

# Characterization of carbapenem-resistant *Pseudomonas aeruginosa* in Canadian hospitals: 6 years of the CANWARD study (2018–23)

Melissa McCracken<sup>1\*</sup>, Andrew Walkty<sup>2,3</sup>, Heather J. Adam <sup>2,3</sup>, Melanie Baxter<sup>2</sup>, Joseph Blondeau<sup>4</sup>, Philippe Lagacé-Wiens <sup>2,3</sup>, James A. Karlowsky<sup>2,3</sup>, George R. Golding<sup>1,2</sup> and George G. Zhanel<sup>2</sup>

<sup>1</sup>National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada R3E 3M4; <sup>2</sup>Department of Medical Microbiology/Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Room 543-745 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0J9; <sup>3</sup>Clinical Microbiology, Diagnostic Services, Shared Health, MS673-820 Sherbrook Street, Winnipeg, Manitoba, Canada R3A 1R9; <sup>4</sup>Department of Clinical Microbiology, Royal University Hospital, Saskatoon, Saskatchewan, Canada S7N 5E5

\*Corresponding author. E-mail: melissa.mccracken@phac-aspc.gc.ca

**Objectives:** Antimicrobial resistance in *Pseudomonas aeruginosa* is of increasing concern in Canada, leading to limited treatment options and poor clinical outcomes. Herein we characterized carbapenem-resistant *P. aeruginosa* identified through the Canadian national surveillance program CANWARD.

**Methods:** Antimicrobial susceptibility for 1725 *P. aeruginosa* isolates was assessed using broth microdilution and 2024 CLSI breakpoints. WGS of carbapenem-resistant isolates was used to identify STs, resistance and virulence markers. Genetic relatedness was further assessed using cgMLST for select STs.

**Results:** From 2018 to 2023, CANWARD collected 1725 *P. aeruginosa* isolates, of which 371 (21.5%) were carbapenem-resistant. The majority of carbapenem-resistant *P. aeruginosa* were isolated from respiratory specimens of male patients aged 18–65 years living in central Canada. Only 0.8% ( $n=3$ ) of the carbapenem-resistant isolates harboured a carbapenemase gene. WGS identified mutations associated with OprD dysfunction, MexAB–OprM efflux and AmpC overexpression in 73.6%, 1.1% and 4.9% of isolates, respectively. Most isolates (98.1%) harboured at least one of the following class D  $\beta$ -lactamase genes: OXA-2, OXA-5, OXA-10 or OXA-50-like subfamily. Wide genetic diversity was observed with 151 different STs identified. The most common STs were ST17 (4.6%), ST27 (4.6%) and high-risk clones ST235 (4.3%), ST244 (3.5%), ST253 (6.4%) and ST357 (2.7%). cgMLST clusters were identified amongst 34.9% of the high-risk clones, suggesting clonal dissemination.

**Conclusions:** Currently, >20% of clinical isolates of *P. aeruginosa* in Canada are carbapenem-resistant. Genetic evidence indicates that clonal dissemination of high-risk clones is occurring in Canada. High-risk clones are virulent and often MDR. Continued surveillance of *P. aeruginosa* is important.

## Introduction

As one of the six ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter* spp.), *Pseudomonas aeruginosa* is a major cause of nosocomial infections and can demonstrate MDR and XDR phenotypes.<sup>1–3</sup> Resistance to carbapenems is of particular concern, has been reported worldwide and is often associated with limited treatment options for infected patients.<sup>4</sup> The WHO has classified carbapenem-resistant *P. aeruginosa* as a high priority pathogen because it frequently causes difficult-to-treat infections.<sup>5</sup>

There are multiple mechanisms by which *P. aeruginosa* can exhibit resistance to carbapenems. Resistance can arise from

mutations that cause the overexpression of MexAB–OprM efflux pumps,<sup>6</sup> alterations of outer membrane porins<sup>7</sup> or overexpression of chromosomal AmpC.<sup>8</sup> Resistance can also be in the form of carbapenemase genes acquired through horizontal transfer of mobile genetic elements.<sup>9</sup> Lastly, the presence of class D  $\beta$ -lactamases have also been shown to contribute to carbapenem resistance.<sup>10,11</sup> To add to this complexity, the precise genetic cause of resistance is often difficult to determine as the cause is often multifactorial.

Carbapenem-resistant *P. aeruginosa* have been associated with several common high-risk clones, including, ST111, ST175, ST233, ST235, ST244, ST253, ST277, ST298, ST308, ST357 and ST654.<sup>9,12,13</sup> High-risk clones are distributed globally; they are frequently MDR, described as highly pathogenic and can cause severe

infections.<sup>9,14</sup> Thus far in Canada, the incidence of high-risk clones has been very low.<sup>15</sup> We previously found wide genetic diversity among the carbapenem-resistant *P. aeruginosa* from 2007 to 2016, with only ST357 as the high-risk clone that was represented by multiple isolates.<sup>15</sup> The global success of high-risk clones in conjunction with increased resistance highlights the importance of continued surveillance in Canada. In this study, carbapenem-resistant *P. aeruginosa* were examined from 2018 to 2023, as part of the Canadian national surveillance program, CANWARD.

## Materials and methods

### Bacterial isolates

From January 2018 to December 2023, tertiary-care medical centre sites in 8 of the 10 Canadian provinces submitted bacterial pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards and ICUs to a central clinical microbiology laboratory (Health Science Centre, Winnipeg, Canada). Sites were requested to submit specific numbers of clinically significant isolates (consecutive, one per patient per infection site per year) as defined by their local hospital criteria, from respiratory, urine, wound and bloodstream infections. Centre participation varied slightly from year-to-year as did the numbers of isolates requested by infection source.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using custom-designed 96-well panels prepared in house according to the standard CLSI broth microdilution method.<sup>16</sup> All isolates were tested against 13 antimicrobials: amikacin, aztreonam, cefepime, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, ciprofloxacin, colistin, imipenem, imipenem-relebactam, meropenem, meropenem-vaborbactam, piperacillin-tazobactam and tobramycin. MICs were interpreted according to CLSI breakpoints,<sup>16</sup> with the exception of meropenem-vaborbactam, which was interpreted using EUCAST breakpoints because the CLSI does not publish breakpoints for this organism/antimicrobial agent combination.<sup>17</sup> Carbapenem-resistant *P. aeruginosa* were defined as resistant to meropenem, imipenem or both. CLSI-resistant breakpoints for meropenem and imipenem was  $\geq 8$  mg/L.<sup>16</sup>

### Whole-genome sequencing and assembly

All carbapenem-resistant *P. aeruginosa* isolates from 2018 to 2023 were sequenced with Illumina platforms. Genomic DNA was extracted using the DNeasy 96 Blood & Tissue kit (QIAGEN, Hilden, Germany) for Illumina from 2018 to 2021, and the Mag-Bind® Universal Pathogen DNA 96 kit (Omega Bio-Tek, Norcross, Georgia, USA) for Illumina from 2022 to 2023. Short-read libraries were created with TruSeq Nano DNA HT sample preparation kits (Illumina, San Diego, CA, USA) from 2017 to 2021 or the NexteraXT library preparation kits (Illumina) from 2022 to 2023. 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 and RefSeq Masher confirmed organism identity. Contigs were assembled using Shovill v1.1.0 on an internal Galaxy platform (<https://github.com/tseemann/shovill>). All WGS data can be found on NCBI under BioProject PRJNA1225611.

### Analysis of WGS data

Staramr v0.10.0<sup>18</sup> was used to detect antimicrobial resistance genes using the ResFinder database v2022-05-24.<sup>19</sup> ResFinder, screened for the following types of carbapenemase genes, GES, KPC, NDM, IMP, VIM, OXA-48. Sequence type using the MLST database v2.23.0 was also

obtained from staramr.<sup>20</sup> ABRicate v1.0.1<sup>21</sup> was used to detect appropriate virulence genes using the virulence factor database (VFDB).<sup>22</sup> Only antimicrobial resistance and virulence genes with 100% coverage and >90% identity were considered for analysis. Blastn+v2.15.0 was used to extract the *oprD* gene nucleotide sequences from the query genomes using a minimum length cut-off of 100 bases and minimum nucleotide identity of 85%. 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 *oprD* gene from *P. aeruginosa* PAO1 (CP053028.1) was used as reference. AMRFinderPlus version 3.11.11 with database version 2024-12-18.1<sup>23</sup> was used to identify: point mutations in *oprD* that may lead to a decrease in outer membrane permeability;<sup>7</sup> mutations in *mexR*, *nalD* and *nalC* that affect the expression of MexAB-OprM efflux pump<sup>6</sup> and mutations in *ampR*, *ampD* and *dacB* that affect the expression of chromosomal cephalosporinase AmpC.<sup>8</sup> A previously published list of specific mutations in the aforementioned genes, which have been associated with carbapenem resistance, was used to screen our data.<sup>13</sup> Mutations identified from our sample set are listed in Table S1 (available as [Supplementary data](#) at JAC Online). SeqSphere+ software (Ridom, Muenster v9.0.10) was used to assess genetic relatedness using the *P. aeruginosa* cgMLST scheme comprised of 3867 alleles.<sup>24</sup> Data were visualized with minimum spanning trees created in SeqSphere or using Microreact.<sup>25</sup>

## Results

### Characteristics of all carbapenem-resistant *P. aeruginosa*

The CANWARD study collected 1725 *P. aeruginosa* isolates from 2018 to 2023; 371 (21.5%) of these isolates were meropenem-resistant (carbapenem-resistant). The annual proportions of *P. aeruginosa* isolates resistant to meropenem were: 2018 (23.7%,  $n=67$ ), 2019 (23.4%,  $n=81$ ), 2020 (20.4%,  $n=58$ ), 2021 (29.4%,  $n=89$ ), 2022 (13.4%,  $n=39$ ) and 2023 (17.0%,  $n=37$ ). The decrease in the proportion of carbapenem-resistant *P. aeruginosa* from 2021 (29.7%) to 2022 (13.4%) was statistically significant ( $P=0.0001$ ; chi-square test).

Carbapenem-resistant *P. aeruginosa* isolates were associated with patient ages ranging from 1 to 94 years with a median age of 58 years (Table 1). Males contributed 64.4% ( $n=239$ ) of the isolates. The majority of isolates were obtained from respiratory specimens (82.5%;  $n=306$ ), followed by bloodstream (7.8%;  $n=29$ ), wound (7.8%;  $n=29$ ) and urine (1.9%;  $n=7$ ) specimens. Most isolates were obtained from patients in an ICU (35.8%,  $n=133$ ) or on a medical ward (48.2%,  $n=179$ ). The majority of isolates were submitted by hospitals in central Canada (70.4%,  $n=261$ ), followed by western Canada (24.8%,  $n=92$ ) and eastern Canada (4.9%,  $n=18$ ).

Amikacin (84.1%) and ceftolozane-tazobactam (78.4%) demonstrated the highest percent susceptible values of all agents tested (Table 2). Percent susceptible values for newer  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combination agents were 63.3% for imipenem-relebactam, 60.1% for ceftazidime-avibactam and 39.1% for meropenem-vaborbactam; 2.4% of isolates were colistin-resistant.<sup>16</sup> Thirty-five (9.4%) of the 371 isolates of carbapenem-resistant *P. aeruginosa* were XDR (resistant to ciprofloxacin, piperacillin-tazobactam, ceftazidime, meropenem and tobramycin) as defined by published guidelines.<sup>26</sup>

All carbapenem-resistant *P. aeruginosa* were screened for potential carbapenem resistance markers, including  $\beta$ -lactamase

**Table 1.** Patient demographic information associated with carbapenem-resistant *P. aeruginosa* isolated in Canadian hospitals in 2018–23

| Demographic parameter    | Number (%) of isolates per year |           |           |           |           |           |
|--------------------------|---------------------------------|-----------|-----------|-----------|-----------|-----------|
|                          | 2018                            | 2019      | 2020      | 2021      | 2022      | 2023      |
| Biological sex           |                                 |           |           |           |           |           |
| female                   | 26 (38.8)                       | 36 (44.4) | 21 (36.2) | 27 (30.3) | 10 (25.6) | 12 (32.4) |
| male                     | 41 (61.2)                       | 45 (55.6) | 37 (63.8) | 62 (69.7) | 29 (74.4) | 25 (67.6) |
| Hospital ward type       |                                 |           |           |           |           |           |
| clinic/office            | 11 (16.4)                       | 5 (6.2)   | 2 (3.4)   | 2 (2.2)   | 3 (7.7)   | 3 (8.1)   |
| emergency room           | 3 (4.5)                         | 3 (3.7)   | 1 (1.7)   | 2 (2.2)   | 2 (5.1)   | 0 (0)     |
| ICU                      | 22 (32.8)                       | 23 (28.4) | 16 (27.6) | 44 (49.4) | 12 (30.8) | 16 (43.2) |
| medicine                 | 28 (41.8)                       | 45 (55.6) | 34 (58.6) | 37 (41.6) | 19 (48.7) | 16 (43.2) |
| surgery                  | 3 (4.5)                         | 5 (6.2)   | 5 (8.6)   | 4 (4.5)   | 3 (7.7)   | 2 (5.4)   |
| Specimen type            |                                 |           |           |           |           |           |
| blood                    | 3 (4.5)                         | 6 (7.4)   | 6 (10.3)  | 3 (3.4)   | 6 (15.4)  | 5 (13.5)  |
| urine                    | 1 (1.5)                         | 2 (2.5)   | 1 (1.7)   | 1 (1.1)   | 1 (2.6)   | 1 (2.7)   |
| wound                    | 3 (4.5)                         | 5 (6.2)   | 3 (5.2)   | 10 (11.2) | 4 (10.3)  | 4 (10.8)  |
| respiratory              | 60 (89.6)                       | 68 (84.0) | 48 (82.8) | 75 (84.3) | 28 (71.8) | 27 (73.0) |
| Geographic region        |                                 |           |           |           |           |           |
| central                  | 45 (67.2)                       | 61 (75.3) | 42 (72.4) | 61 (68.5) | 26 (66.7) | 26 (70.3) |
| east                     | 4 (6.0)                         | 2 (2.5)   | 2 (3.4)   | 4 (4.5)   | 2 (5.1)   | 4 (10.8)  |
| west                     | 18 (26.9)                       | 18 (22.2) | 14 (24.1) | 24 (27.0) | 11 (28.2) | 7 (18.9)  |
| Patient age group, years |                                 |           |           |           |           |           |
| ≤17                      | 1 (1.5)                         | 1 (1.2)   | 2 (3.4)   | 1 (1.1)   | 1 (2.6)   | 1 (2.7)   |
| ≥66                      | 20 (29.9)                       | 21 (25.9) | 22 (37.9) | 28 (31.5) | 14 (35.9) | 16 (43.2) |
| 18–65                    | 46 (68.7)                       | 59 (72.8) | 34 (58.6) | 60 (67.4) | 24 (61.5) | 20 (54.1) |
| Total number of isolates | 67                              | 81        | 58        | 89        | 39        | 37        |

genes and mutations associated with porin inactivation, efflux pump overproduction and AmpC alteration (Figure 1).<sup>6,8,13</sup> Carbapenemase genes were only identified in 0.8% of isolates; two isolates carried *bla*<sub>IMP-26</sub> and one isolate carried *bla*<sub>GES-5</sub>. All isolates were also screened for class D β-lactamase (oxacillinase) genes that are associated with reduced carbapenem activity in *P. aeruginosa*. OXA genes belonging to the intrinsic *bla*<sub>OXA-50</sub>-like family were detected in 98.1% of isolates. Only one *bla*<sub>OXA-50</sub>-like variant was found per isolate, with the following distribution, *bla*<sub>OXA-50</sub> (32.6%; *n*=121), *bla*<sub>OXA-395</sub> (11.1%; *n*=41), *bla*<sub>OXA-396</sub> (5.7%; *n*=21), *bla*<sub>OXA-486</sub> (7.8%; *n*=29), *bla*<sub>OXA-488</sub> (19.4%; *n*=72) and *bla*<sub>OXA-494</sub> (21.6%; *n*=80). The remaining 1.9% (*n*=7) carbapenem-resistant *P. aeruginosa* did not match any known variant of the *bla*<sub>OXA-50</sub>-like family, but showed the closest similarity to *bla*<sub>OXA-395</sub>, suggesting the presence of a potential novel variant. Other OXA genes identified included *bla*<sub>OXA-10</sub> (0.8% of isolates, *n*=3), *bla*<sub>OXA-2</sub> (0.5%, *n*=2) and *bla*<sub>OXA-5</sub> (0.3%, *n*=1). At least one mutation known to be related to porin inactivation, efflux overexpression or AmpC alteration was identified in 78.5% (*n*=291) of isolates (Table S1). Mutations affecting the functionality of OprD occurred frequently (73.5%, *n*=273) and were largely driven by a premature stop codon. Mutations causing overexpression of the cephalosporinase AmpC were found in 3.8% (*n*=14) of all isolates and were primarily in *ampD*, *ampR* or *dacB* genes. Mutations that affect efflux pump MexAB-OprM expression were found in the *mexR* gene in only 1.1% (*n*=4) of all isolates. Situations in which more than

one of the described chromosomal markers of resistance was present were observed in 4.9% (*n*=18) of all isolates.

The seven target MLST scheme revealed wide genetic diversity with 151 unique STs identified among the 371 isolates of carbapenem-resistant *P. aeruginosa* (Figure 2). The most common STs identified were ST17 (4.6%, *n*=17), ST27 (4.6%, *n*=17), and high-risk clones ST253 (6.4%, *n*=24), ST235 (4.3%, *n*=16), ST244 (3.5%, *n*=13) and ST357 (2.7%, *n*=10). Although no statistically significant changes in STs were observed over the time period, an increase in ST357 was observed from 2022 (5.1%, *n*=2) to 2023 (16.2%, *n*=6).

**Characteristics of the six most commonly identified STs of carbapenem-resistant *P. aeruginosa***

Similar to the entire carbapenem-resistant dataset, the majority of the top six STs were cultured from respiratory specimens of male patients aged 18–65 years located in central Canada. ST357 was the only one of the top six STs limited to a single region of Canada (Figure 2). When stratified by antimicrobial percent susceptible values, the six most common STs exhibited different profiles (Figure 3(a)). Amikacin was most active against ST17 (88.2% susceptible, *n*=17), ST235 (87.5%, *n*=16), ST244 (100%, *n*=13), ST253 (100%, *n*=24) and ST357 (100%, *n*=10). All isolates of ST253 were also susceptible to tobramycin. The most active β-lactam/β-lactamase inhibitor combination was ceftolozane-tazobactam, against ST17 (82.4% susceptible),

**Table 2.** Antimicrobial susceptibility profiles of 371 carbapenem-resistant *P. aeruginosa* isolated in Canadian hospitals in 2018–23

| Antimicrobial agent                | MIC, mg/L         |                   |              | MIC breakpoint interpretation |                 |             |
|------------------------------------|-------------------|-------------------|--------------|-------------------------------|-----------------|-------------|
|                                    | MIC <sub>50</sub> | MIC <sub>90</sub> | MIC range    | % susceptible                 | % intermediate  | % resistant |
| Amikacin                           | 8                 | 32                | ≤1 to >64    | 84.1                          | 6.1             | 9.7         |
| Aztreonam                          | 64                | 64                | 1 to >64     | 10.5                          | 12.7            | 76.8        |
| Cefepime                           | 16                | 64                | 1 to >64     | 34.5                          | 26.7            | 38.8        |
| Ceftazidime                        | 32                | 32                | 1 to >32     | 29.1                          | 16.2            | 54.7        |
| Ceftazidime–avibactam              | 8                 | 16                | 1 to >16     | 60.1                          | NA <sup>a</sup> | 39.9        |
| Ceftolozane–tazobactam             | 2                 | 16                | 0.5 to >64   | 78.4                          | 11.3            | 10.2        |
| Ciprofloxacin                      | 1                 | 16                | ≤0.06 to >16 | 42.6                          | 10.2            | 47.1        |
| Colistin                           | 1                 | 2                 | ≤0.06–16     | NA                            | 97.6            | 2.4         |
| Imipenem                           | 16                | 32                | 1 to >32     | 2.4                           | 3.2             | 94.4        |
| Imipenem–relebactam                | 2                 | 8                 | 0.25 to >32  | 63.3                          | 21.0            | 15.6        |
| Meropenem                          | 16                | 32                | 8 to >32     | 0                             | 0               | 100         |
| Meropenem–vaborbactam <sup>b</sup> | 16                | 32                | 0.5 to >32   | 39.1                          | NA              | 60.9        |
| Piperacillin–tazobactam            | 64                | 512               | ≤1 to >512   | 29.6                          | 19.4            | 50.9        |
| Tobramycin                         | 1                 | 32                | ≤0.5 to >64  | 70.6                          | 8.9             | 20.5        |

<sup>a</sup>NA, not applicable.<sup>b</sup>EUCAST MIC interpretations were used for meropenem–vaborbactam.

ST27 (94.1%, *n*=17), ST235 (87.5%), ST244 (84.6%) and ST357 (80.0%); imipenem–relebactam was the most active β-lactam/β-lactamase inhibitor combination ST253 (91.7%). ST357 displayed a non-susceptible phenotype to 10 of the 12 antimicrobials tested.

The correlation between ST and mechanism of carbapenem resistance was also investigated (Figure 3(b)). When stratified by ST all carbapenemases genes identified in this study were associated with ST17 (*bla*<sub>GES-5</sub>; *n*=1) or ST235 (*bla*<sub>IMP-26</sub>; *n*=2). All of the top six STs harboured a *bla*<sub>OXA-50</sub>-like gene. The distribution of other OXA genes were as follows: *bla*<sub>OXA-2</sub> was found in ST235 (1.0%, *n*=1), *bla*<sub>OXA-5</sub> in ST17 (1.0%, *n*=1) and *bla*<sub>OXA-10</sub> in ST244 (1.0%, *n*=1) and ST235 (2.1%, *n*=2). OprD dysfunction occurred most frequently in ST27 (100%; *n*=17), ST17 (82.4%; *n*=14), ST235 (75%, *n*=12) and ST357 (80%; *n*=8) (Figure 3(b)). AmpC mutations were only found in ST27 (1.0%, *n*=1), ST253 (1.0%, *n*=1) and ST357 (1.0%, *n*=1), whereas no efflux mutations were identified in any of the top six STs.

All six STs were screened for four virulence genes (*exoS*, *exoT*, *exoU* and *exoY*) that encode for exotoxins that are part of the type III secretion system.<sup>27</sup> The presence of *exoU* was exclusive to high-risk clones ST235, ST253 and ST357 (Figure 3(b)). In contrast, ST17, ST27 and ST244 were negative for *exoU* and positive for the less virulent *exoS*. The *exoT* gene was present in all isolates, whereas *exoY* was found in all but four isolates.

cgMLST was used to further assess relatedness among the four high-risk clones ST235, ST244, ST253 and ST357, and determined that 34.9% (22/63) of high-risk clones were grouped within seven clusters (Figure 4). Isolates were grouped into a cluster if they shared ≤12 allele differences. Cluster 1, the largest cluster, belonged to ST357 (CT3751 [70%, *n*=7]); clusters 2, 3, 5 and 6 belonged to ST253 (CT3800 [16.7%, *n*=4], CT3569 [12.5%, *n*=3], CT3804 [8.3%, *n*=2] and CT3799 [8.3%, *n*=2], respectively); cluster 4 belonged to ST235 (CT3665 [12.5%, *n*=2]) and cluster 7 belonged to ST244 (CT3774 [15.4%, *n*=2]).

## Discussion

The overall proportion of carbapenem-resistant *P. aeruginosa* amongst Canadian patients over the 6-year (2018–23) study period was 21.5%, which is an increase from the 12.1% reported from 2007 to 2016 in Canada in a previous CANWARD study report<sup>15</sup> and from 3.3% reported by another 2009 national Canadian surveillance study.<sup>28</sup> However, we noted a significant decrease (*P*=0.0001) in the proportion of carbapenem-resistant *P. aeruginosa* from 2021 (29.7%) to 2022 (13.4%) in the current study that cannot be explained by study collection parameters or site participation changes. Similar to previous Canadian and international studies, most isolates were from respiratory samples collected from the ICU or from hospital medical units.<sup>15,28,29</sup> Furthermore, it has been suggested that admission to an ICU stay is a risk factor for carbapenemase-producing *P. aeruginosa* infection.<sup>29</sup> There were no significant differences in demographic information between the current study and the previously published 2007–16 CANWARD study.<sup>15</sup>

In the overall sample dataset, we found the proportion of carbapenem-resistant *P. aeruginosa* isolates susceptible to the newer β-lactam/β-lactamase inhibitor combinations was lower than previously reported in studies from Canada and European countries.<sup>15,30,31</sup> The higher susceptibility to ceftazidime–avibactam (83.3%) observed in Turkey<sup>30</sup> suggests it may be an effective treatment for *P. aeruginosa* infections in that region, however, the 60.1% susceptibility rate in our study points to potentially more limited effectiveness in our population. This discrepancy may reflect regional differences in resistance patterns, possibly due to factors like the prevalence of resistant strains, differences in antibiotic use or variations in infection control practices. Although ceftolozane–tazobactam was one of the more active agents in our current study, with 78.4% susceptibility, this marks a significant decline compared with the 92.5% susceptibility observed in our 2007–16 Canadian study.<sup>15</sup> Additionally, a recent

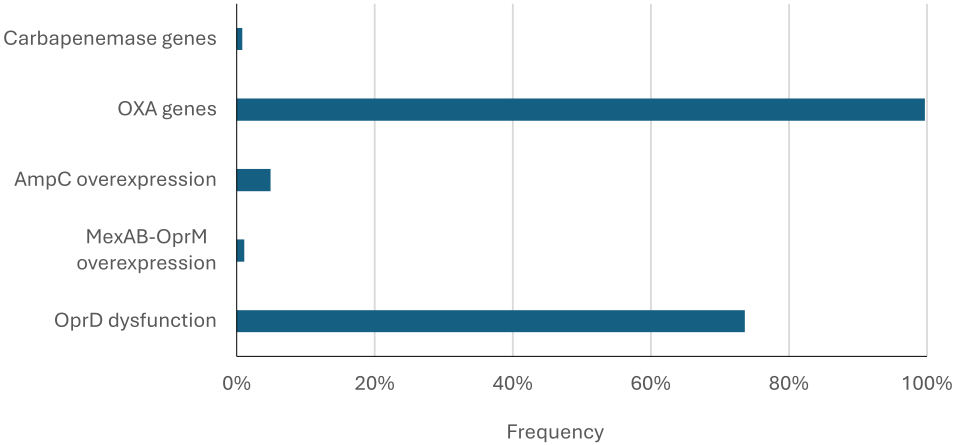


**Figure 1.** Distribution of possible mechanisms of resistance to carbapenems in 371 carbapenem-resistant *P. aeruginosa* isolated in Canadian hospitals in 2018–23.

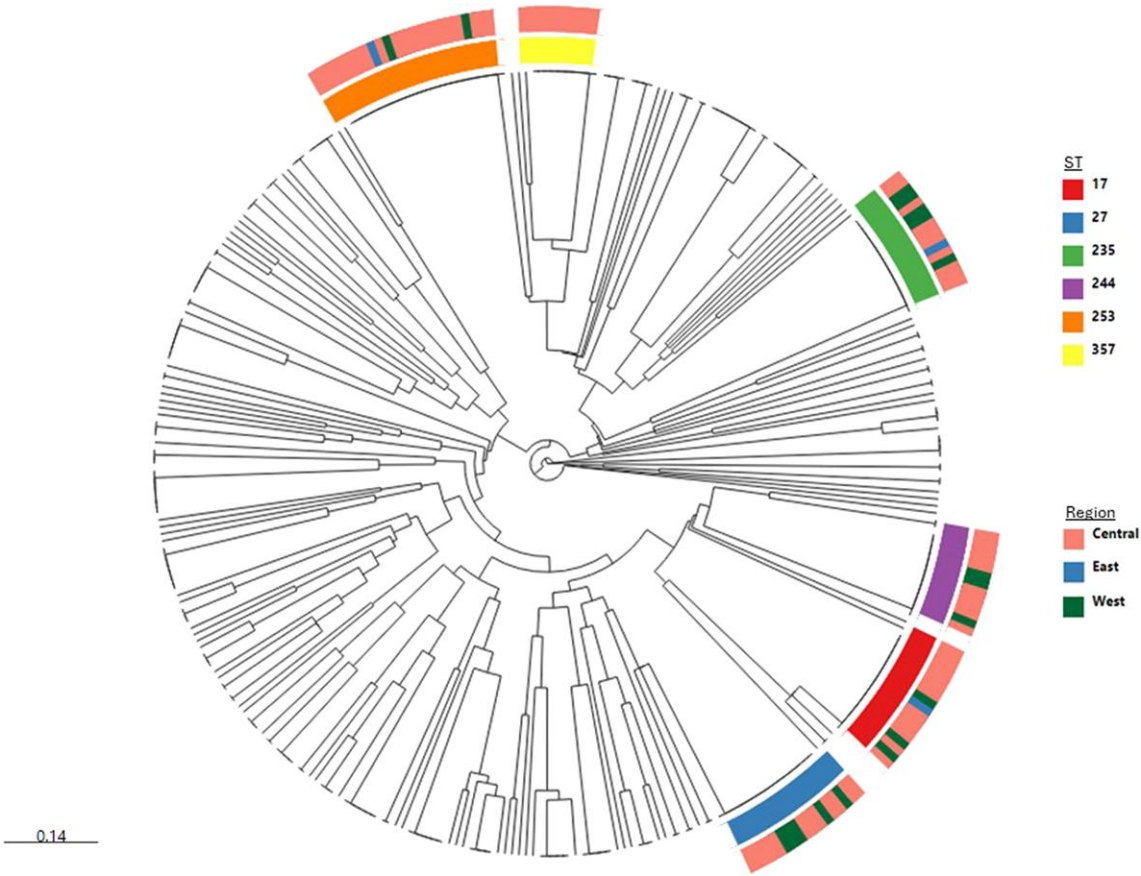


**Figure 2.** Phylogenetic tree illustrating ST distribution of 371 carbapenem-resistant *P. aeruginosa* isolated in Canadian hospitals in 2018–23. The tree is made up of 151 different STs. The six most common STs are highlighted in the inner ring as ST17 (red,  $n=17$ ), ST27 (blue,  $n=17$ ), ST235 (green,  $n=16$ ), ST244 (purple,  $n=13$ ), ST253 (orange,  $n=24$ ) and ST357 (yellow,  $n=10$ ). The outer ring indicates isolates from central, eastern and western regions of Canada.

study from Israel found that only ~48% of their *P. aeruginosa* isolates were susceptible to ceftolozane–tazobactam, further highlighting the regional variability in susceptibility rates for this agent.<sup>32</sup> The susceptibility of 63.3% of carbapenem-resistant

*P. aeruginosa* isolates to imipenem–relebactam in our study is relatively consistent with previous findings in Canada (68.6%) and Israel (50%).<sup>31,32</sup> In contrast, meropenem–vaborbactam, which is not yet recommended for treatment of carbapenem-resistant

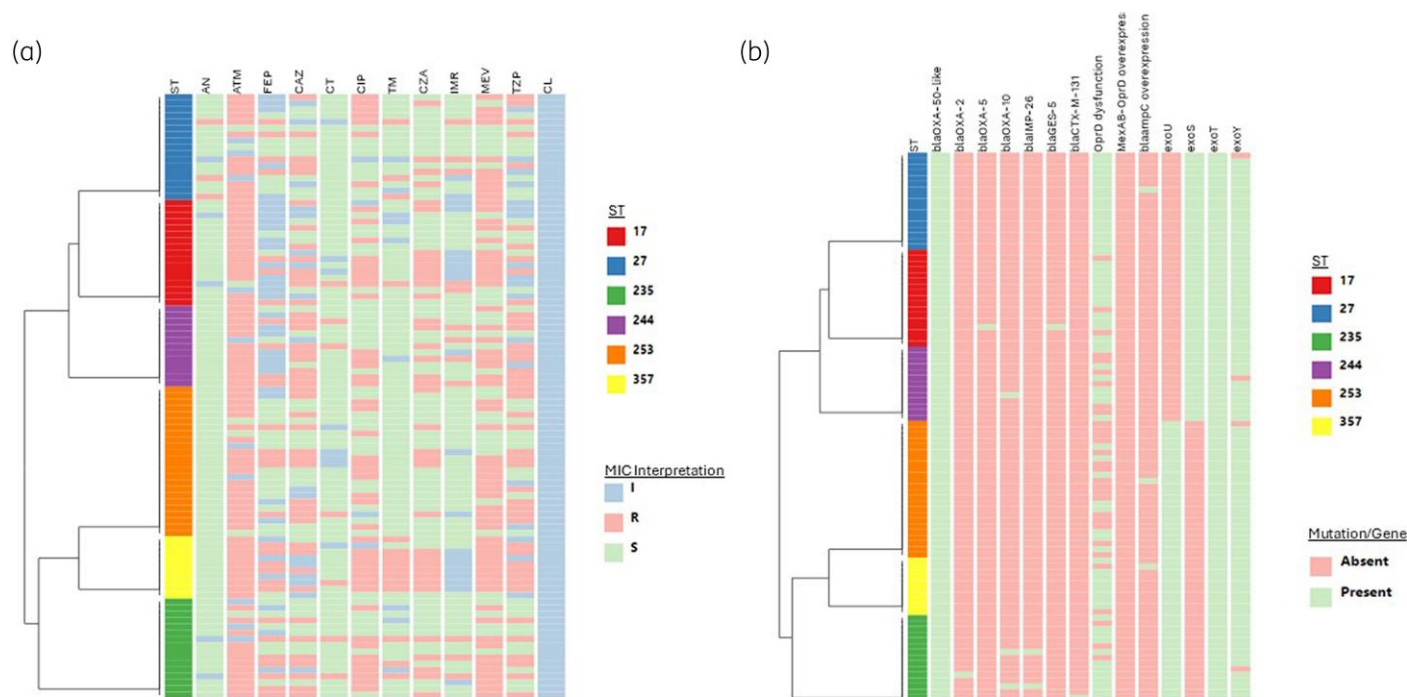


**Figure 3.** (a) Phylogenetic tree of the six most common STs ( $n=97$ ) stratified by MIC interpretation data for amikacin (AN), aztreonam (ATM), ceftipime (FEP), ceftazidime (CAZ), ceftazidime–avibactam (CZA), ceftolozone–tazobactam (CT), ciprofloxacin (CIP), colistin (CL), imipenem–relebactam (IMR), meropenem (MEM), meropenem–vaborbactam (MEV), piperacillin–tazobactam (TZP) and tobramycin (TM). (b) Phylogenetic tree of the six most common STs ( $n=97$ ) stratified by potential carbapenem-resistant mechanisms and exo virulence factors.

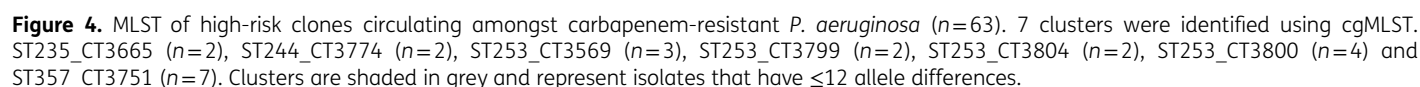
*P. aeruginosa* infections, demonstrated a notably low susceptibility of 39.1% in our study, which is much lower than data reported from Israel (56.5%).<sup>32</sup> Lastly, although aminoglycosides like amikacin and tobramycin are not commonly used due to their potential toxicity (e.g. nephrotoxicity and ototoxicity), they still show relatively high *in vitro* activity against carbapenem-resistant *P. aeruginosa*, with susceptibility rates of >84% for amikacin and >70% for tobramycin. Results published by Kon *et al.*, support our findings, as they showed >90% susceptibility to amikacin and >50% susceptibility to tobramycin tested against clinical isolates of carbapenem-resistant *P. aeruginosa*.

Carbapenem resistance in *P. aeruginosa* can be complex and comprised of multiple different mechanisms such as acquisition of a  $\beta$ -lactamase, porin inactivation, efflux pump overproduction and AmpC overexpression.<sup>6,8,13</sup> Previous studies have shown increases in the proportion of *P. aeruginosa* isolates harbouring carbapenemases,<sup>32,33</sup> which was not observed in this study where only a small portion of carbapenem-resistant isolates carried a carbapenemase gene (0.8%). This is similar to findings in a US-based study from 2016 to 2018 where only 0.1% of isolates were reported to harbour a carbapenemase gene.<sup>13</sup> However, our current observation is considerably lower than reported from CANWARD in 2007–16 and CNISP 2009, where 4.3% and 3.3% of isolates harboured a carbapenemase, respectively.<sup>15,28</sup> In contrast, the prevalence of carbapenemase genes are significantly different internationally. Israel reported 9.4% of carbapenem-resistant isolates carried a carbapenemase,<sup>32</sup>

whereas China and South America reported 32% and 69% carried a carbapenemase gene, respectively.<sup>34</sup> In the current study, the majority of carbapenem resistance in *P. aeruginosa* is likely attributable to the presence of intrinsic class D OXA genes<sup>35</sup> (98.1%) in combination with inactivation of the porin gene OprD (73.5%). Other rarely described class D  $\beta$ -lactamase genes like bla<sub>OXA</sub>-2 and bla<sub>OXA</sub>-10 are also suspected to cause carbapenem resistance particularly in the presence of low outer membrane permeability.<sup>11,36</sup> Reduced membrane permeability can occur from the inactivation of porins, which, as observed in our findings, is one of the most frequently reported mechanisms associated with carbapenem resistance in the USA (87.1%) and Israel (67%).<sup>13,32</sup> A potential limitation of our study is, oprD, efflux and ampC mutations may have been undercounted as we only included previously identified genetic markers and did not explore novel mutations potentially associated with resistance.

Genetic analysis revealed a diverse population of 151 different STs amongst the 371 *P. aeruginosa* isolates tested. The observed diversity is not unusual in *P. aeruginosa* collected from nosocomial infections and reflects what has been observed previously.<sup>13,15</sup> Although we observed some evidence of clonal spread among the four high-risk clones identified in this study, these clones have been responsible for widespread dissemination globally.<sup>9,27,32,37</sup>

ST253 was the most frequently identified high-risk clone in our dataset, despite being the least commonly reported high-risk clone globally.<sup>38–40</sup> Imipenem–relebactam, tobramycin and



within four cgMLST clusters, suggesting the propensity of dissemination of multiple clones within this ST.

The most relevant and widespread high-risk clone, ST235, likely emerged in Europe around 1984.<sup>43</sup> It has a strong association with many class A and B carbapenemases and has an affinity to acquire and maintain foreign DNA through horizontal gene transfer at a greater rate than other clones.<sup>43</sup> In our dataset, it was the second most prevalent high-risk clone at 4.3%, whereas in the CANWARD 2007–16 study, it was the most prevalent clone at 3.6%.<sup>15</sup> ST235 harboured both IMP-26 carbapenemases identified in the study and all were positive for *exoU*. Similar to

ST253, the presence of *exoU* in ST235 may contribute to the pathogenicity of this high-risk clone.<sup>41</sup> Ceftolozane–tazobactam and amikacin were the most active agents (>85%) against our collection of ST235 isolates. In this study, there was no significant evidence of clonal dissemination within ST235, as only 2/16 isolates (12.5%) were related through cgMLST.

ST244 was the third most commonly identified high-risk clone in our study, although it has been reported as the most prevalent clone in other studies.<sup>44,45</sup> Unlike other high-risk clones, ST244 is not always associated with antimicrobial resistance, even though a wide diversity of  $\beta$ -lactamases have been associated with this clone.<sup>9,44</sup> Our findings support these observations as ST244 was susceptible to the most antimicrobials (>80% susceptible to amikacin, aztreonam, ceftazidime–tazobactam and tobramycin) compared with other STs and did not harbour a carbapenemase gene. Furthermore, in line with previous reports, all ST244 in our study harboured the less virulent cytotoxin, *exoS*.<sup>44</sup> As with ST235, only 2/13 (15.4%) of the isolates were related through cgMLST, suggesting no significant clonal dissemination within ST244.

ST357 was the least common high-risk clone identified in our study. Frequently found in Asia, ST357, has also been reported in Japan, Europe and South America.<sup>9,46</sup> From 2007 to 2016, ST357 accounted for 3.1% of the total isolates,<sup>15</sup> similar to the 2.7% observed in the current study. Although ST357 has not significantly increased in Canada over the last 16 years, a small rise in ST357 isolates was noted in 2023, which can be attributed to a possible outbreak at a single hospital. Although previous studies have found an association between ST357 and *bla*<sub>IMP</sub> and *bla*<sub>OXA</sub> variants, this was not observed in our study.<sup>47,48</sup> Compared to the other sequence types discussed, ST357 exhibited a remarkably non-susceptible phenotype while *in vitro* activity of ceftazidime–tazobactam and amikacin remained high. Furthermore, ST357 was the most clonal ST found in our study where 7/10 (70%) of the isolates were grouped within one cgMLST cluster. When examined more closely, the highly resistant AST profile identified among the ST357 s belonged to the highly related cluster, CT3751. The MDR profile, *exoU*-positive genotype, and low genetic diversity are characteristics of successful high-risk clones.

Other commonly reported high-risk clones globally include, ST111, ST175, ST233, ST277, ST298, ST308 and ST654.<sup>9,49</sup> The distribution of these additional high-risk clones in this study was either very low or non-existent (ST111, *n*=3; ST175, *n*=5; ST233, *n*=0; ST277, *n*=3; ST298, *n*=8; ST308, *n*=2; ST654, *n*=0), further supporting previous data on the limited prevalence of high-risk clones in Canada.<sup>15,44</sup>

In conclusion, while the proportion of *P. aeruginosa* isolates resistant to carbapenems is increasing in Canada, carbapenemase genes do not play a significant role in this resistance. Genetic evidence for the clonal dissemination of multiple high-risk clones in this study, which are often associated with MDR and increased virulence, highlight the importance of continued surveillance of this top priority antimicrobial-resistant pathogen in Canada.

## Acknowledgements

We thank Nicole Lermineaux for bioinformatic support as well as the participating centres, investigators and laboratory site staff from the

CANWARD sites for their continued support and cooperation. CANWARD sites are detailed in Zhanel *et al.*<sup>49</sup>

CANWARD data can also be found at [www.can-r.ca](http://www.can-r.ca), the official website of the Canadian Antimicrobial Resistance Alliance (CARA).

## Funding

The CANWARD Study was supported in part by the University of Manitoba, Shared Health, the National Microbiology Laboratory, Avir, Iterum, Paladin Labs, Pfizer, Sandoz, Shionogi, Sunovion, Venatorx, Verity and Xediton. This study was also supported in part by a research grant from Investigator-Initiated Studies Program of Merck Canada Inc. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Canada Inc.

## Transparency declarations

G.G.Z. has received research grants from Avir Pharma Inc., Iterum Therapeutics, Merck Canada Inc., Paladin Labs, Pfizer, Sandoz Canada, Shionogi Inc., Sunovion Pharmaceuticals Canada Inc., Venatorx Pharmaceuticals Inc., Verity Pharmaceuticals and Xediton Pharmaceuticals Inc. All other authors have no declarations.

## Disclaimer

The opinions expressed in this paper are those of the authors, and do not necessarily represent those of Avir Pharma Inc., Iterum Therapeutics, Merck Canada Inc., Paladin Labs, Pfizer, Sandoz Canada, Shionogi Inc., Sunovion Pharmaceuticals Canada Inc., Venatorx Pharmaceuticals Inc., Verity Pharmaceuticals and Xediton Pharmaceuticals Inc.

## Supplementary data

Table S1 is available as [Supplementary data](#) at [JAC Online](#).

## References

- 1 Bgaguidi-Haore H, Varin A, Cholley P *et al.* A bundle of measures to control an outbreak of *Pseudomonas aeruginosa* associated with P-trap contamination. *Infect Control Hosp Epidemiol* 2018; **39**: 164–9. <https://doi.org/10.1017/ice.2017.304>
- 2 Mulani MS, Kamble EE, Kumkar SN *et al.* Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Front Microbiol* 2019; **10**: 539. <https://doi.org/10.3389/fmicb.2019.00539>
- 3 Pandey D, Singhal N, Kumar M. Investigating the OXA variants of ESKAPE pathogens. *Antibiotics (Basel)* 2021; **10**: 1539. <https://doi.org/10.3390/antibiotics10121539>
- 4 Giovagnorio F, De Vito A, Madeddu G *et al.* Resistance in *Pseudomonas aeruginosa*: a narrative review of antibiogram interpretation and emerging treatments. *Antibiotics (Basel)* 2023; **12**: 1621. <https://doi.org/10.3390/antibiotics12111621>
- 5 World Health Organization. *WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance*, 2024. <https://iris.who.int/handle/10665/376776>.
- 6 Pan Y-P, Xu Y-H, Wang Z-X *et al.* Overexpression of MexAB-OprM efflux pump in carbapenem-resistant *Pseudomonas aeruginosa*. *Arch Microbiol* 2016; **198**: 565–71. <https://doi.org/10.1007/s00203-016-1215-7>

- 7 Angeletti S, Cella E, Prosperi M et al. Multi-drug resistant *Pseudomonas aeruginosa* nosocomial strains: molecular epidemiology and evolution. *Microb Pathog* 2018; **123**: 233–41. <https://doi.org/10.1016/j.micpath.2018.07.020>
- 8 Cabot G, Bruchmann S, Mulet X et al. *Pseudomonas aeruginosa* ceftolozane-tazobactam resistance development requires multiple mutations leading to overexpression and structural modification of AmpC. *Antimicrob Agents Chemother* 2014; **58**: 3091–9. <https://doi.org/10.1128/AAC.02462-13>
- 9 Del Barrio-Tofiño E, López-Causapé C, Oliver A. *Pseudomonas aeruginosa* epidemic high-risk clones and their association with horizontally-acquired  $\beta$ -lactamases: 2020 update. *Int J Antimicrob Agents* 2020; **56**: 106196. <https://doi.org/10.1016/j.ijantimicag.2020.106196>
- 10 Arca-Suárez J, Lasarte-Monterrubbio C, Rodiño-Janeiro BK et al. Molecular mechanisms driving the in vivo development of OXA-10-mediated resistance to ceftolozane/tazobactam and ceftazidime/avibactam during treatment of XDR *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother* 2021; **76**: 91–100. <https://doi.org/10.1093/jac/dkaa396>
- 11 Alonso-García I, Vázquez-Ucha JC, Lasarte-Monterrubbio C et al. Simultaneous and divergent evolution of resistance to cephalosporin/ $\beta$ -lactamase inhibitor combinations and imipenem/relebactam following ceftazidime/avibactam treatment of MDR *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother* 2023; **78**: 1195–200. <https://doi.org/10.1093/jac/dkac062>
- 12 Deshpande LM, Vega S, Tinoco JC et al. Endemicity of *Pseudomonas aeruginosa* producing IMP-18 and/or VIM-2 MBLs from the high-risk clone ST111 in Central America. *JAC Antimicrob Resist* 2023; **5**: dlad092. <https://doi.org/10.1093/jacamr/dlad092>
- 13 Stanton RA, Campbell D, McAllister GA et al. Whole-genome sequencing reveals diversity of carbapenem-resistant *Pseudomonas aeruginosa* collected through CDC's Emerging Infections Program, United States, 2016–2018. *Antimicrob Agents Chemother* 2022; **66**: e0049622. <https://doi.org/10.1128/aac.00496-22>
- 14 Roer L, Overballe-Petersen S, Hansen F et al. *Escherichia coli* sequence type 410 is causing new international high-risk clones. *mSphere* 2018; **3**: e00337-18. <https://doi.org/10.1128/mSphere.00337-18>
- 15 McCracken MG, Adam HJ, Blondeau JM et al. Characterization of carbapenem-resistant and XDR *Pseudomonas aeruginosa* in Canada: results of the CANWARD 2007–16 study. *J Antimicrob Chemother* 2019; **74** Suppl 4: iv32–8. <https://doi.org/10.1093/jac/dkz285>
- 16 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirty-Fourth Edition: M100*. 2024.
- 17 The European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 14.0*, 2024. <https://www.eucast.org>
- 18 Bharat A, Petkau A, Avery BP et al. Correlation between phenotypic and in silico detection of antimicrobial resistance in *Salmonella enterica* in Canada using Staramr. *Microorganisms* 2022; **10**: 292. <https://doi.org/10.3390/microorganisms10020292>
- 19 Bortolaia V, Kaas RS, Ruppe E et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; **75**: 3491–500. <https://doi.org/10.1093/jac/dkaa345>
- 20 Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 2018; **3**: 124. <https://doi.org/10.12688/wellcomeopenres.14826.1>
- 21 Seemann T. *ABRicate: Mass Screening of Contigs for Antimicrobial Resistance or Virulence Genes*, v1.0.1. 2016 <https://github.com/tseemann/abricate>.
- 22 Chen LH, Zheng DD, Liu B et al. VFDB 2016: hierarchical and refined dataset for big data analysis-10 years on. *Nucleic Acids Res* 2016; **44**: D694–7. <https://doi.org/10.1093/nar/gkv1239>
- 23 Feldgarden M, Brover V, Gonzalez-Escalona N et al. AMRFinderPlus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci Rep* 2021; **11**: 12728. <https://doi.org/10.1038/s41598-021-91456-0>
- 24 Tönnies H, Prior K, Harmsen D et al. Establishment and evaluation of a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Pseudomonas aeruginosa*. *J Clin Microbiol* 2021; **59**: e01987-20. <https://doi.org/10.1128/JCM.01987-20>
- 25 Argimón S, Abudahab K, Goater RJE et al. Microreact: visualizing and sharing data for genomic epidemiology and phylogeography. *Microb Genom* 2016; **2**: e000093. <https://doi.org/10.1099/mgen.0.000093>
- 26 German GJ, Gilmour M, Tipples G et al. Canadian recommendations for laboratory interpretation of multiple or extensive drug resistance in clinical isolates of Enterobacteriaceae, *Acinetobacter* species and *Pseudomonas aeruginosa*. *Can Commun Dis Rep* 2018; **44**: 29–34. <https://doi.org/10.14745/ccdr.v44i01a07>
- 27 Fischer S, Dethlefsen S, Klockgether J et al. Phenotypic and genomic comparison of the two most common ExoU-positive *Pseudomonas aeruginosa* clones, PA14 and ST235. *mSystems* 2020; **5**: e01007-20. <https://doi.org/10.1128/mSystems.01007-20>
- 28 Mataseje LF, Bryce E, Roscoe D et al. Carbapenem-resistant Gram-negative bacilli in Canada 2009–10: results from the Canadian Nosocomial Infection Surveillance Program (CNISP). *J Antimicrob Chemother* 2012; **67**: 1359–67. <https://doi.org/10.1093/jac/dks046>
- 29 Lucena A, Dalla Costa LM, Nogueira KS et al. Nosocomial infections with metallo- $\beta$ -lactamase producing *Pseudomonas aeruginosa*: molecular epidemiology, risk factors, clinical features and outcomes. *J Hosp Infect* 2014; **87**: 234–40. <https://doi.org/10.1016/j.jhin.2014.05.007>
- 30 Mirza HC, Hortaç E, Koçak AA et al. In vitro activity of ceftolozane-tazobactam and ceftazidime-avibactam against clinical isolates of meropenem-non-susceptible *Pseudomonas aeruginosa*: a two-centre study. *J Glob Antimicrob Resist* 2020; **20**: 334–8. <https://doi.org/10.1016/j.jgar.2019.09.016>
- 31 Karlowsky JA, Walkty AJ, Baxter MR et al. In vitro activity of cefiderocol against extensively drug-resistant *Pseudomonas aeruginosa*: CANWARD, 2007 to 2019. *Microbiol Spectr* 2022; **10**: e0172422. <https://doi.org/10.1128/spectrum.01724-22>
- 32 Kon H, Lurie-Weinberger MN, Bechor M et al. bla<sub>GES</sub>-producing ST654 comprises a quarter of all carbapenem-resistant *Pseudomonas aeruginosa* in blood isolates from 15 hospitals. *Antimicrob Agents Chemother* 2024; **68**: e0096524. <https://doi.org/10.1128/aac.00965-24>
- 33 Castanheira M, Mills JC, Farrell DJ et al. Mutation-driven  $\beta$ -lactam resistance mechanisms among contemporary ceftazidime-nonsusceptible *Pseudomonas aeruginosa* isolates from U.S. hospitals. *Antimicrob Agents Chemother* 2014; **58**: 6844–50. <https://doi.org/10.1128/AAC.03681-14>
- 34 Reyes J, Komarow L, Chen L et al. Global epidemiology and clinical outcomes of carbapenem-resistant *Pseudomonas aeruginosa* and associated carbapenemases (POP): a prospective cohort study. *Lancet Microbe* 2023; **4**: e159–70. [https://doi.org/10.1016/S2666-5247\(22\)00329-9](https://doi.org/10.1016/S2666-5247(22)00329-9)
- 35 Girlich D, Naas T, Nordmann P. Biochemical characterization of the naturally occurring oxacillinase OXA-50 of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2004; **48**: 2043–8. <https://doi.org/10.1128/AAC.48.6.2043-2048.2004>
- 36 Antunes NT, Lamoureaux TL, Toth M et al. Class D  $\beta$ -lactamases: are they all carbapenemases? *Antimicrob Agents Chemother* 2014; **58**: 2119–25. <https://doi.org/10.1128/AAC.02522-13>
- 37 Rath A, Kieninger B, Fritsch J et al. Whole-genome sequencing reveals two prolonged simultaneous outbreaks involving *Pseudomonas*

- aeruginosa* high-risk strains ST111 and ST235 with resistance to quaternary ammonium compounds. *J Hosp Infect* 2024; **145**: 155–64. <https://doi.org/10.1016/j.jhin.2024.01.009>
- 38** Zurita J, Sevillano G, Solís MB et al. *Pseudomonas aeruginosa* epidemic high-risk clones and their association with multidrug-resistant. *J Glob Antimicrob Resist* 2024; **38**: 332–8. <https://doi.org/10.1016/j.jgar.2024.07.003>
- 39** Oliver A, Mulet X, López-Causapé C et al. The increasing threat of *Pseudomonas aeruginosa* high-risk clones. *Drug Resist Updat* 2015; **21–22**: 41–59. <https://doi.org/10.1016/j.drug.2015.08.002>
- 40** Botelho J, Grosso F, Quinteira S et al. Two decades of blaVIM-2-producing *Pseudomonas aeruginosa* dissemination: an interplay between mobile genetic elements and successful clones. *J Antimicrob Chemother* 2018; **73**: 873–82. <https://doi.org/10.1093/jac/dkx517>
- 41** Peña C, Cabot G, Gómez-Zorrilla S et al. Influence of virulence genotype and resistance profile in the mortality of *Pseudomonas aeruginosa* bloodstream infections. *Clin Infect Dis* 2015; **60**: 539–48. <https://doi.org/10.1093/cid/ciu866>
- 42** Peirano G, Matsumara Y, Nobrega D et al. Population-based genomic surveillance of *Pseudomonas aeruginosa* causing bloodstream infections in a large Canadian health region. *Eur J Clin Microbiol Infect Dis* 2024; **43**: 501–10. <https://doi.org/10.1007/s10096-024-04750-w>
- 43** Treepong P, Kos VN, Guyeux C et al. Global emergence of the widespread *Pseudomonas aeruginosa* ST235 clone. *Clin Microbiol Infect* 2018; **24**: 258–66. <https://doi.org/10.1016/j.cmi.2017.06.018>
- 44** Zhao X, Qin J, Chen G et al. Whole-genome sequencing, multilocus sequence typing, and resistance mechanism of the carbapenem-resistant *Pseudomonas aeruginosa* in China. *Microb Pathog* 2024; **192**: 106720. <https://doi.org/10.1016/j.micpath.2024.106720>
- 45** Mihara T, Kimura T, Momiyama K et al. Secondary in-hospital epidemiological investigation after an outbreak of *Pseudomonas aeruginosa* ST357. *J Infect Chemother* 2020; **26**: 257–65. <https://doi.org/10.1016/j.jiac.2019.09.014>
- 46** Kos VN, Déraspe M, McLaughlin RE et al. The resistome of *Pseudomonas aeruginosa* in relationship to phenotypic susceptibility. *Antimicrob. Agents Chemother* 2015; **59**: 427–36. <https://doi.org/10.1128/AAC.03954-14>
- 47** Kainuma A, Momiyama K, Kimura T et al. An outbreak of fluoroquinolone-resistant *Pseudomonas aeruginosa* ST357 harboring the *exoU* gene. *J Infect Chemother* 2018; **24**: 615–22. <https://doi.org/10.1016/j.jiac.2018.03.008>
- 48** Kocsis B, Gulyás D, Szabó D. Diversity and distribution of resistance markers in *Pseudomonas aeruginosa* international high-risk clones. *Microorganisms* 2021; **9**: 359. <https://doi.org/10.3390/microorganisms9020359>
- 49** Zhanel GG, Adam HJ. Seventeen years of the CANWARD study (2007–23). *J Antimicrob Chemother* 2025; **80**(Suppl 2): ii2–4. <https://doi.org/10.1093/jac/dkaf216>