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# Genetic and Phenotypic Characterization of the First Canadian Case of Ambler Class A Carbapenemase FRI-8

Laura F. Mataseje,[1] Florence Doualla-Bell,[2] David A. Boyd,[1] Ken Fakharuddin,[1] Hector Felipe Garcia Jeldes,[3] Valerie Plante,[3] Marie-Claude Beaudoin,[3] Alexandre Godbout,[3] Simon Wong,[2] Judith Fafard,[2] and Michael R. Mulvey[1]

This study investigated the mechanism of carbapenem resistance in an *Enterobacter cloacae* complex positive by the modified carbapenem inactivation method (mCIM) but negative by the Rosco Neo-Rapid Carb Kit, β CARBA, and conventional PCR for common carbapenemase genes (KPC, NDM, OXA-48, IMP, VIM, GES, and IMI/NMC). Using whole genome sequencing (WGS) data we confirmed the identification of *Enterobacter asburiae* (ST1639) and the presence of $bla_{\text{FRI-8}}$ located on a 148kb IncFII(Yp) plasmid. This is the first occurrence of a clinical isolate harboring the FRI-8 carbapenemase and the second occurrence of FRI in Canada. This study highlights the need to use both WGS and phenotypic screening methods for detection of carbapenemase-producing strains if we consider the growing diversity of carbapenemases.

**Keywords:** carbapenemase, Canada, FRI, *Enterobacter* sp

**Brief Report**

*Enterobacter cloacae* complex (ECC) are common nosocomial pathogens and often associated with multidrug resistance including the carbapenems. Carbapenem resistance in ECC may be related to presence of or overexpression of a β-lactamase combined with decreased outer membrane permeability.[1] Alternatively, resistance to carbapenems in ECC can be due to the acquisition of genes expressing carbapenemases.[1] According to a recent report from the Canadian Nosocomial Surveillance Program (CNISP), ECC is among the top three most prevalent organisms harboring carbapenemase genes in Canada.[2]

The first description of the novel class A carbapenemase FRI-1 (French imipenemase) isolated from *E. cloacae* was reported in 2015. It shared the closest amino acid identity with the chromosome-encoded Ambler class A carbapenemases NMC-A and IMI-1.[3] FRI-1 showed significant hydrolysis of carbapenems, confers resistance to aztreonam, but does not confer significant resistance to broad-spectrum cephalosporins.[3]

Limited reports on FRI-type carbapenemases have been published since its description in 2015 and include those from the United Kingdom (FRI-2),[4] Germany (FRI-3),[5] Japan (FRI-4, FRI-5, FRI-8),[6–8] and Canada (FRI-6).[9] In addition, a report from 2019 described a novel carbapenemase FLC-1 (FRI-like carbapenemase) that shared 87% amino acid similarity to FRI-5[10] and 99% similarity to FRI-8.[8]

In this report, an 87-year-old patient with no known travel history was hospitalized in medicine/palliative care with complaints of increasing low-back pain. In the context of an ongoing KPC outbreak, a rectal swab was taken and an *E. cloacae*, later identified by whole genome sequencing (WGS) using FastANI as *Enterobacter asburiae*, was isolated on ChromID CARBA medium (BioMérieux) and designated N21-01785. The submitting hospital

[1]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada.
[2]Laboratoire de santé Publique du Québec, Ste-Anne-de-Bellevue, Canada.
[3]Centre Hospitalier de l'Université Laval, Québec City, Canada.

provided initial susceptibilities by standard broth microdilution which indicated this isolate was resistant to imipenem (8 mg/L), ertapenem (8 mg/L), and meropenem (8 mg/L).

Additional routine testing at the National Microbiology Laboratory showed variable carbapenem-resistant profiles according to CLSI M100 ED31 breakpoints (Table 1). Initially, PCR for carbapenemases KPC, NDM, OXA-48-type, GES, IMP, VIM, and NMC-1/IMI-type was conducted, and all targets were negative. Phenotypic detection of carbapenemase production was conducted using Rosco Neo-Rapid Carb Kit, β CARBA (Bio-Rad), and modified carbapenem inactivation method (mCIM). Only mCIM was positive where no inhibition zone was observed. The isolate was sent for WGS using a hybrid of NextSeq Illumina and Oxford Nanopore MinION platforms.

Sequence read data were deposited at DDBJ/ENA/GenBank under BioProject PRJNA804546 and *de novo* assemblies under accession JAKRWQ000000000. Resistance genes, plasmid typing, and multilocus sequence typing were extracted from WGS data using StarAMR (github.com/phac-nml/staramr). Analysis indicated N21-01785 was a novel sequence type ST1639 and harbored a novel intrinsic $bla_{\text{ACT-105}}$ class C beta-lactamase, in addition to *fosA*, *oqxA/B*, and *qnrE1* on the chromosome. The Ambler class A carbapenemase $bla_{\text{FRI-8}}$ and a partial *mcr-10* were found on a 148 kb plasmid (pN21-01785).

Activity of *mcr-10* is unlikely as 350 base pairs of the gene was deleted likely by insertion of an IS*Ec36*-like element. There are currently no published clinical reports of FRI-8, however, several environmental $bla_{\text{FRI-8}}$ harboring plasmids have been deposited in GenBank, all of which

Table 1. Antimicrobial Susceptibilities (mg/L) for the Clinical and Transformant Isolates Studied in This Report

| | Enterobacter asburiae *N21-01785* | Escherichia coli *TOP10 (pFRI-8TF)*[a] | E. coli *TOP10 (pblaFRI-8)*[b] | E. coli *TOP10* |
|---|---|---|---|---|
| Sensititre CAN1MSF | | | | |
| Amikacin | ≤8 | ≤8 | ≤8 | ≤8 |
| Aztreonam | 2 | **>16** | **16** | ≤1 |
| Cefepime | ≤1 | ≤1 | ≤1 | ≤1 |
| Ceftazidime | ≤4 | 8 | ≤4 | ≤4 |
| Ceftazidime/avibactam | ≤4/4 | ≤4/4 | ≤4/4 | ≤4/4 |
| Ceftolozone/tazobactam | ≤1/4 | **8/4** | ≤1/4 | ≤1/4 |
| Ceftriaxone | ≤1 | **4** | ≤1 | ≤1 |
| Ciprofloxacin | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| Colistin | **>8** | ≤1 | ≤1 | ≤1 |
| Doxycycline | ≤4 | ≤4 | ≤4 | ≤4 |
| Ertapenem | **>2** | **>2** | ≤0.25 | ≤0.25 |
| Gentamicin | ≤2 | ≤2 | ≤2 | ≤2 |
| Imipenem/relebactam | **4/4** | ≤1/4 | ≤1/4 | ≤1/4 |
| Levofloxacin | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 |
| Meropenem | 2 | **4** | ≤0.06 | ≤0.06 |
| Meropenem/vaborbactam | ≤1/8 | ≤1/8 | ≤1/8 | ≤1/8 |
| Minocycline | ≤4 | ≤4 | ≤4 | ≤4 |
| Piperacillin/tazobactam | **>64/4** | **>64/4** | **>64/4** | **>64/4** |
| Plazomicin | ≤1 | ≤1 | ≤1 | ≤1 |
| Tigecycline | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 |
| Tobramycin | ≤2 | ≤2 | ≤2 | ≤2 |
| Trimethoprim/sulfamethoxazole | ≤2/38 | ≤2/38 | ≤2/38 | ≤2/38 |
| Broth microdilution[c] | | | | |
| Meropenem | **8** | | | |
| Imipenem | **8** | | | |
| Ertapenem | **8** | | | |
| Etest | | | | |
| Cefoxitin | **>256** | 16 | 8 | 8 |
| Ertapenem | 0.25 | 0.25 | 0.016 | 0.003 |
| Meropenem | 0.25 | 0.25 | 0.064 | 0.016 |
| Imipenem | **16** | 1 | 0.25 | 0.125 |
| Doripenem | 0.25 | 0.25 | 0.064 | 0.032 |
| Agar dilution | | | | |
| Fosfomycin | 64 | | | |

Bold formatting indicates resistant phenotype according to CLSI (M100 ED31:2021).

[a]*E. coli* TOP10 electrotransformed with pFRI-8

[b]$bla_{\text{FRI-8}}$ and upstream region amplified from N21-01785 with primers FRI8 (FW-ATTGTTAGCTGAAAGGTAATC) and FRI8 (RV-GCTTACACAGGTAATACCTC), cloned into pCR21.TOPO (ThermoFisher Scientific). pCR2.TOPO contains an ampicillin resistance gene ($bla_{\text{TEM-1}}$) and kanamycin resistance gene (*aphII*).

[c]Standard broth microdilution was conducted at the submitting laboratory in Quebec

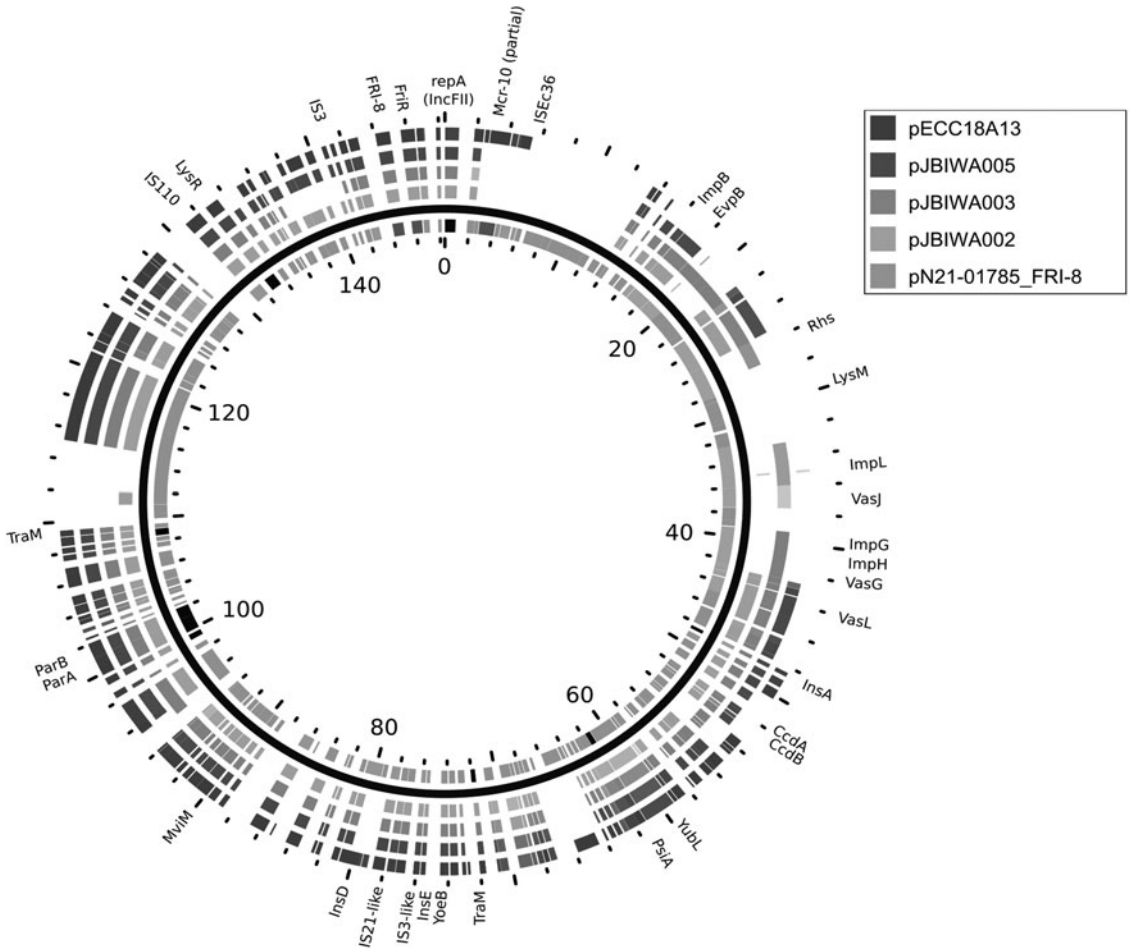


**FIG. 1.** Blast atlas generated by the Gview Server using pN21-01785 as the reference and comparing with pECC18A13 (AP019635.1), pJBIWA002 (CP074179.1), pJBIWA003 (CP074171.1), and pJBIWA005 (CP074160.1). *Inner circle* represents open reading frames of pN21-01785 where proteins are marked by color (*red*—resistance and associated resistance, *black*—replicative functions, *orange*—secretion system associated, *gray*—transposon/insertion sequence associated, *green*—all other). Plasmid was annotated using the genome annotation pipeline DFast (dfast.ddbj.nig.ac.jp).

have been isolated from *Enterobacter* spp. from water sources.[8] Previously reported FRI-type plasmids have been shown to be associated with replicon types IncFII(pECLA)/IncR or IncFII(Yp)-type.[4–7,9] Consistent with reports from FRI-5, FRI-8 and FLC-1 pN21-01785 harbored the IncFII(Yp)-type *repA* gene at 91% identity.

When examining known $bla_{\text{FRI-8}}$ containing plasmids available on GenBank [pECC18A13 (AP019635.1), pJBIWA002 (CP074179.1), pJBIWA003 (CPO74171.1), pJBIWA005 (CPO074160.1)], these plasmids all share numerous regions of homology with each other and pN21-01785 (Fig. 1). Of note was the occurrence of numerous proteins associated with the type IV secretion system (EvpB, ImpB/L/G/H, VasJ/G/L) that may aid in this plasmid's ability to acquire and transfer resistance and virulence genes. In addition, we observed open reading frames showing >97% amino acid identity using BLASTp on NCBI (blast.ncbi.nlm.nih.gov) to several previously deposited virulence factors (*vagC*, *mviM*) and toxin–antitoxin systems (*ccdA/B*, *hok/gef*, *yoeB*) on pN21-01785.

Further investigation of these factors is needed to determine whether they contribute to enhanced virulence of the host and plasmid stability. Similar to our previous report,[9] we were able to successfully transform pN21-01785 into *Escherichia coli* TOP10 (labeled pFRI-8TF), however, conjugation experiments with *Escherichia coli* J53AzR were not successful. We cloned $bla_{\text{FRI-8}}$ into pCR2.1TOPO and subsequently transformed this plasmid into *E. coli* TOP10 (p*bla*FRI-8). Antimicrobial susceptibilities of the transformant are given in Table 1.

Inconsistency in carbapenem MIC between Etest and broth microdilution platforms for this carbapenemase type has been previously observed,[9,11] the reason is unknown. There was no mutation in *mgrB* known to be associated with colistin resistance, however, a recent study showed colistin heteroresistance due to the presence of the *ecr* gene,[12] indeed we found this gene in our isolate that could explain its phenotype.

To our knowledge, this is the first published report of $bla_{\text{FRI-8}}$ identified in a clinical isolate and only the second report of the FRI carbapenemase outside of Europe or Japan.

This study highlights the need for more accurate biochemical methods for the screening of isolates exhibiting elevated MICs to carbapenems due to the diversity of carbapenemases.

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### Authors' Contributions

L.F.M. was involved in investigation, writing—original draft, visualization, supervision, and project administration. F.D.-B. was involved in conceptualization, resources, writing—review and editing, and supervision. D.A.B. carried out investigation, writing—review and editing. K.F. was involved in investigation. H.F.G.J., V.P., M.-C.B., A.G., S.W., and J.F carried out writing—review and editing. M.R.M. was involved in resources, writing—review and editing, and funding acquisition.

### Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
*Laura F. Mataseje, MSc*
*National Microbiology Laboratory*
*Public Health Agency of Canada*
*1015 Arlington Street*
*Winnipeg R3E 3R2*
*Canada*

*E-mail:* laura.mataseje@phac-aspc.gc.ca