

Characterization of ertapenem-resistant Enterobacterales in Canadian hospitals: 17 years of the CANWARD study (2007–23)

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Objectives: To review phenotypic and genotypic characteristics of ertapenem-resistant Enterobacterales isolates identified by the CANWARD study from 2007 to 2023.

Methods: Bacterial isolates were collected as part of the CANWARD surveillance study from 2007 to 2023. CLSI M7 broth microdilution antimicrobial susceptibility testing (12th edition, 2024) was performed. MICs were interpreted by CLSI M100 breakpoints (34th edition, 2024). WGS was performed to identify antimicrobial resistance markers.

Results: Only 134 (0.7%) of the 19 642 Enterobacterales were ertapenem-resistant. Carbapenemase producing Enterobacterales (CPE) accounted for 17.9% ($n = 24$) of ertapenem-resistant isolates and were predominantly *Klebsiella pneumoniae* (54.2%) and *Escherichia coli* (20.8%). KPC was the most common carbapenemase identified (62.5%). *K. pneumoniae* ST834, ST16 and ST258, and *E. coli* ST131 were the most frequent STs detected. CPE percent resistant values ranged from 20.8% to 25.0% for ceftazidime/avibactam, imipenem/relebactam and meropenem/vaborbactam. Most non-CPE ertapenem-resistant isolates were *Enterobacter* spp. (51.8%), *E. coli* (15.5%) and *K. pneumoniae* (10.9%). Non-CPE STs were most commonly *E. cloacae* ST108, ST50 and ST133, and *E. coli* ST131. Non-CPE percent resistant values ranged from 2.7% to 4.5% for ceftazidime/avibactam, imipenem/relebactam and meropenem/vaborbactam. At least one of an AmpC, an ESBL or porin alterations were observed in most non-CPE isolates.

Conclusions: In Canada, ertapenem-resistant Enterobacterales remain uncommon (0.7%). CPE isolates demonstrated higher percent resistant values for multiple antimicrobial classes and harboured more antimicrobial resistance genes than non-CPE isolates. Continued monitoring for ertapenem-resistant Enterobacterales, particularly CPE, is important given their associated multidrug resistance to both established and newer agents.

Introduction

Carbapenem-resistant Enterobacterales (CRE) continue to top the WHO's bacterial pathogen priority list, which ranks human pathogens based upon their importance and need for research and development of new antibiotics.¹ Similarly, the CDC's 2021–22 threats report classified CRE as an urgent threat associated with an increasing trend in reported infections over time in the USA (www.cdc.gov/antimicrobial-resistance/data-research/threats).

CRE have been well documented from various infection sources, including bloodstream, respiratory tract, intra-abdominal and urinary tract infections.² Patients who are infected by carbapenem-resistant pathogens have an increased likelihood of morbidity and mortality compared with those infected by carbapenem-susceptible pathogens.² Such infections impose a significant burden globally, with limited treatment options, due mainly to their associated high antimicrobial resistance rates.^{3,4} The mechanism of carbapenem resistance in CRE isolates can vary by Enterobacterales species and may also differ by

geographic location. CRE are frequently categorized into carbapenemase-producing Enterobacterales (CPE) and non-CPE isolates.⁵ Non-CPE isolates arise due to alterations in porin channels and possibly efflux pumps resulting in low-level carbapenem resistance.^{5–7} Non-CPE isolates are often associated with combinations of an ESBL or AmpC β -lactamase and porin loss.^{6–8} Ertapenem was the most affected carbapenem when plasmids coding AmpC or ESBLs were introduced into a porin-deficient strain of *Klebsiella pneumoniae*.⁹ CPE isolates are associated with high-level resistance to carbapenems (MIC $\geq$ 32 mg/L).⁵ Carbapenemases may be chromosomal or, more frequently and of greater concern, carried on highly transmissible plasmids.^{10,11} Carbapenemases can be classified into three of the four Ambler classification system categories (A, B and D) based on their central catalytic domain and substrate preference.^{11,12}

Herein we describe the phenotypic and genotypic characteristics of all ertapenem-resistant Enterobacterales isolates collected between 2007 and 2023 in Canada by the CANWARD study, and highlight the occurrence of carbapenemase-associated ertapenem resistance.

Materials and methods

Bacterial isolates

The bacterial isolates tested in this study were collected as part of the CANWARD surveillance study from January 2007 to December 2023. CANWARD is an ongoing Canadian national multicentre surveillance study that monitors the *in vitro* activities of antimicrobial agents tested against bacterial pathogens isolated by clinical laboratories in tertiary care hospitals. Tertiary care hospitals in 8 of the 10 Canadian provinces participated in the CANWARD study. Each isolate accepted into CANWARD was deemed clinically significant by protocols in place at the submitting laboratory. Isolates are collected consecutively, one per patient, per site of infection, from both inpatients and outpatients attending emergency rooms, hospital clinics, medical/surgical wards and ICUs each year. Each participating laboratory was asked to submit annual quotas of respiratory, wound, urine and bloodstream isolates. All isolates were shipped to the CANWARD coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), where their identities are confirmed by colonial appearance, spot testing and/or MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA). Isolates were then stored in skimmed milk and stored at -80°C until antimicrobial susceptibility testing was performed.

CANWARD receives annual approval by the University of Manitoba Research Ethics Board (H2009:059). Geographical analysis of CANWARD isolates groups participating laboratories into three regions: western Canada, comprising laboratories in British Columbia, Alberta, Saskatchewan and Manitoba; central Canada, comprising laboratories in Ontario and Quebec; and eastern Canada, comprising laboratories in New Brunswick and Nova Scotia.

Antimicrobial susceptibility testing

Broth microdilution antimicrobial susceptibility testing was performed following the CLSI standard method.¹³ Custom-designed 96-well microtitre panels were prepared in-house, as previously described.¹³ MICs were interpreted using CLSI interpretative breakpoints when available.¹⁴ FDA MIC interpretative breakpoints were used for ceftobiprole (susceptible $\leq$ 0.25 mg/L; intermediate = 0.5 mg/L; resistant $\geq$ 1 mg/L) and tigecycline (susceptible $\leq$ 2 mg/L; intermediate = 4 mg/L; resistant $\geq$ 8 mg/L) (<https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria>).

Genomic characterization of ertapenem-resistant Enterobacterales

Genomic DNA was extracted using QIAGEN DNeasy 96 Blood and Tissue Kits (Hilden, Germany). Short-read libraries were created with TruSeq Nano DNA HT sample preparation kits (Illumina, San Diego, CA, USA). Isolates were sequenced using an Illumina NextSeq™ platform. Paired-end, 151 bp indexed reads were generated using the Illumina NextSeq™ platform. FastQC v0.11.9 (<https://github.com/s-andrews/FastQC>) was used to assess quality control metrics for Illumina reads. Contigs were assembled using Shovill v1.1.0 (<https://github.com/tseemann/shovill>). Bacterial species were confirmed using the RefSeq Masher Matches tool (https://github.com/phac-nml/refseq_masher). staramr v0.9.1¹⁵ was used to detect antimicrobial resistance genes using the ResFinder database v2022-05-24¹⁶ and ST using the MLST database v2.23.0.¹⁷ Outer membrane protein (OMP) presence and mutations were determined using Blastn+ v2.15.0 to extract the *omp* gene nucleotide sequences from the query genomes using a minimum length cut-off of 100 bases and minimum nucleotide identity of 92%. Nucleotide hits were translated to amino acid using gcode 11 with SeqKit v2.8.2 (<https://doi.org/10.1371/journal.pone.0163962>) and screened for stop codons. The following OMPs and genome accessions were used as references: *Enterobacter cloacae* CP001918.1 *ompC*; *E. cloacae* CP001918.1 *ompF*; *Enterobacter kobei* CP017181.1 *ompC*; *E. kobei* CP017181.1 *ompF*; *Escherichia coli* NC_000913.3 *ompF*; *E. coli* NC_000913.3 *ompC*; *Klebsiella aerogenes* KT894105.1 *omp35*; *K. aerogenes* KT894106.1 *omp36*; *K. pneumoniae* AP006725.1 *ompK35*; *K. pneumoniae* AP006725.1 *ompK36*; *K. pneumoniae* AJ011502.1 *ompK37*; *Serratia marcescens* CP041233.1 *ompC*; *S. marcescens* L24960.1 *ompC*; and *S. marcescens* U81967.1 *ompF*.

Statistical analysis

Statistical significance was calculated by the Fisher's exact test (<https://www.graphpad.com>). Statistical significance was defined as a *P* value of $\leq$ 0.05.

Results

A total of 19 642 Enterobacterales were collected by the CANWARD study between 2007 and 2023, of which 134 (0.7%) were ertapenem resistant. Ertapenem-resistant Enterobacterales were more commonly isolated from males (63.4%) than females (36.6%) ($P < 0.001$) (Table 1). Isolates were more common in patients aged 19–64 years (45.5%) and > 64 years (46.3%) than in younger patients. Ertapenem-resistant Enterobacterales were more commonly isolated from respiratory specimens (46.3%) than from blood (31.3%), wound specimens (12.7%) and urine (9.7%), and from patients in ICUs (40.3%) than those on medical wards (29.1%), in the emergency room (11.9%), on surgical wards (11.9%) and clinics and doctor's offices (outpatients).

Among the 134 ertapenem-resistant Enterobacterales isolates, 24 (17.9% of ertapenem-resistant Enterobacterales and 0.1% of all Enterobacterales) harboured a known carbapenemase gene (Figure 1). Carbapenemase genes were reported relatively consistently throughout the study (from 2009 to 2023; no CPE isolates were identified in 2007, 2008, 2013 or 2016) with a frequency of 0–5 isolates per year (annual average of 1.4 isolates per year). Among the 134 ertapenem-resistant Enterobacterales isolates, there was no significant difference in the distribution of CPE and non-CPE based upon patient biological sex, patient age or patient location or specimen source (Table 1). Non-CPE isolates were mainly from central Canada (47.8%) and western Canada

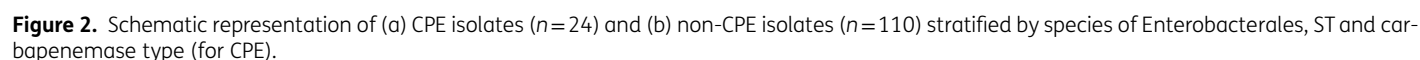
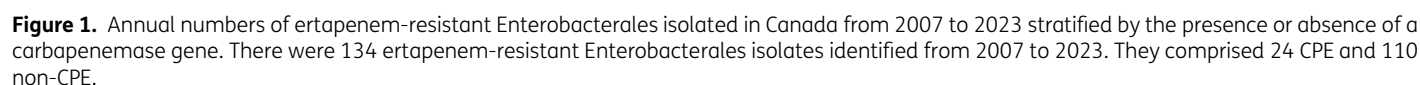
Table 1. Demographic parameters associated with ertapenem-resistant Enterobacterales in Canada 2007–23

Demographic parameter	Isolate type, n (%) ^a			Fisher's test P value (CPE versus non-CPE)
	Ertapenem resistant	CPE	non-CPE	
Patient biological sex ^b				
Male	85 (63.4)	15 (62.5)	70 (63.6)	1
Female	49 (36.6)	9 (37.5)	40 (36.4)	1
Patient age group (years)				
1–18	11 (8.2)	2 (8.3)	9 (8.2)	1
19–64	61 (45.5)	9 (37.5)	52 (47.3)	0.5
>64	62 (46.3)	13 (54.2)	49 (44.5)	0.5
Isolate specimen source				
Respiratory	62 (46.3)	11 (45.8)	51 (46.4)	1
Blood	42 (31.3)	8 (33.3)	34 (30.9)	0.8
Wound	17 (12.7)	4 (16.7)	13 (11.8)	0.4
Urine	13 (9.7)	1 (4.2)	12 (10.9)	0.5
Patient location/ward type				
ICU	54 (40.3)	7 (29.2)	47 (42.7)	0.2
Medical ward	39 (29.1)	10 (41.7)	29 (26.4)	0.1
Emergency room	16 (11.9)	2 (8.3)	14 (12.7)	0.7
Surgical ward	16 (11.9)	4 (16.7)	12 (10.9)	0.5
Clinic/doctor's office	9 (6.7)	1 (4.2)	8 (7.3)	1

^aThere were 134 ertapenem-resistant Enterobacterales isolates comprising 24 CPE and 110 non-CPE.
^bMale versus female, *P* < 0.001.

(38.9%), while CPE isolates were primarily from central Canada (87.5%) compared with 12.5% from western Canada (data not shown). The distribution of carbapenemase genes among CPE isolates, stratified by bacterial species and ST, is shown in Figure 2(a). In total, 28 carbapenemase genes were identified in the 24 CPE isolates: 15 KPC (10 KPC-3, 5 KPC-2), three OXA-48-like (OXA-181, OXA-301 and OXA-48), one NDM (NDM-5) and one SME (SME-3). Interestingly, OXA-301 has only been described in *Acinetobacter* spp.¹⁸ Additionally, four isolates harboured both an NDM and an OXA-48-like carbapenemase (two isolates with NDM-1 and OXA-232; one isolate with NDM-5 and OXA-232; one isolate with NDM-5 and OXA-181). Carbapenemase genes were mainly identified in *K. pneumoniae* (*n* = 13; 54.2%) followed by *E. coli* (*n* = 5; 20.8%). Due to the low number of CPE isolates, assessing trends in STs was not possible; however, we did note that *K. pneumoniae* ST834 (*n* = 4) were all from the same Canadian province and that 42% of all CPE isolates were associated with known or emerging high-risk clones, namely *K. pneumoniae* ST258, ST101 and ST16, and *E. coli* ST131, ST405 and ST361.

Among non-β-lactam agents tested, >50% of CPE isolates were resistant to ciprofloxacin (79.2%), nitrofurantoin (54.2%), tobramycin (58.3%) and trimethoprim/sulfamethoxazole (70.8%) (Table 2). Resistance rates for newer β-lactam/β-lactamase inhibitor combination agents were 20.8% for ceftazidime/avibactam and meropenem/vaborbactam, and 25.0% for imipenem/relebactam. Resistance to these newer agents was associated with the presence of NDM genes. Tigecycline was the most active agent *in vitro* against CPE isolates (4.2% resistant); 12.5% of isolates were tigecycline intermediate. We also observed that CPE isolates contained 2–22 AMR genes with an average of 12 AMR genes per isolate. The most common AMR genes targeted aminoglycosides, β-lactams, sulphonamides and trimethoprim; multiple different copies of genes with activity against each of these classes were observed (Figure 3). When examining total plasmid content in CPE isolates we found that replicon types FIB, FII and Col-like were most commonly observed (Figure S1, available as [Supplementary data](#) at JAC Online). We observed between 1 and 11 replicons per isolate with an average of 5.6 replicons per isolate. Non-CPE isolates (*n* = 110) were most commonly *Enterobacter* spp. [51.8% (*n* = 57), of which 77% (44/57) were *E. hormaechei*], followed by *E. coli* (15.5%), *K. pneumoniae* (10.9%), *K. aerogenes* (8.1%), *S. marcescens* (6.4%) and other species (7.2%) (Figure 2b). STs among non-CPE species were diverse, with the exception of *E. coli* ST131 and *E. cloacae* STs ST108, ST50 and ST133, which were observed for ≥5 isolates. Among non-β-lactam agents tested, percent resistant values for non-CPE isolates were elevated but generally slightly lower than for CPE isolates (Table 2). Percent resistant values were <5% for meropenem/vaborbactam (2.7%), ceftazidime/avibactam (4.5%) and imipenem/relebactam (4.5%). Although all isolates were ertapenem resistant, cross-resistance to meropenem (13.6%) and imipenem (18.2%) was infrequent. We determined that non-CPE isolates contained between 1 and 24 AMR genes with an average of 6.5 AMR genes per isolate (Figure S2). Similar to CPE, the most common AMR genes conferred resistance to aminoglycosides, β-lactams and sulphonamides, as well as fosfomycin; again, multiple different copies of genes active against agents in each of these antimicrobial classes were identified. Outer membrane porin gene sequences were examined for non-CPE isolates of *E. cloacae*, *E. coli*, *K. aerogenes*, *K. pneumoniae* and *S. marcescens* (*n* = 106). The absence of a referenced OMP or a premature stop codon (observed >10 amino acids into OMPs) were considered to be indicators of OMP loss of function. Thirty-one percent of isolates (33/106) showed OMP potential loss of functionality; 16 of those also contained either an ESBL or mobile AmpC. Of the remaining 73 isolates, no major OMP mutation was observed; however, 21 (28.8%) isolates harboured an ESBL or mobile AmpC. Among these ESBL/mobile AmpC producers (*n* = 37), CTX-M-15 (*n* = 17; 45.9%) and SHV-12 (*n* = 8; 21.6%) were observed the most frequently. Of all 110 non-CPE isolates, overall, 56 (50.1%) did not contain a known ESBL, mobile AmpC or OMP mutation (for species mentioned above). Of those, 47 isolates harboured chromosomal AmpCs, which when derepressed can result in elevated ertapenem MICs; mainly *Enterobacter* spp. (*n* = 40) and *S. marcescens* (*n* = 7). The remaining nine isolates (seven *K. aerogenes* and two *Proteus mirabilis*) did not have β-lactamase genes detected (Table S1).



CRE are currently uncommon in Canada (~1%). The current study reports an occurrence of 0.7% CRE among all collected Enterobacterales from 2007 to 2023. Data from the ECDC showed 0.2% of *E. coli* and 11.7% of *K. pneumoniae* as carbapenem resistant in 2021.¹⁹ A recent report by Sabour *et al.*²⁰ indicated the incidence of CRE harbouring a carbapenemase in the USA was 35%. This is twice as high than we observed in the

current study our report (17.9%). We observed distinct differences in the genera associated with CPE and non-CPE (Figure 2). Herein we describe *K. pneumoniae* as the dominant CPE species and *E. cloacae* as the most frequent non-CPE species, which was also observed by Sabour *et al.*²⁰ Although we did not observe notable differences in the demographic parameters associated with CPE and non-CPE, we did observe significantly more males than females harbouring CRE; this observation has also been recently reported for CRE in Europe.²¹ In addition,

Table 2. Antimicrobial susceptibility profiles of ertapenem-resistant isolates stratified by the presence or absence of a carbapenemase gene^a

Isolate genotype Antimicrobial agent	No. of isolates tested	mg/L		MIC interpretation			
		MIC ₅₀	MIC ₉₀	% Susceptible	% SDD	% Intermediate	% Resistant
CPE							
Amikacin	19	4	>64	63.2	–	5.3	31.6
Amoxicillin/clavulanate	24	>32	>32	0	–	0	100
Aztreonam	11	64	>64	9.1	–	0	90.9
Cefazolin	24	>128	>128	0	–	0	100
Cefepime	24	64	>64	8.3	8.3	–	83.3
Cefoxitin	24	>32	>32	8.3	–	16.6	75.0
Ceftazidime/avibactam	24	1	>16	79.2	–	0	20.8
Ceftazidime	24	>32	>32	4.2	–	0	95.8
Ceftobiprole ^b	24	>32	>32	0	–	8.6	91.7
Ceftolozane/tazobactam	24	64	>64	12.5	–	0	87.5
Ceftriaxone	24	>64	>64	4.2	–	0	95.8
Ciprofloxacin	24	>16	>16	12.5	–	8.3	79.2
Colistin	24	0.5	16	–	–	75.0	25.0
Ertapenem	24	16	>32	0	–	0	100
Gentamicin	24	2	>32	54.2	–	0	45.8
Imipenem/relebactam	24	0.5	16	66.7	–	8.6	25.0
Imipenem	24	8	>32	4.2	–	4.2	91.7
Meropenem/vaborbactam	24	0.06	>32	79.2	–	0	20.8
Meropenem	24	8	>32	20.8	–	8.6	70.8
Nitrofurantoin	24	128	>512	29.2	–	16.7	54.2
Tigecycline ^c	24	1	4	83.3	–	12.5	4.2
Tobramycin	24	16	>64	37.5	–	4.2	58.3
Trimethoprim/sulfamethoxazole	24	>8	>8	29.2	–	0	70.8
Non-CPE							
Amikacin	93	2	8	83.9	–	6.5	9.7
Amoxicillin/clavulanate	110	>32	>32	4.5	–	3.6	91.8
Aztreonam	54	64	>64	9.3	–	0	90.7
Cefazolin	110	>128	>128	0.9	–	0	99.1
Cefepime	110	4	>64	35.5	31.8	–	32.7
Cefoxitin	110	>32	>32	1.8	–	2.7	95.5
Ceftazidime/avibactam	110	1	8	95.5	–	0	4.5
Ceftazidime	110	>32	>32	14.5	–	4.5	80.9
Ceftobiprole ^b	110	16	>32	9.1	–	8.2	82.7
Ceftolozane/tazobactam	110	16	>64	22.7	–	9.1	68.2
Ceftriaxone	110	>64	>64	3.6	–	1.8	94.5
Ciprofloxacin	110	0.5	>16	47.3	–	3.6	49.1
Colistin	110	0.5	>16	–	–	83.6	16.4
Doxycycline	110	16	32	33.6	–	10.0	56.4
Ertapenem	110	4	32	0	–	0	100
Gentamicin	110	≤0.5	>32	80.0	–	1.8	18.2
Imipenem/relebactam	110	0.25	2	89.1	–	6.4	4.5
Imipenem	110	1	4	66.4	–	15.5	18.2
Meropenem/vaborbactam	110	0.12	2	95.5	–	1.8	2.7
Meropenem	110	0.5	4	80.0	–	6.4	13.6
Nitrofurantoin	110	128	>512	25.5	–	16.4	58.2
Tigecycline ^c	110	1	4	68.2	–	23.6	8.2
Tobramycin	109	1	32	66.1	–	5.5	28.4
Trimethoprim/sulfamethoxazole	110	1	>8	57.3	–	0	42.7

SDD, susceptible, dose-dependent.

^aThere were 134 ertapenem-resistant Enterobacterales isolates comprising 24 CPE and 110 non-CPE.^bCeftobiprole MICs were interpreted using FDA breakpoints.^cTigecycline MICs were interpreted using FDA breakpoints.

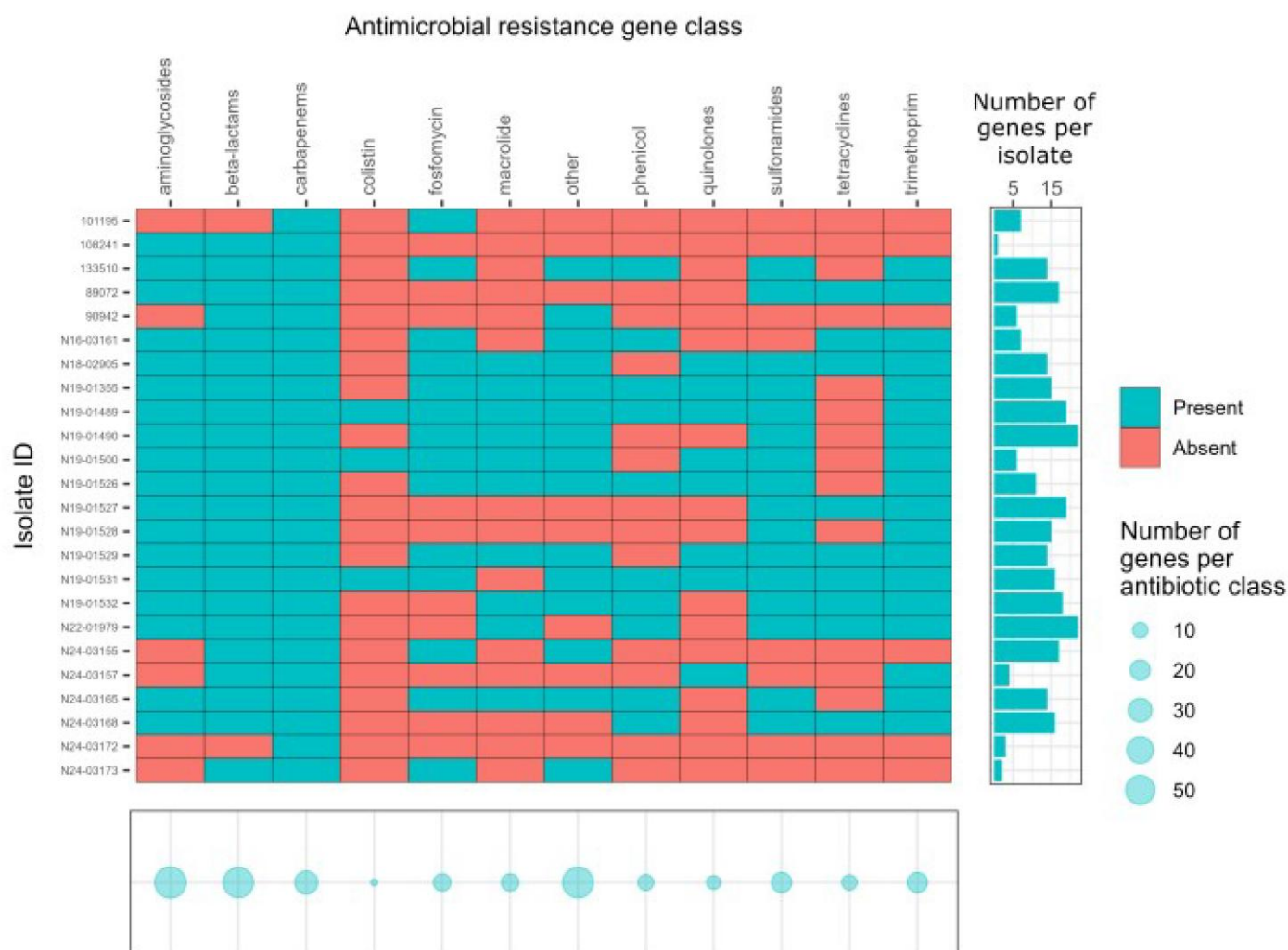


Figure 3. Schematic representation of the distribution of AMR resistance gene category classes for CPE isolates ($n=24$). Multiple genes per category may be present; however, duplicated genes per isolate were not counted.

both CPE and non-CPE CRE were most often isolated from the respiratory tract (Table 1).

The global dominance of the carbapenemase KPC has been well described as a result of clonal expansion and horizontal gene transfer, mainly within *K. pneumoniae*.^{22,23} KPC is endemic in many countries and global regions, including the USA, Middle East countries, Greece, Italy and China.²² Our data show the dominance of KPC in Canada, which is consistent with other Canadian reports.^{24,25} Although the distribution of ertapenem-resistant Enterobacterales was relatively equal across western and central Canadian regions, it is of note that 87.5% of CPE were from the central region. This is perhaps attributed to differences in carbapenemase screening and infection control practices.

As expected, non-CPE isolates harboured fewer AMR genes and resistance rates were generally lower than for CPE isolates, including the newer β -lactam/ β -lactamase inhibitor combination agents ceftazidime/avibactam, imipenem/relebactam and meropenem/vaborbactam, where resistance was below 5% for non-CPE isolates. Consistent with earlier literature,^{26–28} resistance to β -lactam/

β -lactamase inhibitor combination agents in CPE was attributed to the presence of an MBL (NDM) and/or a class D oxacillinase (OXA-48-like; note, ceftazidime/avibactam inhibits OXA-48-like), as all isolates carrying only a class A (KPC) were susceptible.

Numerous publications have identified an association between carbapenem resistance in numerous species of Enterobacterales and the presence of a combination of ESBL, AmpC and porin loss.^{6–8,29} One study specifically assessed *E. cloacae* and the contribution of ESBLs, AmpC overproduction, active efflux and OMP expression as contributors to ertapenem-resistant Enterobacterales.³⁰ Over 50% of our non-CPE isolates were *E. cloacae* and were shown to have a combination of β -lactamases and possible porin deficiencies.

Overall, we have observed that the prevalence of ertapenem-resistant Enterobacterales in Canada from 2007 to 2023 was low (0.7%), with a CPE genotype responsible for 17.9% of ertapenem-resistant Enterobacterales isolates and a non-CPE genotype accounting for the remaining 82.1% of isolates. *E. coli* and *K. pneumoniae* are the drivers of CPE in Canada, while *E. cloacae*

was the most common species associated with non-CPE isolates. Given the global landscape of CPE and its recognition as a top pathogen threat, we must continue to monitor CRE, including both CPE and non-CPE, in Canada.

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Transparency declarations

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Supplementary data

Figures S1 and S2 and Table S1 are available as [Supplementary data](#) at JAC Online.

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