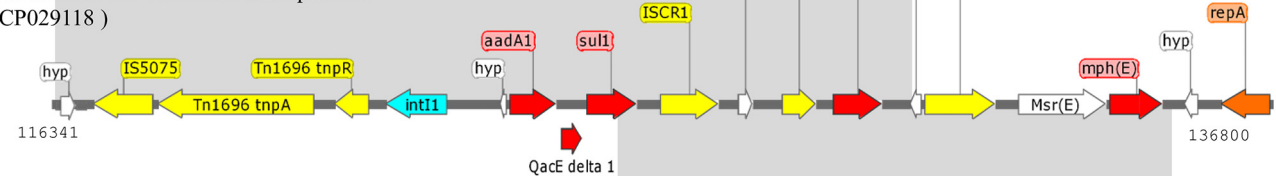
Providencia rettgeri AR_0082 IncC plasmid (CP029737)



Shewanella algae p18064-CSB-B-B IncC plasmid (CP047421)



Escherichia coli unnamed5 IncC plasmid (CP029118)



Acinetobacter baumannii chromosome (CP045528)

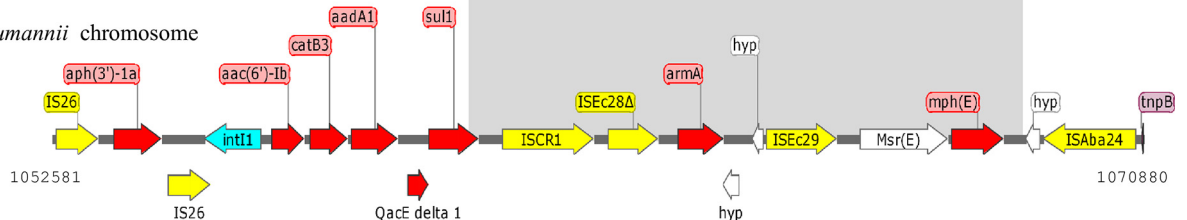


FIG 1 Linear map of the *armA* resistance region of p18064-CSB-B-B found in *S. algae* (image generated with SnapGene Viewer 5.0.4). Comparison with similar IncC plasmids identified by BLASTn analysis in *Providencia rettgeri* and *E. coli* and in the chromosome of several *A. baumannii* strains (strain 6507, isolated in India and sequenced in 2018, was selected as the prototypic genome among others found with BLASTn analysis). Arrows, open reading frames: red, resistance genes; yellow, transposase genes; turquoise, class 1 integrase; white, other genes.

Several IncC plasmids showed a high level of identity with p18064-CSB-B-B, although none of them possessed both the *bla*_{CTX-M-15} and *armA* regions (see Fig. S1). p18064-CSB-B-B was compared with 263 deposited IncC plasmids with 100% nucleotide identity to the replicase gene of reference plasmids pR148 and pR55 (8). The phylogeny tree indicated that p18064-CSB-B-B belonged to a node containing 4 plasmids (see Fig. S3 in the supplemental material). The p18064-CSB-B-B backbone was related to that of the NDM-1 plasmid (GenBank accession no. LN831185) identified in *Vibrio cholerae* in New Delhi (9). The other 2 IncC plasmids were from *Escherichia coli* strains isolated in the United States (GenBank accession no. CP029118) and Canada (GenBank accession no. CP012902) (10).

The circular 4,744,471-bp chromosome of 18064-CSB-B-B contained the carbapenemase *bla*_{OXA-55} in a genetic environment already reported in two *S. algae* isolates (see Fig. S4 in the supplemental material). In this context, we note that *Shewanella* species isolates are suggested to be the natural progenitors of several *bla*_{OXA} genes (2, 11–13). The same accounts for *qnrA3*, which was the only additional ARG present on the chromosome of 18064-CSB-B-B (14).

We described the first IncC plasmid cohabiting *bla*_{CTX-M-15} extended-spectrum β -lactamase and *armA* 16S rRNA methylase ARGs. Thanks to its great ability to be mobilized, the *bla*_{CTX-M-15} *ISEc1* was already observed in several IncC plasmids (7, 15). On the other hand, the aminoglycoside resistance gene *armA* was located in a genetic context that is rare on IncC plasmids but frequent in *A. baumannii* isolates.

IncC plasmids have not been described in *Acinetobacter* species isolates, and we did not identify complete IncC plasmids in GenBank. However, the region comprised

between 1 to 24,748 and 135,839 to 149,553 nucleotide positions of p18064-CSB-B-B, encoding the transfer locus, hypothetical proteins, restriction methylase, and partitioning protein ParB but not the replicase gene, is present in the chromosome of *A. baumannii* PB364 (GenBank accession no. [CP040425](https://doi.org/10.1007/s10096-017-2962-3)). This is important and novel information suggesting that IncC plasmids can enter *Acinetobacter* spp. but are probably not stable.

Accession number(s). Plasmid p18064-CSB-B-B and the chromosome of *S. algae* strain 18064-CSB-B-B were deposited under GenBank accession no. [CP047421](https://doi.org/10.1007/s10096-017-2962-3) and [CP047422](https://doi.org/10.1007/s10096-017-2962-3), respectively.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

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