AMERICAN SOCIETY FOR MICROBIOLOGY | mSphere®

RESEARCH ARTICLE

Emergence of *Morganellaceae* Harboring bla_{IMP-27} Metalloenzyme in Canada

David A. Boyd,[a] Laura F. Mataseje,[a] Tanis Dingle,[b] Linda Hoang,[c] Brigitte Lefebvre,[d] Allison McGeer,[e] Roberto G. Melano,[f] Ian Stuart,[g] Andrew Walkty,[h] Amanda Wilmer,[i] Michael R. Mulvey[a]

[a]Antimicrobial Resistance & Nosocomial Infections, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada
[b]University of Alberta, Provincial Laboratory for Public Health, Edmonton, Alberta, Canada
[c]Bacteriology & Mycology, BCCDC Public Health Laboratory, Vancouver, British Columbia, Canada
[d]Laboratoire de Santé Publique du Québec, Institut National de Santé Publique du Québec, Sainte-Anne-de-Bellevue, Québec, Canada
[e]Department of Microbiology, Mount Sinai Hospital, Toronto, Ontario, Canada
[f]Public Health Ontario Laboratories, Toronto, Ontario, Canada
[g]Horizon Health Network, Fredricton, New Brunswick, Canada
[h]Departments of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada
[i]Department of Laboratory Medicine, Kelowna General Hospital, Kelowna, British Columbia, Canada

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_{IMP-27} within a chromosomal Tn*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 carbapenems are considered the last line of defense against these organisms. The ability of carbapenemases to hydrolyze most β-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, β-lactamases that hydrolyze carbapenem β-lactams, have been found globally among clinically significant members of the *Enterobacterales* (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-β-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

Citation Boyd DA, Mataseje LF, Dingle T, Hoang L, Lefebvre B, McGeer A, Melano RG, Stuart I, Walkty A, Wilmer A, Mulvey MR. 2021. Emergence of *Morganellaceae* harboring bla_{IMP-27} metalloenzyme in Canada. mSphere 6:e00048-21. https://doi.org/10.1128/mSphere.00048-21.

Editor Patricia A. Bradford, Antimicrobial Development Specialists, LLC

Address correspondence to David A. Boyd, david.boyd@canada.ca.

Received 15 January 2021
Accepted 27 April 2021
Published 19 May 2021

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 bla_{IMP-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 cloacae* isolate harboring bla_{IMP-13} and an *Acinetobacter pittii* isolate harboring bla_{IMP-26} (4, 5). IMP-27 was first reported in 2011 from *Proteus mirabilis* PM185, isolated in 2009, with further studies determining that bla_{IMP-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 bla_{IMP-27} was reported from a patient in Ontario, Canada, in 2012 (9). Another study reported bla_{IMP-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 bla_{IMP-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 bla_{IMP} 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 bla_{IMP} 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 bla_{IMP-27}, while among the *P. aeruginosa* isolates, one harbored bla_{IMP-7}, one bla_{IMP-62}, and three bla_{IMP-26}. The bla_{IMP-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 bla_{IMP-27}.

Antimicrobial susceptibility and detection of bla_{IMP-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,

TABLE 1 Antimicrobial susceptibilities of the isolates in this study

Isolate	MIC (μg/ml)[a]																
	Etest[b]			Sensititre GNXF2[c]													
	IPM[d]	MEM	ETP	IPM	MEM	ETP	DOR	FEP	CTX	CAZ	TIM	GEN	CIP	LVX	TGC	DOX	MIN	SXT
P. mirabilis IMP-27:																		
GN855	>32CZ	0.75	0.125	4	4	4	>2	8	32	4	≤16	≤1	0.5	2	8	>16	>16	≤0.5
N18-00201	3	0.75	1CZ	2	2	1	>2	4	32	2	≤16	2	≤0.25	≤1	2	>16	>16	≤0.5
N18-00931	8	6	1.5	4	4	2	>2	8	32	4	≤16	2	≤0.25	≤1	8	>16	>16	≤0.5
N18-02940	4	0.38	0.047	2	4	2	>2	4	32	4	≤16	2	≤0.25	≤1	4	>16	>16	≤0.5
N18-03414	4	0.38	0.047	4	2	2	>2	4	32	2	≤16	2	≤0.25	≤1	4	>16	>16	≤0.5
N18-04196	4	1.5	2CZ	4	2	1	>2	4	32	2	≤16	2	≤0.25	≤1	4	>16	>16	≤0.5
N19-02040	>32	0.75	0.19	4	4	4	>2	4	32	8	≤16	4	≤0.25	≤1	2	>16	>16	≤0.5
N19-02041	24	0.25	0.047	4	2	2	>2	4	32	4	≤16	2	≤0.25	≤1	4	>16	>16	≤0.5
N19-02665	>32	0.38	0.047	2	2	2	>2	8	32	4	≤16	≤1	≤0.25	≤1	4	>16	>16	≤0.5
N19-02708	>32	0.5	0.047	4	4	4	>2	8	32	4	≤16	≤1	≤0.25	≤1	4	>16	>16	≤0.5
N19-02786	4	0.38	0.094	4	4	4	>2	4	32	4	≤16	2	≤0.25	≤1	2	>16	>16	≤0.5
N19-03602	>32	0.38	0.047	4	4	2	>2	8	32	4	≤16	≤1	≤0.25	≤1	4	>16	>16	≤0.5
N19-03729	>32	0.5	0.125	8	8	4	>2	16	32	4	≤16	2	≤0.25	≤1	4	>16	>16	≤0.5
N19-04409	4	0.5	0.125	4	4	2	>2	4	32	2	≤16	2	≤0.25	≤1	4	>16	>16	≤0.5
N19-05885	6	0.38	0.032	4	2	4	>2	8	32	2	≤16	≤1	≤0.25	≤1	4	>16	>16	≤0.5
P. mirabilis non-CPO:																		
N18-02761	1.5	0.032	0.008	≤1	≤1	≤0.25	0.25	≤2	≤1	≤1	≤16	≤1	≤0.25	≤1	4	>16	>16	≤0.5
N18-02763	0.25	0.125	0.016	≤1	≤1	≤0.25	≤0.12	≤2	≤1	≤1	≤16	≤1	≤0.25	≤1	1	>16	>16	≤0.5
N18-02764	1	0.047	0.004	2	≤1	≤0.25	0.25	≤2	≤1	≤1	≤16	≤1	≤0.25	≤1	8	>16	>16	≤0.5
M. morganii IMP-27:																		
N18-00103	4	0.25	0.032	4	≤1	≤0.25	0.5	≤2	8	≤1	≤16	>8	≤0.25	≤1	2	>16	>16	>4
N18-01877	2	0.19	0.032	2	≤1	≤0.25	0.5	≤2	8	≤1	≤16	≤1	≤0.25	≤1	2	4	4	≤0.5
N18-02673	3	0.38	0.047	4	≤1	≤0.25	1	≤2	8	≤1	≤16	≤1	≤0.25	≤1	1	>16	16	≤0.5
N18-02869	4	0.25	0.047	4	≤1	≤0.25	0.5	≤2	8	2	≤16	≤1	≤0.25	≤1	1	>16	16	≤0.5
N19-00225	4	0.19	0.047	2	≤1	≤0.25	0.5	≤2	16	≤1	≤16	≤1	≤0.25	≤1	1	>16	8	2
N19-00598		0.19	0.016	2	≤1	≤0.25	0.5	≤2	8	2	≤16	≤1	≤0.25	≤1	0.5	>16	>16	≤0.5
N19-05814	2	0.25	0.047	2	≤1	≤0.25	0.5	≤2	8	≤1	≤16	≤1	≤0.25	≤1	1	>16	>16	≤0.5
M. morganii non-CPO:																		
N18-00856	4	0.094	0.016	2	≤1	≤0.25	0.25	≤2	16	16	32	4	≤0.25	≤1	4	>16	>16	≤0.5
N18-03607	1.5	0.064	0.004	2	≤1	≤0.25	0.25	≤2	2	≤1	≤16	2	≤0.25	≤1	2	8	16	≤0.5
P. rettgeri IMP-27:																		
N18-03642	3	0.75	0.125	4	4	4	>2	8	32	4	≤16	≤1	0.5	2	8	>16	>16	≤0.5

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

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 2 Resistome, plasmid types, and results of phenotypic tests for carbapenemase activity for the isolates in this study[a]

Isolate	Resistome[b]	Plasmid type	Phenotypic test for carbapenemase activity[c]				
			mCIM[d]	β-Carba	Carba-NP	Neo-Rapid Carb	NG-Test CARBA 5[e]
P. mirabilis IMP-27							
GN855	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	NEG
N18-00201	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	NEG
N18-00931	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N18-02940	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N18-03414	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N18-04196	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-02040	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-02041	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-02665	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-02708	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-02786	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-03602	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-03729	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-04409	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
N19-05885	bla_{IMP-27}, *aadA1*, *cat*, *tet*(J)	No hits	POS	NEG	NEG	NEG	Not done
P. mirabilis non-CPO							
N19-02761	*cat*, *tet*(J)	No hits	NEG	NEG	NEG	NEG	NEG
N19-02763	*cat*, *tet*(J)	No hits	NEG	NEG	NEG	NEG	Not done
N19-02764	*cat*, *tet*(J)	No hits	NEG	NEG	NEG	NEG	Not done
M. morganii IMP-27							
N18-00103	bla_{IMP-27}, bla_{DHA-14}, bla_{TEM-1B}, *aadA1*, *aadA2*, *aac(3)-Iid*, *aph(6)Id*, *aph(3'')Ib*, *aph(3')Ia*, *mph(A)*, *catA1*, *tet*(B), *sul1*, *sul2*, *dfrA12*	IncQ1	POS	POS	Invalid	wPOS	wPOS
N18-01877	bla_{IMP-27}, bla_{DHA-1}, *aadA1*, *catA2*	ColI (RGK), ColI440I	POS	POS	Invalid	POS	Not done
N18-02673	bla_{IMP-27}, bla_{DHA-1}, *aadA1*, *catA2*, *tet*(B)	No hits	POS	POS	NEG	wPOS	Not done
N18-02869	bla_{IMP-27}, bla_{DHA-1}, *aadA1*, *catA2*, *tet*(B)	No hits	POS	POS	NEG	wPOS	wPOS
N19-00225	bla_{IMP-27}, bla_{DHA-16}, *aadA1*, *catA2*, *tet*(D), *dfrA1*	No hits	POS	POS	NEG	wPOS	Not done
N19-00598	bla_{IMP-27}, bla_{DHA-1}, *aadA1*, *catA2*, *tet*(B)	No hits	POS	POS	POS	POS	Not done
N19-05814	bla_{IMP-27}, bla_{DHA-1}, *aadA1*, *catA2*, *tet*(B)	No hits	POS	POS	POS	POS	Not done
M. morganii non-CPO							
N18-00856	bla_{DHA-1}, *tet*(B), *catA2*	No hits	NEG	NEG	NEG	NEG	Not done
N18-03607	bla_{DHA-1}, *tet*(B), *catA2*	IncX2, *repA* (FII)	NEG	NEG	NEG	NEG	NEG
P. rettgeri IMP-27:							
N18-03642	bla_{IMP-27}, *aadA1*	No hits	POS	POS	POS	NEG	Not done

[a]Resistome and plasmid types were determined by ResFinder and PlasmidFinder, respectively.
[b]The *sat-2* gene was not in the ResFinder database. The bla_{DHA} gene is the intrinsic *ampC* gene of *M. morganii*.
[c]POS, 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.
[d]All mCIM-positive isolates were also positive in the eCIM test.
[e]Immunochromatographic 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 (ENO022CAR/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).

TABLE 3 Specific activities of crude lysates against imipenem from some isolates in this study

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

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*$_{IMP-27}$ is found within a Tn*7* element located in the chromosome.** WGS analysis showed that the *bla*$_{IMP-27}$ gene was located in the class 2 integron In2-71 (http://integrall.bio.ua.pt/?), which was integrated into a Tn*7* element (Fig. 1). This structure, labeled Tn*7*[In2-71], was inserted into the chromosome of all isolates via the *att*Tn*7* site at the 3′ end of the *glmS* gene, the canonical bacterial Tn*7* insertion site (11), and each element was flanked by direct repeats, indicating acquisition by transposition. Tn*7*[In2-71] 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*7*[In2-71] 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

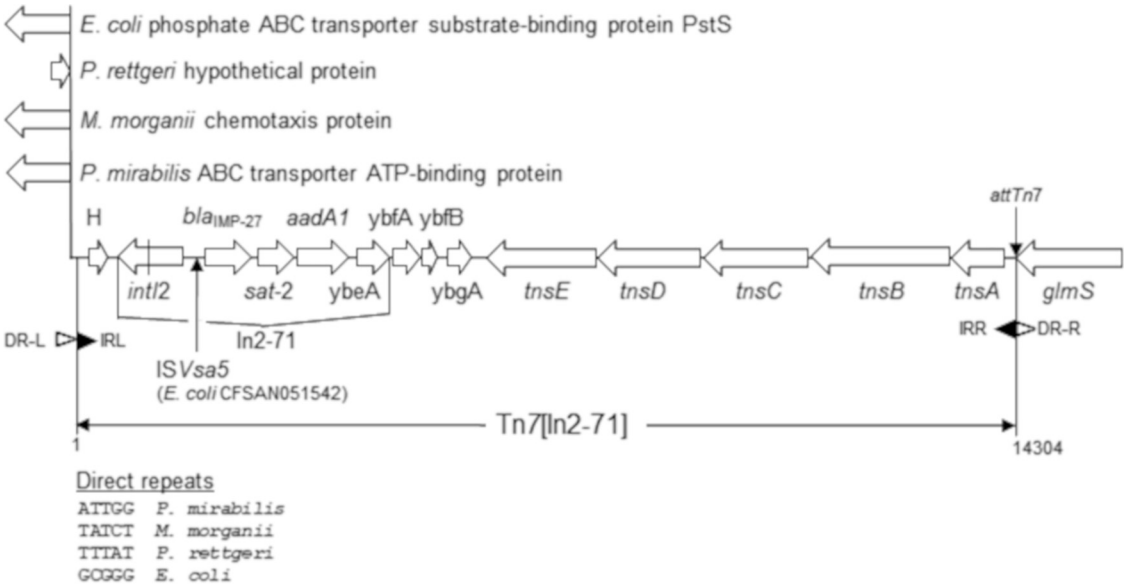

FIG 1 Schematic diagram depicting Tn*7*[In2-71] and its position in the chromosome. The Tn*7*[In2-71] element in *E. coli* CFSAN051542 is 15,642 bp, as it harbors an ISVsa5 element between the *intI2* and *bla*$_{IMP-27}$ genes. The *intI2* gene contains an internal stop codon, indicated by a vertical line. The coordinates for Tn*7*[In2-71] in the genomes are as follows: *E. coli* CFSAN05142, 4844260 to 4859901 (accession no. CP020835); *P. rettgeri* N18-03642, 27829 to 42132 (accession no. JAAOIA010000015); *M. morganii* N18-00103, 19322 to 33625 (accession no. CP048275); *P. mirabilis* N18-00201, 3732335 to 3746638 (accession no. CP048404).

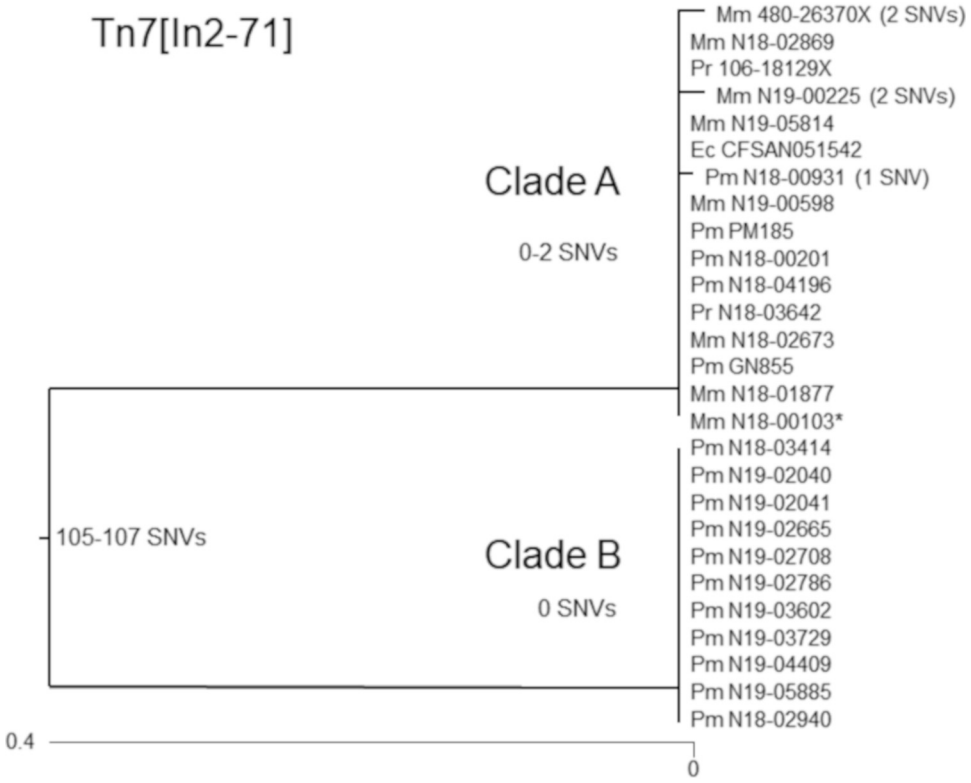

FIG 2 Phylogenetic tree of Tn*7*[In2-71] elements based on a multiple-sequence alignment. The SNV differences are from using the Tn*7*[In2-71] from *M. morganii* N18-00103 as the reference (indicated by an asterisk). The Tn*7* [In2-71] from *E. coli* CFSAN051542 was analyzed after removal of the IS*Vsa5* sequence and one of its target site duplications.

tnsA-tnsB region indicating a region of recombination (data not shown). No plasmid replicons 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_{IMP-27} (10), the IncQ1 replicon in N18-00103 was found to be integrated into the chromosome and not linked to Tn*7*[In2-71].

Limited clonality revealed by core genome SNV analysis. We carried out core genome SNV analysis on all *P. mirabilis* and all *M. morganii* to determine strain relatedness (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*7*[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 isolates can be postulated to have derived from a common ancestor that has spread to multiple locations in western Canada.

DISCUSSION

Morganellaceae isolates harboring bla_{IMP-27} have emerged in Canada since 2018. These isolates may be difficult to detect as CPOs, as they can exhibit susceptibility to

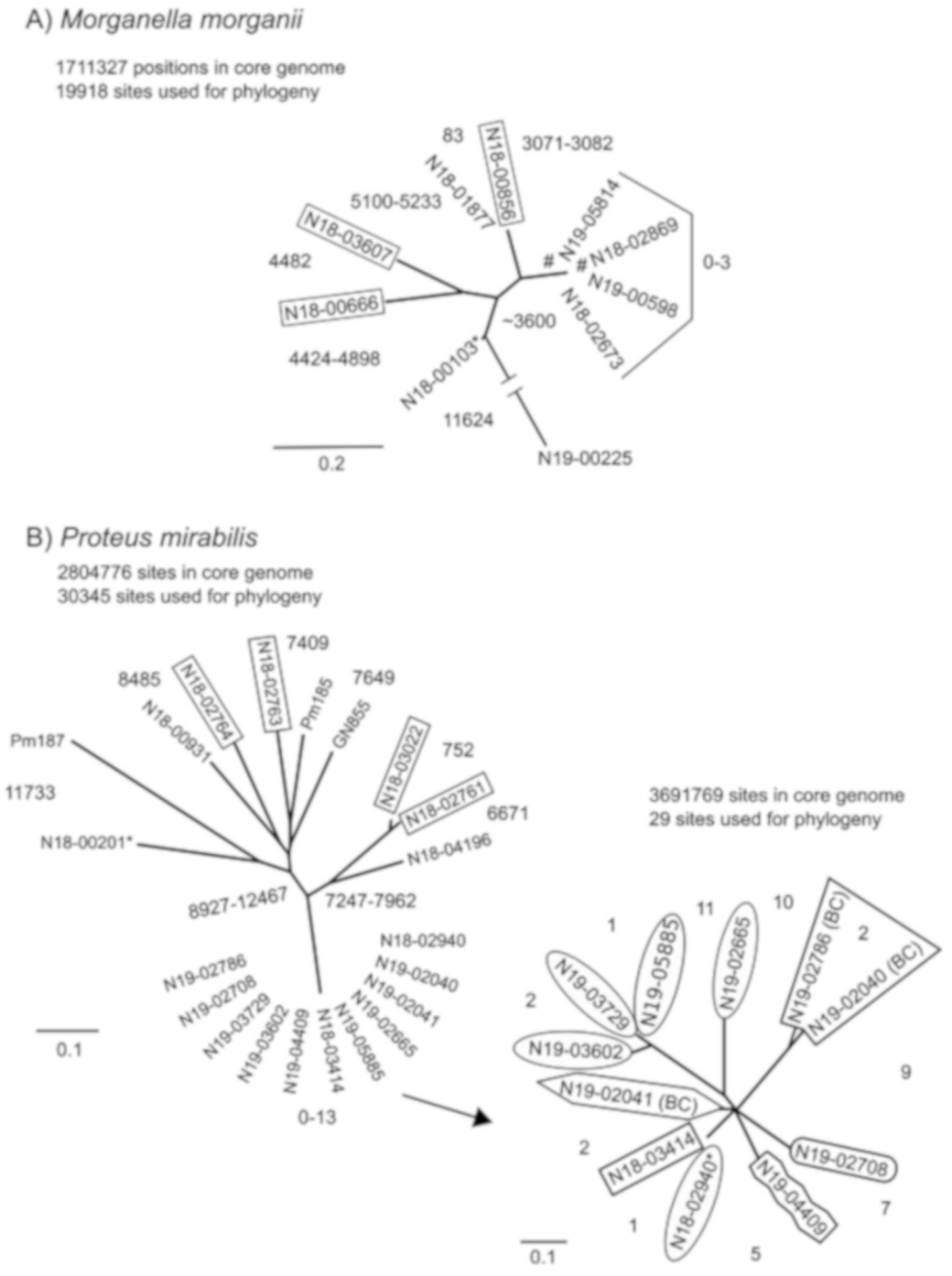

FIG 3 Phylogenetic trees of the (A) *M. morganii* and (B) *P. mirabilis* isolates in this study as generated by the SNVPhyl Pipeline, which generates an alignment of high-quality valid SNVs through PhyML using the GTR+γ model (15). Reference genomes used are indicated by an asterisk and were the closed 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 bla_{IMP-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 bla_{IMP-27} was exclusively chromosomally located here, its dissemination may be facilitated by being

harbored within a mobile Tn*7* transposon. Isolates were diverse but phylogenomics revealed clones harboring bla_{IMP-27} 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_{IMP-27} isolates may be underestimated. In addition, due to bla_{IMP} 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′-CTTGAMGARGGYGTTTATGTTCATAC), which pairs with IMP-2, and IMI-Dr (5′-TCATTTGCMGTACCGTATGC), 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.genomicepidemiology.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 JAAOIA000000000, respectively. The sequence of Tn*7*[In2-71] from N18-02940 has been assigned accession no. MT226801.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Ken Fakharuddin and Romeo Hizon as well as the staff of the Genomics Core Facility of the NML.

REFERENCES

1. Nordmann P, Poirel L. 2014. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 20:821–830. https://doi.org/10.1111/1469-0691.12719.
2. Matsumura Y, Peirano G, Motyl MR, Adams MD, Chen L, Kreiswirth B, DeVinney R, Pitout JD. 2017. Global molecular epidemiology of IMP-producing Enterobacteriaceae. Antimicrob Agents Chemother 61:e02729-16. https://doi.org/10.1128/AAC.02729-16.
3. Gibb AP, Tribuddharat C, Moore RA, Louie TJ, Krulicki W, Livermore DM, Palepou M-FI, Woodford N. 2002. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla_{IMP} allele, bla_{IMP-7}. Antimicrob Agents Chemother 46:255–258. https://doi.org/10.1128/aac.46.1.255-258.2002.
4. Mataseje LF, Abdesselam K, Vachon J, Mitchel R, Bryce E, Roscoe D, Boyd DA, Embree J, Katz K, Kibsey P, Simor AE, Taylor G, Turgeon N, Langley J, Gravel D, Amaratunga K, Mulvey MR. 2016. Results from the Canadian Nosocomial Infection Surveillance Program on Carbapenemase-Producing Enterobacteriaceae, 2010 to 2014. Antimicrob Agents Chemother 60:6787–6794. https://doi.org/10.1128/AAC.01359-16.
5. Boyd DA, Mataseje LF, Pelude L, Mitchell R, Bryce E, Roscoe D, Embree J, Katz K, Kibsey P, Lavallee C, Simor AE, Taylor G, Turgeon N, Langley JM, Amaratunga K, Mulvey MR, Wong A, McGeer A, Simor A, Lee B, Frenette C, Ellis C, Lavallee C, Mertz D, Bryce E, Henderson E, Taylor G, German G, Davis I, de Heer J, Minion J, Embree J, Langley J, Srigley J, Embil J, Vayalumkal J, Suh K, Katz K, Johnston L, Lefebvre M-A, John M, Blackburn MM, Bridger N, Turgeon N, Thampi N, Kibsey P, Stagg P, Richardson S, Hota S, Pelletier S, members of the Canadian Nosocomial Infection Surveillance Program, et al. 2019. Results from the Canadian Nosocomial Infection Surveillance Program for detection of carbapenemase-producing *Acinetobacter* spp. in Canadian hospitals, 2010–16. J Antimicrob Chemother 74:315–320. https://doi.org/10.1093/jac/dky416.
6. Moulds NM, Thomson KS, Hanson ND. 2011. IMP-27, a novel metallo-β-lactamase (MBL) associated with a class II integron identified in an isolate of *Proteus mirabilis*, abstr C1-1212. Abstr 51st Intersci Conf Antimicrob Agents Chemother.
7. Dixon N, Fowler RC, Yoshizumi A, Horlyama T, Ishii Y, Harrison L, Geyer CN, Moland ES, Thomson K, Hanson ND. 2016. IMP-27, a unique metallo-β-lactamase identified in geographically distinct isolates of *Proteus mirabilis*. Antimicrob Agents Chemother 60:6418–6421. https://doi.org/10.1128/AAC.02945-15.
8. Potter RF, Wallace MA, McMullen AR, Prusa J, Stallings CL, Burnham CAD, Dantas G. 2018. bla_{IMP-27} on transferable plasmids in *Proteus mirabilis* and *Providencia rettgeri*. Clin Microbiol Infect 24:1019.E5–1019.E8. https://doi.org/10.1016/j.cmi.2018.02.018.
9. Tiget N, Siebert H, MacNeill M, Rawte P, Farrell DJ, Low DE, Patel SN, Melano RG. 2012. Detection of IMP-27 metallo-β-lactamase in *Proteus mirabilis*, ON, Canada, abstr C2-090. Abstr 52st Intersci Conf Antimicrob Agents Chemother.
10. Mollenkopf DF, Stull JW, Mathys DA, Bowman AS, Feicht SM, Grooters SV, Daniels JB, Wittum TE. 2017. Carbapenemase-producing *Enterobacteriaceae* recovered from the environment of a swine farrow-to-finish

operation in the United States. Antimicrob Agents Chemother 61:e01298-16. https://doi.org/10.1128/AAC.01298-16.
11. Peters JE. 2014. Tn*7*. Microbiol Spectr 2:MDNA3-0010-2014. https://doi.org/10.1128/microbiolspec.MDNA3-0010-2014.
12. Lowe CF, Matic N, Champagne S, Romney MG, Leung V, Ritchie G. 2020. The Brief Case: IMP, the uncommonly common carbapenemase. J Clin Microbiol 58:e01094-19. https://doi.org/10.1128/JCM.01094-19.
13. Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing: M100, 30th ed. CLSI, Wayne, PA, USA.
14. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
15. Petkau A, Mabon P, Sieffert C, Knox NC, Cabral J, Iskander M, Iskander M, Weedmark K, Zaheer R, Katz LS, Nadon C, Reimer A, Taboada E, Beiko RG, Hsiao W, Brinkman F, Graham M, Van Domselaar G. 2017. SNVPhyl: a single nucleotide variant phylogenomics pipeline for microbial genomic epidemiology. Microb Genom 3:e000116. https://doi.org/10.1099/mgen.0.000116.