Emergence of *Morganellaceae* Harboring *bla*\textsubscript{IMP-27} Metalloenzyme in Canada

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ABSTRACT In 2018 to 2019, PCR for carbapenemases in routine Gram-negative isolates submitted to the National Microbiology Laboratory revealed an increase in IMP-type metalloenzyme-positive isolates, mostly among *Morganellaceae*. Whole-genome sequencing revealed that 23 *Morganellaceae* harbored *bla*\textsubscript{IMP-27} within a chromosomal Tn\textsubscript{7} element. Phylogenomics indicated diversity of isolates but also the presence of a few clonal isolates dispersed geographically. These isolates may be difficult to detect due to carbapenem susceptibility and false-negative results in phenotypic testing.

IMPORTANCE Over the last decade or so, the frequency of isolation of clinical carbapenemase-producing organisms (CPOs) has increased among health care-associated infections. This may seriously compromise antimicrobial therapy, as carbapenemases are considered the last line of defense against these organisms. The ability of carbapenemases to hydrolyze most \(\beta\)-lactams in addition to the co-occurrence of mechanisms of resistance to other classes of antimicrobials in CPOs can leave few options for treating infections. The class B metalloenzymes are globally distributed carbapenemases, and the most commonly found include the NDM, VIM, and IMP types. Our study describes a sudden emergence of IMP-27-harboring *Morganellaceae* during 2018 to 2019 in Canada. There is a paucity of literature on IMP-27 isolates, and our data bolster the information on the genetic context, antimicrobial profiles, and phylogenomics of this group of CPOs.

KEYWORDS *Morganellaceae*, antimicrobial resistance, metallo-\(\beta\)-lactamase

Carbapenemases, \(\beta\)-lactamases that hydrolyze carbapenem \(\beta\)-lactams, have been found globally among clinically significant members of the *Enterobacteriales* (e.g., *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp.) and *Pseudomonadales* (e.g., *Pseudomonas aeruginosa* and *Acinetobacter* spp.) (1). The most prevalent carbapenemases are the so-called “big 5,” namely, KPC (class A), NDM, VIM, IMP (class B metallo-\(\beta\)-lactamases), and OXA-48 (class D). Though they are internationally distributed, some enzyme groups tend to be more prevalent in specific countries or areas (1, 2). The class B IMP enzymes, though found worldwide, tend to be more successfully established in Southeast Asia and the South Pacific regions and have occurred only sporadically in North America (2). Currently, 73
variants of IMP have been assigned (https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources/). In Canada, the first IMP carbapenemase identified was blaIMP-7 from an outbreak of nosocomial *P. aeruginosa* isolated from 1995 to 1997 in a single region (3). The Canadian Nosocomial Infections Surveillance Program identified only two IMP producers among 615 carbapenemase-producing *Enterobacterales* collected from 2010 to 2016, an *Enterobacter cloaceae* isolate harboring blaIMP-13 and an *Acinetobacter pittii* isolate harboring blaIMP-26 (4, 5). IMP-27 was first reported in 2011 from *Proteus mirabilis* PM185, isolated in 2009, with further studies determining that blaIMP-27 was on the chromosome in PM185, on an IncX8 plasmid and the chromosome in *P. mirabilis* PM187, and on a plasmid of unknown Inc type isolated from *Providencia rettgeri* PR1 (6–8). *P. mirabilis* GN855 harboring blaIMP-27 was reported from a patient in Ontario, Canada, in 2012 (9). Another study reported blaIMP-27 located on an IncQ1 plasmid found in multiple species of *Enterobacterales*, including *P. mirabilis*, *Morganella morganii*, and *P. rettgeri*, isolated from the environment of a swine operation in the United States (10).

**RESULTS**

**Bacteria harboring blaIMP-27.** In 2018 and 2019 the National Microbiology Laboratory (NML) screened 2270 Gram-negative isolates by PCR for the most common carbapenemase gene groups, KPC, OXA-48, NDM, VIM, IMP, GES, and NMC/IMI. Twenty-eight isolates (1.2%) were positive by PCR for a blaIMP gene, including one *P. rettgeri*, 15 *P. mirabilis*, seven *M. morganii*, and five *P. aeruginosa* isolates. In 2017, of 242 *P. aeruginosa* and 30 *Morganellaceae* isolates received for routine carbapenemase PCR, four *P. aeruginosa* isolates and one *M. morganii* isolate (N17-03220) harbored an IMP gene. *M. morganii* N17-03220 was later found to be indistinguishable by pulsed-field gel electrophoresis (PFGE) from *M. morganii* N18-00103 received 56 days later (January 2018) and in fact was from the same patient, and it was no longer studied. Thus, there was a significant increase of *Morganellaceae* harboring blaIMP received by the NML after 2017. The 28 IMP-harboring isolates from 2018 to 2019 were from central (n = 9), western (n = 18), or eastern (n = 1) Canada and were isolated mainly from urine (n = 17), wounds (n = 4), or rectal swabs (n = 4). Whole-genome sequencing (WGS) analysis of all 2018–2019 isolates and *P. mirabilis* GN855 determined that all *M. morganii* isolates, 14 of the *P. mirabilis* isolates, and the *P. rettgeri* isolate harbored blaIMP-27, while among the *P. aeruginosa* isolates, one harbored blaIMP-27, one blaIMP-62, and three blaIMP-26. The blaIMP-27 gene could not be identified from the WGS data of one IMP PCR-positive *P. mirabilis* isolates and was presumed lost after subculture; therefore, this isolate was not further studied. Thus, among all the *Morganellaceae* received by the NML in 2018 to 2019 (n = 82) 26.8% (n = 22) were confirmed to harbor blaIMP-27.

**Antimicrobial susceptibility and detection of blaIMP-27-harboring isolates.** Antimicrobial susceptibilities were determined for all IMP-harboring isolates as well as a few non-carbapenemase-producing organisms (CPOs) for comparative purposes (Table 1). As expected for *Morganellaceae*, most were intermediate (I) or resistant (R) to imipenem regardless of the presence/absence of IMP-27, confirming that this is not a suitable phenotype for indicating the possible presence of a carbapenemase. Gradient diffusion was poor for indicating IMP-27 presence, as most isolates were susceptible (S) to meropenem and ertapenem. By Sensititre testing, all IMP-27 *P. mirabilis* isolates and the *P. rettgeri* isolate were I or R to all carbapenems, while the non-CPOs were S to the three nonimipenem carbapenems. However, all of the *M. morganii* isolates were S to all nonimipenem carbapenems by Sensititre testing.

Full antibiograms were in congruence with the resistomes (Table 2). Among the phenotypic tests (Table 2), the modified carbapenem inactivation method (mCIM) test was 100% specific and sensitive for carbapenemase presence/absence. All mCIM-positive isolates were also positive by EDTA-modified CIM (eCIM), correctly indicating the presence of a class B enzyme. The β-Carba test was 100% sensitive and specific for *M. morganii* and *P. rettgeri*, but all IMP-27-producing *P. mirabilis* isolates were falsely negative. The Carba-NP and Neo-Rapid Carb test, which work on the same principle,
performed poorly, and all IMP-27-producing *P. mirabilis* isolates and the *P. rettgeri* isolate were falsely negative. Among IMP-27 *M. morganii* isolates, results for the Carba-NP and Neo-Rapid Carb tests were variable, with some exhibiting false-negative, invalid, or weakly positive results.

### TABLE 1 Antimicrobial susceptibilities of the isolates in this study

| Isolate   | MIC (µg/ml) | Sensitivity GNXF2 |
|-----------|-------------|-------------------|
| **P. mirabilis IMP-27:** | | |
| GN855     | >32 CZ      | 0.76               | 0.125              | 0.25                | 0.25                | >8 | 32 | ≤16 | ≤16 | >16 | >16 | ≤0.5 |
| N18-00201 | 3           | 0.75               | 1 CZ               | 2                  | 2                  | 1              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-00931 | 8           | 6                  | 1.5               | 4                  | 4                  | 2              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-02940 | 4           | 0.38               | 0.047             | 4                  | 4                  | 2              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-03414 | 4           | 0.38               | 0.047             | 4                  | 2                  | 2              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-04196 | 4           | 1.5                | 2 CZ              | 4                  | 2                  | 1              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-02040 | >32         | 0.75               | 0.19              | 4                  | 4                  | 4              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-02041 | 24          | 0.25               | 0.047             | 4                  | 4                  | 2              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-02665 | >32         | 0.38               | 0.047             | 4                  | 2                  | 2              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-02708 | >32         | 0.5                | 0.047             | 4                  | 4                  | 4              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-02786 | 4           | 0.38               | 0.094             | 4                  | 4                  | 4              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-03602 | 32          | 0.38               | 0.047             | 4                  | 4                  | 2              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-03729 | >32         | 0.5                | 0.125             | 8                  | 8                  | 4              | >2 | 16 | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-04409 | 4           | 0.5                | 0.125             | 4                  | 4                  | 2              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| N18-05885 | 6           | 0.38               | 0.032             | 4                  | 2                  | 4              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |
| **P. mirabilis nonCPO:** | | |
| N18-02761 | 1.5         | 0.032              | 0.008             | ≤1                | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-02763 | 0.25        | 0.125              | 0.016             | ≤1                | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N16-02764 | 1           | 0.047              | 0.004             | 2                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| **M. morganii IMP-27:** | | |
| N18-00103 | 4           | 0.25               | 0.032             | 4                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | >8 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-01877 | 2           | 0.19               | 0.032             | 4                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-02673 | 3           | 0.38               | 0.047             | 4                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-02669 | 4           | 0.25               | 0.047             | 4                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-00225 | 4           | 0.19               | 0.047             | 2                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-00598 | 0.19        | 0.016              | 0.25              | 2                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-05814 | 2           | 0.25               | 0.047             | 2                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | ≤16 | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| **M. morganii nonCPO:** | | |
| N18-00856 | 4           | 0.094              | 0.016             | 2                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | 32 | 4    | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| N18-03607 | 1.5         | 0.064              | 0.004             | 2                  | ≤1                | ≤0.25          | 0.25 | ≤1 | ≤16 | ≤16 | 32 | 2    | ≤0.25 | ≤1 | ≤16 | >16 | >16 | ≤0.5 |
| **P. rettgeri IMP-27:** | | |
| N18-03642 | 3           | 0.75               | 0.125             | 4                  | 4                  | 4              | >2 | 8  | 32  | 4    | ≤16 | ≤16 | ≤0.5 |

aCell color indicates antimicrobial susceptibility category: yellow indicates resistance, green indicates intermediate or dose-dependent susceptibility (cefepime), and no color indicates susceptibility.
bEtest values are as read, but for categorization, they are rounded up to the nearest doubling dilution. CZ, colonies in the zone.
cAztreonam, piperacillin-tazobactam, amikacin, and tobramycin are not listed as all isolates were susceptible.

Abbreviations: AMK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; DOR, doripenem; DOX, doxycycline; ETP, ertapenem; FEP, cefepime; GEN, gentamicin; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; MIN, minocycline; TGC, tigecycline; TIM, ticarcillin-clavulanate; TOB, tobramycin; TZP, piperacillin-tazobactam; SXT, sulfamethoxazole-trimethoprim.
The mCIM results indicate that IMP-27 is produced by all the isolates, though it was observed 5 to 10 min after the recommended test time of repeat testing with cells obtained from Mueller-Hinton containing 100 μg/ml ampicillin, a faint IMP-specific band was observed for the two IMP-27-harboring isolates, though the activities can vary by 2- to 5-fold (Table 3). Nonetheless, we determined specific activity against imipenem for the isolates tested by NG-Test CARBA 5 and confirmed imipenemase activity in the IMP-27-harboring isolates, though the activities can vary by 2- to 5-fold (Table 3).

### TABLE 2 Resistome, plasmid types, and results of phenotypic tests for carbapenemase activity for the isolates in this study

| Isolate        | Resistomea | Plasmid type | mCIMb | β-Carba | Carba-NP | Neo-Rapid Carb | NG-Test CARBA 5c |
|----------------|------------|--------------|-------|---------|----------|----------------|------------------|
| **P. mirabilis IMP-27** |            |              |       |         |          |                |                  |
| GN855          |            |             |       | POS     | NEG      | NEG            | NEG              |
| N18-00201      |            |             |       | POS     | NEG      | NEG            | NEG              |
| N18-00931      |            |             |       | POS     | NEG      | NEG            | NEG              |
| N18-02940      |            |             |       | POS     | NEG      | NEG            | NEG              |
| N18-03414      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N18-04196      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-02040      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-02041      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-02665      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-02708      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-02786      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-03729      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-04409      |            |             |       | POS     | NEG      | NEG            | Not done         |
| N19-05885      |            |             |       | POS     | NEG      | NEG            | Not done         |
| **P. mirabilis non-CPO** |            |              |       |         |          |                |                  |
| N19-02761      |            |             |       | NEG     | NEG      | NEG            | NEG              |
| N19-02763      |            |             |       | NEG     | NEG      | NEG            | Not done         |
| N19-02764      |            |             |       | NEG     | NEG      | NEG            | Not done         |
| **M. morganii IMP-27** |            |              |       |         |          |                |                  |
| N18-00103      |            |             | IncQ1 | POS     | POS      | Invalid        | wPOS             |
| N18-01877      |            |             | Coll (RGK), Coll440I | POS | POS | Invalid | POS | Not done |
| N18-02673      |            |             | No hits | POS | POS | NEG | wPOS | Not done |
| N18-02869      |            |             | No hits | POS | POS | NEG | wPOS | wPOS |
| N19-00225      |            |             | No hits | POS | POS | NEG | wPOS | wPOS |
| N19-00598      |            |             | No hits | POS | POS | POS | Not done |       |
| N19-05814      |            |             | No hits | POS | POS | POS | Not done |       |
| **M. morganii non-CPO** |            |              |       |         |          |                |                  |
| N18-00856      |            |             | No hits | NEG | NEG | NEG | NEG | Not done |
| N18-03607      |            |             | IncX2, repA (Fil) | NEG | NEG | NEG | NEG | Not done |
| **P. rettgeri IMP-27** |            |              |       |         |          |                |                  |
| N18-03642      |            |             | No hits | POS | POS | POS | NEG | Not done |

aResistome and plasmid types were determined by ResFinder and PlasmidFinder, respectively.
bThe sat-2 gene was not in the ResFinder database. The bla_{oxa-1} gene is the intrinsic ampC gene of M. morganii.
cPOS, positive; NEG, negative. "Invalid" means that the no-meropenem control turned orange-yellow. "wPOS" means that an orange color was observed for the Carba-NP or NeoRapid Carb test or that a faint IMP band was observed in the NG-Test CARBA 5 test.
dAll mCIM-positive isolates were also positive in the eCIM test.
eImmunochromatographic assay to detect KPC, OXA-48-like, VIM, IMP, and NDM enzymes.

We also tried the more expensive NG-Test CARBA 5 immunochromatographic assay on a small number of isolates, even though the package insert (EN0022CAR/Rev: 200131) does not list IMP-27 as one of the variants that can be detected by this test (Table 2). When the cells were obtained from tryptic soy agar (TSA)-blood plates (M. morganii) or Mueller-Hinton medium (P. mirabilis), all results were negative. Upon repeat testing with cells obtained from Mueller-Hinton containing 100 μg/ml ampicillin, a faint IMP-specific band was observed for the two IMP-27-harboring M. morganii isolates, though it was observed 5 to 10 min after the recommended test time of 15 min. The mCIM results indicate that IMP-27 is produced by all the bla_{IMP-27}-harboring isolates in the study. Nonetheless, we determined specific activity against imipenem for the isolates tested by NG-Test CARBA 5 and confirmed imipenemase activity in the IMP-27-harboring isolates, though the activities can vary by 2- to 5-fold (Table 3).
Together, the results indicate that *P. mirabilis* is likely recalcitrant to lysis/permeabilization in the non-mCIM phenotypic tests, all of which have a cell suspension/lysis solution. For the *M. morganii* isolates, although results indicate that some lysis does occur, it may be suboptimal, and this, combined with low IMP-27 levels for some isolates and/or technical issues, may account for poor results in the non-mCIM phenotypic tests.

*Bla<sub>IMP-27</sub>* is found within a Tn<sub>7</sub> element located in the chromosome. WGS analysis showed that the *bla<sup>IMP-27</sup>* gene was located in the class 2 integron In<sub>2-71</sub> (http://integrall.bio.ua.pt/), which was integrated into a Tn<sub>7</sub> element (Fig. 1). This structure, labeled Tn<sub>7</sub>[ln2-71], was inserted into the chromosome of all isolates via the attTn<sub>7</sub> site at the 3' end of the glmS gene, the canonical bacterial Tn<sub>7</sub> insertion site [11], and each element was flanked by direct repeats, indicating acquisition by transposition. Tn<sub>7</sub>[ln2-71] elements were identified from the GenBank database (>99% identity) in *P. mirabilis* PM185 (accession no. NOWB01000038), *P. rettgeri* 106-1829X (accession no. KY847874), *M. morganii* 480-26370X (accession no. KY847873), and *E. coli* CFSAN051542 (accession no. CP020835). Sequence analysis divided the Tn<sub>7</sub>[ln2-71] elements into two clades, A (n = 16) and B (n = 11), with the elements in clade A being >99.9% identical and the elements in clade B being 100% identical, but with the clades differing by 105 to 107 bp differences (Fig. 2). The vast majority of base pair differences were found in the

| Isolate       | Sp act (µmol min⁻¹ mg⁻¹) |
|---------------|-------------------------|
| *P. mirabilis*|                         |
| GN 855 (IMP-27)| 70.1 ± 19.6             |
| N18-00201 (IMP-27)| 28.0 ± 5.1             |
| N18-02761 (non-CPO) | None detected            |
| *M. morganii* |                         |
| N18-00103 (IMP-27)| 149.7 ± 22.1            |
| N18-02869 (IMP-27)| 70.7 ± 5.8              |
| N18-03607 (non-CPO) | None detected            |

**FIG 1** Schematic diagram depicting Tn<sub>7</sub>[ln2-71] and its position in the chromosome. The Tn<sub>7</sub>[ln2-71] element in *E. coli* CFSAN051542 is 15,642 bp, as it harbors an ISVsa5 element between the int<sub>2</sub> and *bla<sup>IMP-27</sup>* genes. The int<sub>2</sub> gene contains an internal stop codon, indicated by a vertical line. The coordinates for Tn<sub>7</sub>[ln2-71] in the genomes are as follows: *E. coli* CFSAN05142, 4844260 to 4859901 (accession no. CP020835); *P. rettgeri* N18-03642, 27829 to 42132 (accession no. JAA0100000015); *M. morganii* N18-00103, 19322 to 33625 (accession no. CP048275); *P. mirabilis* N18-00201, 3732335 to 3746638 (accession no. CP048404).
tnsA-tnsB region indicating a region of recombination (data not shown). No plasmid repli-
cons were identified in the *P. mirabilis* or the *P. rettgeri* isolates, whereas two *M. morganii*
isolates harbored replicons (Table 2). Though IncQ1 plasmids have been found to harbor
*bla*<sub>IMP-27</sub> (10), the IncQ1 replicon in N18-00103 was found to be integrated into the chro-
mosome and not linked to Tn<sub>7</sub>[In2-71].

**Limited clonality revealed by core genome SNV analysis.** We carried out core ge-
nome SNV analysis on all *P. mirabilis* and all *M. morganii* to determine strain related-
ness (Fig. 3A and B). Among the *M. morganii* isolates, 6 of 10 isolates are diverse, with
the number of single nucleotide variants (SNVs) between them ranging from 83 to
>14,000 (Fig. 3A). Four isolates clustered at 0 to 3 SNVs, but no strong epidemiological
links could be uncovered between any of the four patients, though two isolates were
from patients who had been in the same hospital but 470 days apart. The analysis of *P.
mirabilis*, which included the U.S. IMP-27 isolates PM185 and PM187, showed that 10
isolates were diverse, differing by 752 to >12,800 SNVs from each other (Fig. 3B).
However, the 11 *P. mirabilis* isolates harboring Tn<sub>7</sub>[In2-71]-B (Fig. 2) clustered together
at 0 to 13 SNVs or 1 to 15 SNVs when reanalyzed separately with an internal reference
and, hence, a larger core genome. Anonymized patient facilities were available for
some isolates, indicating some common facilities, but the limited data make inferring
direct transmission events unfeasible. Nonetheless, this cluster of closely related iso-
lates can be postulated to have derived from a common ancestor that has spread to
multiple locations in western Canada.

**DISCUSSION**

*Morganellaceae* isolates harboring *bla*<sub>IMP-27</sub> have emerged in Canada since 2018.
These isolates may be difficult to detect as CPOs, as they can exhibit susceptibility to
Carbapenemase IMP-27 in Morganellaceae

FIG 3  Phylogenetic trees of the (A) M. morganii and (B) P. mirabilis isolates in this study as generated by the SNVPhyl Pipeline, which generates an alignment of high-quality valid SNVs through PhyML using the GTR+γ model (15). Reference genomes used are indicated by an asterisk and were the closed genomes of M. morganii N18-00103 (CP048275) or P. mirabilis N18-00201 (CP048404) or a pseudogenome (concatenated contigs) of P. mirabilis N18-02940. SNVs or SNV ranges between isolates or groups of isolates are shown. For the main analysis of each group of the same species, boxed isolates do not harbor blaIMP-27. For the subanalysis of the cluster of the closely related P. mirabilis isolates, each unique shape indicates a specific facility from which the bacterium was isolated. The isolates were isolated in Alberta except for the three from British Columbia (BC).

carbapenems depending on which susceptibility testing method is used. The mCIM detected carbapenemase production or lack thereof among all study isolates, as did the β-Carba test for the M. morganii and P. rettgeri isolates. Though blaIMP-27 was exclusively chromosomally located here, its dissemination may be facilitated by being
harbored within a mobile Tn7 transposon. Isolates were diverse but phylogenomics revealed clones harboring bla<sub>IMP-27</sub> have dispersed in Canada. The major limitation of this study was that isolates were voluntarily submitted to the NML, and thus, the prevalence of bla<sub>IMP-27</sub> isolates may be underestimated. In addition, due to bla<sub>IMP</sub> family sequence variation, in-house primers and some commercial assays may yield false-negative results (12).

**MATERIALS AND METHODS**

**Bacterial isolates.** The bacteria in this study were from routine isolates voluntarily sent to the NML for carbapenemase PCR. Typically, organisms are sent because of a suspicion of carbapenemase production due to reduced susceptibility/resistance to a carbapenem and/or a positive result of a phenotypic method that indicates carbapenemase production. For the isolates that test positive, the PCR results are reported, and the carbapenemase gene is not sequenced unless by special request.

**Antimicrobial susceptibilities and phenotypic carbapenemase detection.** Antimicrobial susceptibilities were carried out by Etest (bioMérieux) and Sensititre GNX2F plates (Thermo Fisher Scientific, Toronto, ON, Canada). Categorical interpretations were done using CLSI (13) or FDA guidelines (tigecycline). The β- Carba test (Bio-Rad Laboratories, Mississauga, ON, Canada), Neo-Rapid Carb test (Roscoe Diagnostica, Taastrup, Denmark), and NG-Test CARBA 5 (NG Biotech, Guipry, France) were carried out per the manufacturer’s instructions. The Carba-NP, mCIM, and eCIM tests were carried out as described elsewhere (13).

**Carbapenemase multiplex PCR.** The carbapenemase multiplex PCR was as previously described (4) except with two updated primers, IMP-F2 (5'-CTTGAMGARGGYGTATATTGTCATAC), which pairs with IMP-2, and IMI-Dr (5'-TCAATTCGAGTCACATGTCAT), which pairs with IMI-A.

**Sequencing and bioinformatics.** Whole-genome sequencing (WGS) was carried out on all isolates by NextSeq (Illumina Inc., San Diego, CA), with two isolates (M. morganii N18-00103 and P. mirabilis N18-00201) additionally sequenced by Nanopore technology (Oxford Nanopore Technologies, Oxford, UK). Read assembly was carried out using Unicycler v0.4.4 (14). Single nucleotide variant (SNV) analysis was carried out using the SNVPhyl Pipeline (15). Assemblies were analyzed by the ResFinder and PlasmidFinder tools at the Center of Genomic Epidemiology website (http://www.genomic epidemiology.org).

**Data availability.** Nucleotide sequences and WGS reads have been deposited in NCBI BioProject PRJNA603518. The complete closed genomes of P. mirabilis N18-00201 and M. morganii N18-00103 and the draft genome of P. rettgeri N18-02642 have been assigned accession no. CP048404, CP048275, and JAAOA000000000, respectively. The sequence of Tn7[ln2-71] from N18-02940 has been assigned accession no. MT226801.

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