

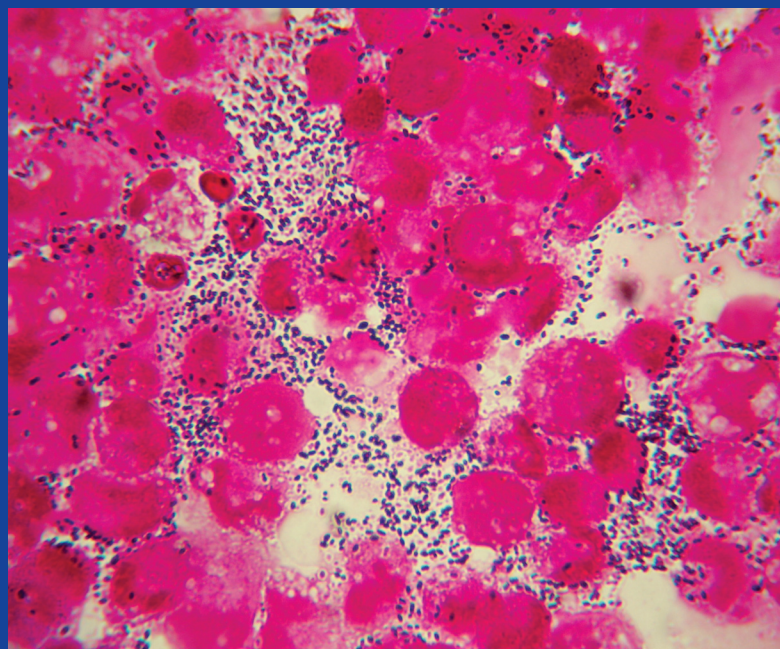
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Journal of Antimicrobial Chemotherapy

Supplement 2

CANWARD 2007-23

Gram stain of *Streptococcus pneumoniae*
in CSF provided by Dr. Andrew Walkty



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The CANWARD studies: Canadian Antimicrobial Resistance Alliance (CARA) Canadian Hospital Ward Antibiotic Resistance Surveillance collection

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This is the third supplement reporting on the Canadian ward surveillance study (CANWARD).^{1,2} CANWARD provides surveillance for commonly tested and recently approved antimicrobials, for bacterial pathogens obtained from more than 15 tertiary care Canadian hospitals representing at least 8 of the 10 Canadian provinces.

This supplement contains nine articles, starting with an introductory commentary by Zhanel and Adam, and provide an overview of the 17 years of CANWARD and some of the notable findings.

The current reports emanating from this surveillance appear in this supplement. It updates surveillance information published in *Journal of Antimicrobial Chemotherapy* in 2019.²

It details targeted surveillance information for Gram-positive pathogens, Gram-negative pathogens and other specific pathogen categories for isolates that were obtained from inpatients and outpatients attending medical wards, emergency rooms, ICUs, hospital clinics and surgical wards in Canadian hospitals. A small proportion were isolates from paediatric patients. The reports detail the inclusion criteria but the aim was to obtain susceptibility data broadly representative of pathogens seen across the breadth of Canada over the surveillance period. The surveillance provides antimicrobial resistance trends since 2007. There is also information of trends in relation to serotype patterns in *Streptococcus pneumoniae*.

The editors hope that this information will serve as a benchmark for antimicrobial resistance in Canada and a reference to

serve as a model for similar surveillance studies elsewhere in the world.

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None to declare.

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Seventeen years of the CANWARD study (2007–23)

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The global crisis of infections caused by antimicrobial-resistant and MDR pathogens continues to increase worldwide.^{1–4} Numerous critical resistant pathogens are recognized, including Gram-positive organisms such as MRSA, both community-associated (CA-MRSA) and healthcare-associated (HA-MRSA), VRE and penicillin-resistant *Streptococcus pneumoniae* and Gram-negative pathogens such as ESBL-producing Enterobacterales, carbapenem-resistant Enterobacterales and MDR *Pseudomonas aeruginosa*.^{5–9} The greatest threat posed by these resistant pathogens is that treatment options can be severely limited, particularly if isolates demonstrate a MDR or XDR phenotype.^{3,10}

Of the various proposed solutions to this global problem of antimicrobial resistance, including infection prevention and control strategies, antimicrobial stewardship and development of new antimicrobials, surveillance has remained the core foundation.⁴ In 2007, we initiated the CANWARD study, which is an ongoing, national, Canadian Antimicrobial Resistance Alliance (CARA)/Health Canada-partnered, multi-year study focused on pathogens obtained from patients seeking care in Canadian hospitals.¹¹ The CANWARD study was designed to assess the epidemiology of pathogens and the degree of antimicrobial resistance within specific hospital wards (medical, surgical, intensive care units, emergency room and clinics) as well as specific infection sites (urine, blood, respiratory and wound). We previously published a CANWARD 2007–16 (10 year) supplement in the *Journal of Antimicrobial Chemotherapy*.^{11–18}

The current supplement documents 17 years of the CANWARD study (2007–23, inclusive). The eight manuscripts in this supplement provide a comprehensive examination of the causative pathogens of various infectious processes and their antimicrobial resistance patterns and trends over a 17-year period. It should be mentioned that the CANWARD study primarily used the North American-based CLSI standards methodology and breakpoint interpretations, not the European-based EUCAST standards. Although the CLSI and EUCAST standards have some differences in methodology and breakpoint interpretations, both are widely used and accepted internationally.^{19–21} The CANWARD data presented herein highlight the ongoing evolution of resistance and provide the current status of

antimicrobial resistance in Canada to healthcare professionals and researchers nationally and globally.

In the first paper, Zhanel *et al.*²² introduce CANWARD, describe the methodology of the study, laboratory protocols, and isolated Gram-positive pathogens with an overview of their susceptibility to various antimicrobials. A total of 25 897 Gram-positive pathogens were received, and antimicrobial susceptibility testing was performed on 22 132 isolates. Patient demographic data and 17 years of antimicrobial susceptibility data on Gram-positive pathogens commonly causing infections in patients attending Canadian hospitals are provided.

In the second paper, Zhanel *et al.*²² describe the evolution of Gram-negative pathogens with an overview of their susceptibility to various antimicrobials. A total of 34 155 Gram-negative pathogens were received and had antimicrobial susceptibility testing was performed. Patient demographic data and 17 years of antimicrobial susceptibility data on Gram-negative pathogens commonly causing infections in patients attending Canadian hospitals are provided.

In the third paper, Brahami *et al.*²³ assess the demographic and molecular characteristics of 12 734 community-associated (CA) and healthcare-associated (HA) MRSA genotypes in Canadian hospitals. They demonstrate that the overall proportion of MRSA decreased significantly from 2007–17 and then increased in 2023. CA-MRSA *spa* types increased while HA-MRSA decreased over the study period. The predominant MRSA *spa* types identified among all isolates were t002, a HA-MRSA genotype, and t008, a CA-MRSA genotype. PVL was detected in most CA-MRSA and very few HA-MRSA. MRSA isolates were ≥99% for ceftobiprole, dalbavancin, daptomycin, linezolid and vancomycin.

Adam *et al.*²⁴ compare the epidemiology and antimicrobial susceptibility patterns of 3111 *S. pneumoniae* collected from respiratory and blood culture samples in the fourth paper. They report significant differences in the serotypes isolated from blood culture and respiratory specimens and demonstrate lower antimicrobial susceptibilities and higher MDR rates in respiratory isolates compared to isolates from blood cultures. Serotypes 19A and 15A were predominant in the MDR/XDR isolates. A greater

proportion of isolates from blood cultures were serotypes covered by current pneumococcal vaccines.

In the fifth paper, Lagacé-Wiens *et al.*²⁵ assess the prevalence, antimicrobial susceptibilities and molecular characteristics of 11 931 ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* infecting patients receiving care at Canadian hospitals. The authors show that the prevalence of both ESBL-producing *E. coli* and *K. pneumoniae* increased significantly in Canada during the study period. Genotypic analysis showed increasing *bla*_{CTX-M-15} in *K. pneumoniae* and *bla*_{CTX-M-27} in *E. coli*. Other β -lactamases including *bla*_{OXA-1} and *bla*_{TEM-1} were associated with first-generation β -lactam/ β -lactamase inhibitor combination resistance.

Mataseje *et al.*²⁶ report on their analysis of 134 (0.7% of all Enterobacterales) ertapenem-resistant Enterobacterales in the sixth paper. Carbapenemase-producing Enterobacterales (CPE) accounted for only a small proportion of ertapenem-resistant isolates and were predominantly KPC-producing *K. pneumoniae* and *E. coli*. Most non-CPE ertapenem-resistant isolates were *Enterobacter cloacae*. CPE isolates demonstrated higher prevalence of resistant values for multiple antimicrobial classes and harboured more antimicrobial resistance genes than non-CPE isolates.

In the seventh paper, McCracken *et al.*²⁷ characterize 371 (21.5% of all *P. aeruginosa*) carbapenem-resistant *P. aeruginosa*. Only 0.8% of the carbapenem-resistant isolates harboured a carbapenemase gene. Whole genome sequencing identified substitutions that have been associated with dysfunction of porin protein OprD, or MexAB-OprM efflux and AmpC overexpression. They report that genetic evidence indicates that clonal dissemination of high-risk clones is occurring in Canada and that these high-risk clones are virulent and often MDR.

In the final paper, Walkty *et al.*²⁸ report on their characterization of 26 067 bloodstream isolates of which the five most common pathogens were *E. coli*, *Staphylococcus aureus*, *K. pneumoniae*, *Staphylococcus epidermidis* and *S. pneumoniae*. They report little variation in pathogen distribution over time; however, some resistant phenotypes (e.g. ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae* and VRE) are being encountered more frequently in recent years.

The CANWARD data described in this supplement discuss resistance patterns by ward type, infection process, patient demographics and time assisting both clinicians and researchers in critically assessing the current status of hospital pathogens in Canada today. From a global perspective, the CANWARD study allows comparisons between countries. CANWARD continues to advocate that surveillance programmes allow us to ‘think globally, act locally’ and provides important information to our continued understanding of antimicrobial resistance. Global and national surveillance studies such as CANWARD can assist in determining antimicrobial resistance trends over specific age groups, geographic areas, time for a variety of infectious syndromes and pathogens and allow comparisons between countries.

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Transparency declarations

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Gram-positive pathogens from Canadian hospitals: 17 years of results from the CANWARD study (2007–23)

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Objectives: CANWARD is a Canadian Antimicrobial Resistance Alliance (CARA)/Health Canada partnered national surveillance study established in 2007 to annually assess antimicrobial susceptibilities for bacterial pathogens isolated from patients receiving care in Canadian hospitals. This paper focuses on Gram-positive pathogens.

Methods: In total, 25 897 Gram-positive pathogens were received and 22 132 underwent CLSI broth microdilution testing providing susceptibility data.

Results: 47.9%, 30.2%, 5.7% and 16.2% of isolates tested were from blood, respiratory, urine and wound specimens, respectively; 29.9%, 24.8%, 19.0%, 18.1% and 8.2% of isolates were from patients in medical wards, emergency rooms, intensive care units, hospital clinics and surgical wards. Patient demographics associated with the isolates were: 58.9% male/41.1% female; 13.5% patients aged ≤17 years, 48.5% 18–64 years and 38.0% ≥65 years. Of the 25 897 pathogens received, the most common were: *Staphylococcus aureus* [20.9% (MSSA 16.5%/MRSA 4.4%)], *Streptococcus pneumoniae* (5.2%) and *Enterococcus* spp. (*Enterococcus faecalis* and *Enterococcus faecium*) (5.0%). MRSA rates (MRSA as a % of all *S. aureus* tested) decreased significantly during 2007–15 from 26.0% to 19.3% ($P < 0.0001$); subsequently they increased significantly through study years 2016–23 (16.9%–24.1%; $P = 0.002$). VRE rates (VRE as a % of all *E. faecium* tested) ranged from 3.8% to 18.4%, with marked fluctuation year-to-year. Susceptibility rates for MRSA were highest with: 100% ceftobiprole, 99.9% linezolid, vancomycin and daptomycin, 99.8% dalbavancin and 99.4% tedizolid, while susceptibility rates for VRE were 98.4% daptomycin and 86.0% with linezolid.

Conclusions: The CANWARD surveillance study has provided 17 years of reference antimicrobial susceptibility testing data on Gram-positive pathogens.

Introduction

Infections caused by antimicrobial-resistant and MDR pathogens are increasing worldwide.^{1–3} Gram-positive pathogens of concern include MRSA, both community-associated (CA-MRSA) and healthcare-associated (HA-MRSA), VRE and penicillin-resistant *Streptococcus pneumoniae*.^{3–7} Treatment options for antimicrobial-resistant organisms can be severely limited as these pathogens frequently display an MDR phenotype.^{3,5,8}

Proposed solutions to the global problem of antimicrobial resistance include surveillance and antimicrobial stewardship programmes.^{2,9} In 2007, we initiated the CANWARD study, which is an ongoing, annual, national, Canadian Antimicrobial Resistance Alliance (CARA)/Health Canada partnered surveillance study.^{9,10} The purpose of CANWARD is to assess the pathogens causing infections in patients seeking care in tertiary-care medical centres across Canada, as well as their susceptibilities to commonly prescribed and newer antimicrobial agents.

In the current report, we summarize the Gram-positive pathogens causing infections and the *in vitro* activities of commonly used antimicrobial agents against these isolates collected from 2007 to 2023, inclusive, from patients seeking care in tertiary-care medical centres across Canada.

Materials and methods

Bacterial isolates

As part of the CANWARD study, tertiary-care medical centres (12 centres in 2007, 10 in 2008, 15 in 2009, 14 in 2010, 15 in 2011, 12 in 2012, 15 in 2013, 13 in 2014, 13 in 2015, 13 in 2016, 14 in 2017, 12 in 2018, 13 in 2019, 10 in 2020, 10 in 2021, 9 in 2022 and 8 in 2023) representing 8 of the 10 Canadian provinces submitted pathogens from patients attending emergency rooms, medical and surgical wards, intensive care units and hospital clinics.^{9,10} The sites were geographically distributed in a population-based fashion. From 2007 through 2023, inclusive, each study site was asked to submit clinically significant isolates as defined by their individual hospital criteria (consecutive, one per patient, per infection site) from inpatients and outpatients attending hospital clinics with respiratory, urine, wound and bloodstream infections. By year, each centre was asked to provide: 2007—200 respiratory, 50 wounds, 100 urinary, 30/month blood isolates; 2008—150 respiratory, 50 wounds, 100 urinary, 20/month blood isolates; 2009–10—100 respiratory, 50 wound, 50 urinary, 15/month (January–November) blood isolates; and 2011–23—100 respiratory, 25 wounds, 25 urinary, 10/month (January to October) blood isolates. Surveillance swabs, eye, ear, nose and throat swabs were excluded. Anaerobic bacteria and fungi were also excluded from the primary CANWARD objectives, except for yeast from bloodstream infections. Yeast from bloodstream infections were included in two components of CANWARD: (i) the assessment of the epidemiology of causative agents of bloodstream infections in Canadian hospitalized patients and (ii) the *Candida*-specific objective in which antifungal susceptibility testing was performed (data were published previously¹¹ and are not presented in this manuscript). Isolate identification was performed by the submitting site and confirmed at the reference site as required, based on morphological characteristics (e.g. Gram stain, colonial morphology and haemolysis patterns) and antimicrobial susceptibility patterns.^{9,10} Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media and stocked in skim milk at –80°C until MIC testing was performed.^{9,10} Isolate viability (including fastidious organisms such as *S. pneumoniae*) using these methods was >99%. Patient demographic information collected with the isolates included gender, age, hospital location and specimen source. From 2007–23, a total of 25 897 Gram-positive isolates: 3503 (2007), 2533 (2008), 2408 (2009), 2225 (2010), 1536 (2011), 1253 (2012), 1504 (2013), 1445 (2014), 1409 (2015), 1289 (2016), 1347 (2017), 1186 (2018), 1165 (2019), 779 (2020), 845 (2021), 777 (2022) and 693 (2023) were collected. The focus of this study was the 25 897 Gram-positive pathogens collected from CANWARD 2007 to 2023, inclusive. The decline in isolates per year from 2007 to 2011 is mostly related to a decrease in the number of isolates requested from each participating institution and not extensive changes to the annual collection protocol.

The CANWARD study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Antimicrobial susceptibility testing

Following two subcultures from frozen stock, the *in vitro* activity of antimicrobial agents was determined by broth microdilution in accordance with CLSI guidelines.¹² Antimicrobial agents were obtained as laboratory grade powders from their respective manufacturers. Stock solutions were

prepared and dilutions made as described by CLSI.¹² MICs were determined using 96-well custom-designed microtitre plates made in-house by our laboratory. Quality control followed CLSI guidelines.¹² MICs were interpreted according to CLSI breakpoints¹³ except for tigecycline, where FDA MIC breakpoints were used [*S. aureus* (MSSA and MRSA), ≤0.5 mg/L susceptible; *Enterococcus faecalis* (vancomycin susceptible) ≤0.25 mg/L susceptible] (<https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria>) and for ceftobiprole, where Health Canada MIC breakpoints were used [*S. aureus* (MSSA and MRSA), ≤4 mg/L susceptible; *S. pneumoniae*, ≤0.5 mg/L susceptible] (<https://health-products.canada.ca/dpd-bdpp/info?lang=eng&code=93212>). Of the 25 897 Gram-positive organisms collected by the CANWARD surveillance study from 2007 to 2023, 22 132 (85.5%) underwent susceptibility testing. Isolates selected for susceptibility testing included the top 20 Gram-positive (coagulase-negative staphylococci and viridans group streptococci were not tested) and Gram-negative pathogens. Development of a centralized database for the CANWARD study results was created by IHMA (Schaumburg, IL, USA).

Characterization of MRSA and VRE

Screening for methicillin resistance was performed by the CLSI-approved disk diffusion with cefoxitin,^{10,14} as well as by growth on MRSA Select chromogenic media (Bio-Rad Laboratories Ltd, Mississauga, Ontario, Canada). Potential MRSA isolates were confirmed by *mecA* PCR as previously described.¹⁴ All isolates of MRSA were typed using staphylococcal protein A (*spa*) DNA sequencing to assess whether the isolates were community-associated (CA) or healthcare-associated (HA).¹⁴ Isolates with a *spa* type associated with C(Canadian)MRSA7 or CMRSA10 were considered CA-MRSA. Isolates with a *spa* type associated with CMRSA1, CMRSA2, CMRSA4, CMRSA5, CMRSA3/6, CMRSA8 or CMRSA9 were considered HA-MRSA.¹⁴ Potential VRE isolates were confirmed by *vanA* and *vanB* PCR, as previously described.¹⁵

Statistical analysis

Annual proportions of MRSA isolates were assessed by the Cochran–Armitage test of trend using XLSTAT (v.2019). Statistical significance was defined as a *P* value of ≤0.05.

Results

Patient demographics and specimen types

A total of 25 897 isolates of Gram-positive bacteria were collected between 2007 and 2023. They were more commonly collected from males (15 255, 58.9%) than females (10 641, 41.1%); a biological sex identifier was not available for one isolate. Isolates were from blood (*n*=12 410; 47.9%), respiratory (*n*=7828; 30.2%), urinary (*n*=1461; 5.7%) and wound (*n*=4198; 16.2%) specimens. By age group, 3505 (13.5%), 12 545 (48.5%) and 9847 (38.0%) isolates were from patients ≤17 years, 18–64 years and ≥65 years, respectively. Regarding hospital location, 7925 (30.6%), 6630 (25.6%), 4816 (18.6%), 4428 (17.1%) and 2098 (8.1%) isolates were received from patients in medical wards, emergency rooms, intensive care units, clinics and surgical wards, respectively. The geographical locations of the referring centres were as follows: British Columbia/Alberta (16.7%), Manitoba/Saskatchewan (17.2%), from Ontario (27.5%), Quebec (25.4%) and New Brunswick/Nova Scotia (13.2%).

Most common organisms isolated

Though the focus of this paper is Gram-positive isolates, for completeness, 61 066 Gram-positive and Gram-negative isolates

Table 1. The 20 most common Gram-positive and Gram-negative bacterial^a pathogens isolated in Canadian hospital laboratories

Rank	Organism	No. of isolates	% of total isolates
1	<i>Staphylococcus aureus</i>	12 734	20.9
	(<i>Staphylococcus aureus</i> , MSSA)	10 037	16.5
	(<i>Staphylococcus aureus</i> , MRSA)	2 697	4.4
2	<i>Escherichia coli</i>	11 932	19.5
3	<i>Pseudomonas aeruginosa</i>	5 961	9.8
4	<i>Klebsiella pneumoniae</i>	3 973	6.5
5	<i>Streptococcus pneumoniae</i>	3 170	5.2
6	<i>Haemophilus influenzae</i>	2 351	3.8
7	<i>Enterococcus faecalis</i>	2 261	3.7
8	CoNS ^b / <i>Staphylococcus epidermidis</i>	2 129	3.5
9	<i>Enterobacter cloacae</i>	1 659	2.7
10	<i>Stenotrophomonas maltophilia</i>	1 210	2.0
11	<i>Klebsiella oxytoca</i>	1 060	1.7
12	<i>Serratia marcescens</i>	1 003	1.6
13	<i>Streptococcus agalactiae</i>	968	1.6
14	<i>Proteus mirabilis</i>	957	1.6
15	<i>Streptococcus pyogenes</i>	866	1.4
16	<i>Enterococcus faecium</i>	810	1.3
17	<i>Moraxella catarrhalis</i>	585	1.0
18	<i>Candida albicans</i>	563	0.9
19	Viridans group streptococci	531	0.9
20	<i>Klebsiella aerogenes</i>	437	0.7
Other ^c		5 906	9.7
Total		61 066	

Organisms isolated from blood, respiratory, urine and wound specimens.

^aList also contains *Candida* spp. collected as part of the CANWARD bloodstream infection objective. The *Candida* spp. collected specifically for the *Candida* objective are not included.¹¹

^bCoNS, coagulase-negative staphylococci.

^cOther: *Abiotrophia* spp., *Achromobacter* spp., *Acinetobacter* spp., *Aerococcus* spp., *Aeromonas* spp., *Aggregatibacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Arcanobacterium* spp., *Bacillus* spp., *Bactobacillus* spp., *Bordetella* spp., *Branhamella* spp., *Brevibacillus* spp., *Brevibacterium* spp., *Burkholderia* spp., *Campylobacter* spp., *Candida* spp., *Capnocytophaga* spp., *Cardiobacterium* spp., *Cedecea* spp., *Chryseobacterium* spp., *Citrobacter* spp., *Clostridium* spp., *Comamonas* spp., *Corynebacterium* spp., *Cronobacter* spp., *Cryptococcus* spp., *Cupriavidus* spp., *Cutibacterium* spp., *Delftia* spp., *Dermabacter* spp., *Diphtheroids*, *Eikenella* spp., *Elizabethkingia* spp., *Empedobacter* spp., *Enterobacter* spp., *Enterococcus* spp., *Escherichia* spp., *Ewingella* spp., *Flavobacterium* spp., *Gemella* spp., *Gordonia* spp., *Granulicatella* spp., *Haemophilus* spp., *Hafnia* spp., *Jeotgaliococcus* spp., *Klebsiella* spp., *Kluyvera* spp., *Kocuria* spp., *Kytococcus* spp., *Lactobacillus* spp., *Lactococcus* spp., *Leclercia* spp., *Legionella* spp., *Leifsonia* spp., *Lelliottia* spp., *Leuconostoc* spp., *Listeria* spp., *Macroccoccus* spp., *Micrococcus* spp., *Moraxella* spp., *Morganella* spp., *Mycobacterium* spp., *Myroides* spp., *Neisseria* spp., *Nocardia* spp., *Ochrobactrum* spp., *Oligella* spp., *Paenibacillus* spp., *Pantoea* spp., *Paracoccus* spp., *Pasteurella* spp., *Phenylobacterium* spp., *Pluralibacter* spp., *Proteus* spp., *Providencia* spp., *Pseudocitrobacter* spp., *Pseudomonas* spp., *Psychrobacter* spp., *Rahnella* spp., *Ralstonia* spp., *Raoultella* spp., *Rhizobium* spp., *Rhodococcus* spp., *Roseomonas* spp., *Rothia* spp., *Saccharomyces* spp., *Salmonella* spp., *Serratia* spp., *Shigella* spp., *Sphingobacterium* spp., *Sphingomonas* spp., *Staphylococcus* spp., *Stenotrophomonas* spp., *Stomatococcus* spp., *Streptococcus* spp., *Trichosporon* spp., *Trueperella* spp., *Vibrio* spp., *Weissella* spp., *Yersinia* spp.

were isolated (Table 1). The 20 most common organisms isolated ($n = 55\,160$, 90.3% of isolates) from hospitals across Canada included 23 469 (42.5%) Gram-positive cocci and 31 128 (56.4%) Gram-negative bacilli. No significant changes occurred over the study period (2007–23) in the proportions of individual Gram-positive cocci causing infections (bacteraemia, respiratory, urinary or wound). For example, *S. pneumoniae* was consistently the first or second most common pathogen causing respiratory tract infections each year.

Of the 25 897 Gram-positive cocci isolated, the 10 most common species accounted for 92.7% ($n = 24\,018$) of isolates (Table 2). *Staphylococcus aureus* (both MSSA and MRSA), *S. pneumoniae*, *Enterococcus* spp. and *Streptococcus* spp. were the most

common Gram-positive organisms (Table 2). MRSA represented 21.2% of all *S. aureus* isolates obtained and tested from 2007 to 2023 (Table 2). VRE were 6.1% of all *Enterococcus* spp. and 23.0% of all *Enterococcus faecium* collected from 2007 to 2023.

Antimicrobial activity against Gram-positive cocci

The *in vitro* activity of various antimicrobials against MSSA, MRSA, including HA-MRSA and CA-MRSA, *Staphylococcus epidermidis*, *S. pneumoniae*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *E. faecalis*, *E. faecium* and VRE is displayed in Table 3. For MSSA, limited resistance was observed with the exception of

Table 2. The 10 most common Gram-positive bacterial^a pathogens isolated in Canadian hospital laboratories

Rank	Organism	No. of isolates	% of total isolates
1	<i>Staphylococcus aureus</i>	12 734	49.2
	(<i>Staphylococcus aureus</i> , MSSA)	10037	38.8
	(<i>Staphylococcus aureus</i> , MRSA)	2697	10.4
2	<i>Streptococcus pneumoniae</i>	3170	12.2
3	<i>Enterococcus faecalis</i>	2261	8.7
4	CoNS ^b / <i>Staphylococcus epidermidis</i>	2129	8.2
5	<i>Streptococcus agalactiae</i>	968	3.7
6	<i>Streptococcus pyogenes</i>	866	3.4
7	<i>Enterococcus faecium</i>	810	3.1
8	Viridans group streptococci	531	2.1
9	<i>Staphylococcus hominis</i>	281	1.1
10	β-Haemolytic streptococci, Group G	268	1.0
Other ^c		1879	7.3
Total		25 897	

Organisms isolated from blood, respiratory, urine and wound specimens.
^aList also contains *Candida* spp. collected as part of the CANWARD blood-stream infection objective. The *Candida* spp. collected specifically for the *Candida* objective are not included.¹¹
^bCoNS, coagulase-negative staphylococci.
^cOther: *Abiotrophia* spp., *Aerococcus* spp., *Arcanobacterium* spp., *Bacillus* spp., *Bactobacillus* spp., *Brevibacillus* spp., *Brevibacterium* spp., *Clostridium* spp., *Corynebacterium* spp., *Cutibacterium* spp., *Dermabacter* spp., *Diphtheroids*, *Enterococcus* spp., *Gemella* spp., *Gordonia* spp., *Granulicatella* spp., *Jeotgalicoccus* spp., *Kocuria* spp., *Kytococcus* spp., *Lactobacillus* spp., *Lactococcus* spp., *Leifsonia* spp., *Leuconostoc* spp., *Listeria* spp., *Macrococcus* spp., *Micrococcus* spp., *Mycobacterium* spp., *Nocardia* spp., *Paenibacillus* spp., *Rhodococcus* spp., *Rothia* spp., *Staphylococcus* spp., *Stomatococcus* spp., *Streptococcus* spp., *Trueperella* spp., *Weissella* spp.

clarithromycin, the fluoroquinolones and clindamycin. Only 23 (0.3%) of the 10037 MSSA tested displayed vancomycin MICs of 2 mg/L. The most active agents tested against MRSA were ceftobiprole (100% susceptible), linezolid, vancomycin and daptomycin (99.9% susceptibility), dalbavancin (99.8%), tedizolid (99.4%) and tigecycline (98.2%), respectively. Resistance rates in MRSA were 76.9%–85.9% to fluoroquinolones, 80.4% to clarithromycin, 39.1% to clindamycin and 5.2% to trimethoprim-sulfamethoxazole. MRSA rates decreased significantly from 2007 (26.0%) to 2015 (19.3%) ($P<0.0001$); however, they increased significantly in study years 2016 (16.9%) to 2023 (24.1%) ($P=0.002$). Thirty-five of the 2697 (1.3%) MRSA tested displayed vancomycin MICs of 2 mg/L, while three (0.11%) isolates of MRSA had vancomycin MICs of 4 mg/L. The proportion of MRSA with vancomycin MICs of 2 mg/L per year from 2007 to 2023 ranged from 0% to 2.9% but did not change significantly over time ($P>0.05$).
Against *S. epidermidis* significant resistance was observed for clarithromycin, clindamycin, gentamicin, fluoroquinolones and

trimethoprim-sulfamethoxazole (Table 3). The most active agents tested against *S. epidermidis* were vancomycin, daptomycin, linezolid and tigecycline. Ceftobiprole, dalbavancin and tedizolid demonstrated excellent activity with MIC₉₀ of ≤ 2 mg/L, 0.06 mg/L and 0.25 mg/L, respectively. Three-hundred and seventy of the 1323 *S. epidermidis* (28.0%) displayed vancomycin MICs of 2 mg/L while 0.15% (two of 1323 isolates) displayed vancomycin MICs of 4 mg/L.
For *S. pneumoniae*, limited resistance was observed with the exception of clarithromycin (18.7%), clindamycin (7.1%), doxycycline (12.6%), penicillin (MIC₉₀ of 0.25 mg/L, with 4.6% resistance) and trimethoprim-sulfamethoxazole (9.1%). Isolates were 100% susceptible to vancomycin, while susceptibility was 99.97% to linezolid, 99.92% to ceftobiprole, 99.4% to ceftriaxone and 97.7% to amoxicillin/clavulanate (Table 3). Dalbavancin and tedizolid were very active against *S. pneumoniae*, with MIC₉₀ values of ≤ 0.03 mg/L and 0.25 mg/L, respectively. Phenotypic (susceptibility) testing with clarithromycin and clindamycin suggested that ~38% of isolates displayed altered target site resistance to macrolides, while ~62% of *S. pneumoniae* demonstrated efflux mediated resistance to macrolides.¹⁰ Both *S. pyogenes* and *S. agalactiae* were highly susceptible to most antimicrobials tested with the exception of clarithromycin, for which resistance was 9.8% and 33.4%, respectively.
For *E. faecalis* and *E. faecium*, resistance was 24.6% and 91.3% to ciprofloxacin and 33.3% and 84.8% to levofloxacin, respectively. *E. faecalis* isolates were 99.9% vancomycin-susceptible and 98.2% daptomycin-susceptible. *E. faecium* isolates were 76.9% vancomycin-susceptible and 99.5% daptomycin-susceptible-dose dependent (Table 3). *E. faecalis* susceptibilities were >90% for dalbavancin (99.8%), tedizolid (99.7%), tigecycline 96.0% and linezolid (92.6%). Linezolid susceptibility with *E. faecium* was 89.3%. Ceftobiprole was very active against *E. faecalis* (MIC₉₀, 0.5 mg/L) but not against *E. faecium* (MIC₉₀, >16 mg/L). Vancomycin-resistant *E. faecium* [23.0% (186/810)] were comprised of 95.2% *vanA*-positive and 4.3% *vanB*-positive isolates; one isolate (0.5%) was positive for both *vanA* and *vanD* genes. The most active agents tested against VRE were daptomycin (98.4% susceptible-dose dependent), and linezolid (86.0%).

Discussion

The CANWARD study is the only national, ongoing, prospective, CARA/Health Canada partnered surveillance study assessing antimicrobial activity against pathogens received from patients accessing care in Canadian hospitals (hospital clinics, emergency rooms, medical and surgical wards and intensive care units).^{9,10} A total of 61066 organisms were collected between 2007 and 2023, inclusive, of which 25 897 were Gram-positive organisms (Table 1).
The most potent antimicrobial agents (depending on the specific pathogen and based upon MIC data only) against Gram-positive bacterial pathogens were vancomycin, linezolid, ceftobiprole, dalbavancin, daptomycin, tedizolid and tigecycline (Table 2). As an *in vitro* susceptibility study, the evaluation of antimicrobial activity was based solely upon population MIC values even though potency, which depends upon an agent's pharmacokinetics along with *in vitro* susceptibility (i.e. pharmacodynamics), is a more clinically relevant measure.^{5,8}

Table 3. Antimicrobial susceptibility testing results for the most common Gram-positive cocci ($n = 22\,132$) isolated from patients attending Canadian hospitals from 2007 to 2023

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% susceptible	% intermediate	% resistant	MIC ₅₀	MIC ₉₀	MIC range
<i>Staphylococcus aureus</i> , MSSA ^b (10 037)						
Cefazolin ^c	100			≤0.5	1	≤0.5 to >128
Ceftobiprole ^d	100			0.5	0.5	≤0.03–2
Ciprofloxacin	87.6	3.0	9.4	0.5	2	≤0.06 to >16
Clarithromycin	75.7	0.7	23.6	0.25	>32	≤0.03 to >32
Clindamycin	94.1	0.4	5.5	≤0.25	≤0.25	≤0.25 to >8
Dalbavancin	100			0.06	0.06	≤0.03–0.25
Daptomycin	99.97		0.03	0.25	0.5	≤0.03–2
Gentamicin	98.3	0.1	1.6	≤0.5	≤0.5	≤0.5–≤0.5
Levofloxacin	90.1	0.3	9.6	0.25	1	≤0.06 to >32
Linezolid	99.99		0.01	2	4	≤0.12–8
Moxifloxacin	90.6	0.8	8.6	≤0.06	0.25	≤0.06 to >16
Nitrofurantoin	99.88	0.02	0.10	16	16	≤0.5 to >512
Tedizolid	99.6	0.4		0.25	0.5	≤0.03–1
Tigecycline ^e	99.1		0.9	0.25	0.25	≤0.03–2
Trimethoprim–sulfamethoxazole	99.4		0.6	≤0.12	≤0.12	≤0.12 to >8
Vancomycin	100			1	1	≤0.25–2
<i>Staphylococcus aureus</i> , MRSA ^b (2697)						
Cefazolin ^c			100	64	>128	≤0.5 to >128
Ceftobiprole ^d	100			1	2	0.25–4
Ciprofloxacin	22.4	0.5	77.1	>16	>16	≤0.06 to >16
Clarithromycin	19.0	0.6	80.4	>32	>32	≤0.03 to >32
Clindamycin	60.8	0.1	39.1	≤0.25	>8	≤0.25 to >8
Dalbavancin	99.8		0.2	0.06	0.06	≤0.03–1
Daptomycin	99.9		0.1	0.25	0.5	0.06–4
Gentamicin	93.4	0.3	6.3	≤0.5	1	≤0.5 to >32
Levofloxacin	14.1		85.9	>32	>32	0.12 to >32
Linezolid	99.9		0.1	2	2	≤0.12 to >16
Moxifloxacin	19.1	4.0	76.9	8	>16	≤0.06 to >16
Nitrofurantoin	99.7	0.1	0.2	16	16	8–128
Tedizolid	99.4	0.4	0.2	0.25	0.5	0.06 to >16
Tigecycline ^e	98.2		1.8	0.25	0.5	≤0.03–2
Trimethoprim–sulfamethoxazole	94.8		5.2	≤0.12	0.25	≤0.12 to >8
Vancomycin	99.9	0.1		1	1	≤0.25–4
<i>Staphylococcus epidermidis</i> (1323)						
Cefazolin				1	32	≤0.5 to >128
Ceftobiprole ^d				1	2	≤0.3–8
Ciprofloxacin	48.1	1.6	50.3	4	>16	≤0.06 to >16
Clarithromycin	34.8	1.8	63.4	32	>32	≤0.03 to >32
Clindamycin	59.7	1.4	38.9	≤0.25	>8	≤0.25 to >8
Dalbavancin				≤0.03	0.06	≤0.03–1
Daptomycin	99.8		0.2	0.25	0.25	≤0.03–2
Gentamicin	62.3	5.4	32.3	≤0.5	>32	≤0.5 to >32
Levofloxacin	44.2	1.6	54.2	4	>32	0.12 to >32
Linezolid	100			0.5	1	≤0.12–4
Moxifloxacin	48.0	8.8	43.2	1	>16	≤0.06 to >16
Nitrofurantoin	99.7	0.3		8	16	≤0.5–64
Tedizolid				0.12	0.25	≤0.03–0.5
Tigecycline ^e	98.2		1.8	0.12	0.5	≤0.03–2
Trimethoprim–sulfamethoxazole	62.3		37.7	0.25	8	≤0.12 to >8

Continued

Table 3. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% susceptible	% intermediate	% resistant	MIC ₅₀	MIC ₉₀	MIC range
Vancomycin	100			1	2	≤0.12–4
<i>Streptococcus pneumoniae</i> (3170)						
Amoxicillin/clavulanate	97.7	1.3	1.0	≤0.06	0.12	≤0.06–16
Ceftobiprole ^d	99.92		0.1	≤0.03	0.06	≤0.03–1
Ceftriaxone ^f	99.4	0.4	0.2	≤0.12	≤0.12	≤0.12–4
Cefuroxime	91.9	1.2	6.9	≤0.25	0.5	≤0.25 to >16
Ciprofloxacin	97.1		2.9	1	2	≤0.06 to >16
Clarithromycin	78.3	3.0	18.7	≤0.03	4	≤0.03 to >32
Clindamycin	92.4	0.5	7.1	≤0.25	≤0.25	≤0.25 to >8
Dalbavancin				≤0.03	≤0.03	≤0.03–0.12
Doxycycline	86.3	1.1	12.6	≤0.25	2	≤0.25 to >16
Ertapenem	98.97	0.99	0.04	≤0.06	0.12	≤0.06–4
Levofloxacin	99.2	0.1	0.7	1	1	≤0.06–32
Linezolid	99.97		0.03	1	1	≤0.12–4
Meropenem	94.9	3.3	1.8	≤0.06	0.12	≤0.06–2
Moxifloxacin	99.2	0.4	0.4	0.12	0.25	≤0.06–8
Penicillin ^f	82.6	12.8	4.6	≤0.03	0.25	≤0.03 to >8
Tedizolid				0.12	0.25	≤0.03–0.5
Telithromycin				0.008	0.06	≤0.002–2
Tigecycline ^e	99.7		0.3	0.03	0.06	≤0.015–0.25
Trimethoprim–sulfamethoxazole	84.9	6.0	9.1	≤0.12	2	≤0.12 to >8
Vancomycin	100			≤0.25	≤0.25	≤0.25–1
<i>Streptococcus agalactiae</i> (968)						
Amoxicillin/clavulanate				≤0.06	0.12	≤0.06–0.25
Ceftobiprole ^d				≤0.03	0.06	≤0.03–0.06
Ceftriaxone	100			≤0.12	≤0.12	≤0.12–0.5
Cefuroxime				≤0.25	≤0.25	≤0.25–0.5
Ciprofloxacin				0.5	1	0.25 to >16
Clarithromycin	63.5	3.1	33.4	≤0.03	>32	≤0.03 to >32
Clindamycin	79.2	0.8	20.0	≤0.25	>8	≤0.25 to >8
Dalbavancin	100			≤0.03	≤0.03	≤0.03–≤0.03
Daptomycin	100			0.25	0.25	≤0.03–1
Doxycycline				8	16	≤0.25 to >16
Ertapenem	100			≤0.06	≤0.06	≤0.06–0.12
Levofloxacin	96.4		3.6	1	1	≤0.06 to >32
Linezolid	98.1		1.9	1	2	≤0.12–4
Meropenem	100			≤0.06	≤0.06	≤0.06–0.12
Moxifloxacin				0.12	0.25	≤0.06–16
Penicillin	100			0.06	0.12	≤0.03–0.12
Tedizolid	100			0.25	0.25	≤0.03–0.5
Telithromycin				0.015	0.06	≤0.002–1
Tigecycline ^e	99.9		0.1	0.06	0.06	≤0.015–1
Trimethoprim–sulfamethoxazole				≤0.12	0.25	≤0.12–1
Vancomycin	100			0.5	0.5	≤0.25–1
<i>Streptococcus pyogenes</i> (866)						
Amoxicillin/clavulanate				≤0.06	≤0.06	≤0.06–0.25
Ceftobiprole ^d				≤0.03	0.06	≤0.03–0.5
Ceftriaxone	100			≤0.12	≤0.12	≤0.12–0.5
Cefuroxime				≤0.25	≤0.25	≤0.25–1
Ciprofloxacin				0.5	1	≤0.06 to >16
Clarithromycin	88.6	1.6	9.8	≤0.03	0.5	≤0.03 to >32

Continued

Table 3. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% susceptible	% intermediate	% resistant	MIC ₅₀	MIC ₉₀	MIC range
Clindamycin	97.5	0.1	2.4	≤0.25	≤0.25	≤0.25 to >8
Dalbavancin	100			≤0.03	≤0.03	≤0.03–0.06
Daptomycin	100			0.06	0.12	≤0.03–0.25
Doxycycline				≤0.25	4	≤0.25 to >16
Ertapenem	100			≤0.06	≤0.06	≤0.06–1
Levofloxacin	99.8	0.1	0.1	0.5	1	≤0.06–16
Linezolid	99.4		0.6	1	2	≤0.12–4
Meropenem	100			≤0.06	≤0.06	≤0.06–0.25
Moxifloxacin				0.12	0.25	≤0.06–4
Penicillin	100			≤0.03	≤0.03	≤0.03–0.12
Tedizolid	100			0.12	0.25	≤0.03–0.5
Telithromycin				0.008	0.015	≤0.002–4
Tigecycline ^e	100			0.03	0.06	≤0.015–0.25
Trimethoprim–sulfamethoxazole				≤0.12	≤0.12	≤0.12 to >8
Vancomycin	100			0.5	0.5	≤0.25–1
<i>Enterococcus faecalis</i> (2261)						
Amoxicillin/clavulanate				0.5	1	≤0.06 to >32
Ceftobiprole ^d				0.5	2	≤0.03–16
Ciprofloxacin	64.6	10.8	24.6	1	>16	≤0.06 to >16
Clarithromycin				2	>32	≤0.03 to >32
Dalbavancin	99.8		0.2	0.06	0.06	≤0.03–1
Daptomycin	98.2	1.8		1	2	≤0.03–4
Ertapenem				16	16	0.25 to >32
Levofloxacin	65.7	1.0	33.3	2	>32	0.25 to >32
Linezolid	92.6	7.4		2	2	0.25–4
Meropenem				4	8	≤0.06 to >32
Moxifloxacin				0.25	16	≤0.06 to >16
Nitrofurantoin	99.3	0.3	0.4	8	16	≤0.5–256
Tedizolid	99.7		0.3	0.25	0.5	0.06–1
Tigecycline ^e	96.0		4.0	0.12	0.25	≤0.03–1
Vancomycin	100			1	2	≤0.25–2
<i>Enterococcus faecium</i> (810)						
Amoxicillin/clavulanate				>32	>32	≤0.06 to >32
Ceftobiprole ^d				>16	>16	0.25 to >16
Ciprofloxacin	7.1	1.6	91.3	>16	>16	0.25 to >16
Clarithromycin				>32	>32	≤0.03 to >32
Dalbavancin				0.12	>16	≤0.03 to >16
Daptomycin ^g	99.5		0.5	1	2	≤0.03–16
Ertapenem				>32	>32	0.5 to >32
Levofloxacin	13.3	1.9	84.8	>32	>32	1 to >32
Linezolid	89.3	9.7	1.0	2	4	≤0.12 to >16
Meropenem				>32	>32	0.25 to >64
Moxifloxacin				>16	>16	0.12 to >16
Nitrofurantoin	31.0	28.2	40.8	64	256	≤0.5 to >512
Tedizolid				0.25	0.5	0.12–4
Tigecycline ^e				0.12	0.25	≤0.03–2
Vancomycin	76.9	0.1	23.0	1	>32	≤0.25 to >32
<i>Vancomycin-resistant enterococci</i> (186) ^h						
Amoxicillin/clavulanate				>32	>32	4 to >32
Ciprofloxacin			100	>16	>16	16 to >16
Clarithromycin				>32	>32	0.06 to >32

Continued

Table 3. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% susceptible	% intermediate	% resistant	MIC ₅₀	MIC ₉₀	MIC range
Daptomycin ^g	98.4		1.6	1	2	≤0.03–16
Ertapenem				>32	>32	32 to >32
Levofloxacin			100	>32	>32	32 to >32
Linezolid	86.0	11.3	2.7	2	4	0.5 to >16
Meropenem				>32	>32	32 to >64
Moxifloxacin				>16	>16	8 to >16
Nitrofurantoin	17.4	21.1	61.5	128	256	4 to >512
Tedizolid				0.25	0.5	0.12–4
Tigecycline ^e				0.12	0.25	≤0.03–0.5
Vancomycin			100	>32	>32	32 to >32

Clindamycin inducibility was not tested for staphylococci or streptococci.

^aOrganism-antimicrobial combinations for which intrinsic resistance is known have been excluded from the table.

^bMSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; HA, healthcare-associated; CA, community-associated.

^cSusceptibility and resistance to cefazolin based upon oxacillin susceptibility testing result.

^dHealth Canada approved breakpoints were used to interpret ceftobiprole MICs.

^eFDA approved breakpoints were used to interpret tigecycline MICs.

^fMICs interpreted by CLSI breakpoints for ceftriaxone (non-meningitis MIC breakpoints) and penicillin (oral penicillin V MIC breakpoints).

^gCLSI susceptible-dose dependent (SDD) breakpoints were used to interpret daptomycin MICs.

^hVancomycin-resistant enterococci were comprised of 177 *vanA* isolates, eight *vanB* isolates and one isolate with both *vanA* and *vanD* genes.

Consistent with other reports, we report that vancomycin was potent against MSSA and MRSA with only three isolates (0.11%) of MRSA displaying a vancomycin MIC of 4 mg/L and no vancomycin MIC creep.^{4,16–19} Vancomycin was less potent against *S. epidermidis* than against MSSA and MRSA, which is consistent with previous reports.^{4,19} In this study, as well as in previous reports, vancomycin remains very active against all *Streptococcus* spp.^{7,16,19} Vancomycin was also less potent against *E. faecium* with 23.0% of isolates demonstrating resistance. Consistent with reports in international studies,^{19,20} the predominant *vanA* genotype continues to be the most common form of VRE in Canada (~95% in this study).

Linezolid was active against MSSA, MRSA, MSSE, MRSE and *Streptococcus* spp., with 86.1%–100% of all isolates demonstrating susceptibility to linezolid. Linezolid's continued high-level *in vitro* activity versus these isolates is consistent with the current literature.^{16,18–21} Linezolid was less active against *E. faecalis* and *E. faecium* with 7.4% and 9.7% of isolates demonstrating an intermediate MIC interpretation (and 1.0% resistance for *E. faecium*), respectively. The observed low rate of linezolid non-susceptibility in *E. faecalis* and *E. faecium* is consistent with previous reports.^{16,19,21} Tedizolid was active against MSSA, MRSA, MSSE, MRSE, *Streptococcus* spp. and *Enterococcus* spp., with 4–8-fold greater activity versus linezolid. Tedizolid's 4–8-fold greater activity versus Gram-positive cocci compared to linezolid has been well described.^{16,21,22}

Daptomycin was active against MSSA, MRSA, methicillin-susceptible *S. epidermidis* (MSSE) and methicillin-resistant *S. epidermidis* (MRSE) with 95.1%–100% susceptibility. Daptomycin's excellent activity against MSSA, MRSA, MSSE and MRSE has been previously documented.^{16,18–21} In addition, it has been reported

that daptomycin displays excellent activity (MIC₉₀ of 0.5 mg/L) and maintains bactericidal activity against MRSA with vancomycin MICs of 2 mg/L, many of which are heteroresistant VISAs (vancomycin-intermediate *S. aureus*).^{5,18} As has been previously reported, daptomycin was active against *Streptococcus* spp.¹⁹ Daptomycin was also very active against *E. faecalis*, *E. faecium* and VRE. Daptomycin-resistant *Enterococcus* spp. continue to be rare in Canada, consistent with previous reports from Canada and the USA.^{15,19,20} From our data, it is clear that daptomycin is a very active agent against all common Gram-positive organisms causing infections in Canadian hospitals.

Dalbavancin was active against MSSA, MRSA, MSSE and MRSE with ≥99.6% susceptibility. Dalbavancin's excellent activity against Gram-positive pathogens has been previously documented.^{18,19,23–25} As has been previously reported, dalbavancin was active against *Streptococcus* spp.^{19,23–25} Dalbavancin was also very active against *E. faecalis*, but not VRE (*vanA* *E. faecium*).^{19,23,24} From our data, it is clear that dalbavancin is a very active agent against common Gram-positive organisms causing infections in Canadian hospitals.

Tigecycline was active against MSSA, MRSA, MSSE and MRSE, which has been previously documented.^{5,26} In addition, tigecycline was very active against *Streptococcus* spp. and also very active against *E. faecalis* and *E. faecium*, including VRE isolates. In Canada, tigecycline continues to be a very active agent against common Gram-positive organisms.

Ceftobiprole was active against MSSA, MRSA, MSSE, MRSE and *Streptococcus* spp. which has been previously documented.^{5,27–29} Unlike other cephalosporins, it was active against *E. faecalis* but not *E. faecium*.^{5,27,28} In Canada, ceftobiprole continues to be the only cephalosporin available for use which is active against MRSA

(100% susceptible using Health Canada breakpoints) as ceftaroline is not approved for usage.^{5,18,28,30}

The data generated by the CANWARD surveillance study have three important limitations. One, each participating medical centre commits to study criteria that encourage the submission of clinically significant specimens from patients with a presumed infectious disease, however, this interpretation cannot be rigorously controlled by the coordinating site. Two, the coordinating site does not have admission date data for each patient/clinical specimen, thus we are not able to provide an accurate description of community versus healthcare onset. Third, antimicrobial susceptibility testing was not performed for all commercially available antimicrobial agents due to the lack of space on the custom-designed susceptibility panels utilized.

In conclusion, *S. aureus*, *Streptococcus* spp. and *Enterococcus* spp. are the most common Gram-positive bacterial pathogens in Canadian hospitals. MRSA rates decreased significantly during 2007–15 from 26.0% to 19.3% ($P < 0.0001$) and subsequently increased significantly through study years 2016–23 (16.9%–24.1%; $P = 0.002$). All isolates of MSSA were susceptible to ceftobiprole, dalbavancin and vancomycin. MRSA susceptibilities were highest with ceftobiprole, dalbavancin, daptomycin, linezolid, tedizolid and vancomycin. *S. pneumoniae* susceptibilities were highest to amoxicillin/clavulanate, ceftobiprole, ceftriaxone, linezolid, tigecycline and vancomycin. VRE rates ranged from 3.8% to 18.4%, with marked fluctuation year-to-year. Daptomycin and linezolid demonstrated the highest susceptibilities for VRE.

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Transparency declarations

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Disclaimer

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Gram-negative pathogens from Canadian hospitals: 17 years of results from the CANWARD study (2007–23)

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Objectives: CANWARD is a Canadian Antimicrobial Resistance Alliance (CARA)/Health Canada partnered national surveillance study established in 2007 to annually assess the *in vitro* activities of commonly tested and recently approved antimicrobial agents for bacterial pathogens isolated from patients receiving care in Canadian hospitals.

Methods: In total, 34 155 Gram-negative pathogens were tested using the CLSI reference broth microdilution method.

Results: In total, 39.4%, 37.1%, 17.7% and 5.8% of isolates tested were from respiratory, blood, urine and wound specimens, respectively; 31.1%, 23.9%, 19.0%, 18.5% and 7.5% of isolates were from patients in medical wards, emergency rooms, ICUs, hospital clinics and surgical wards. In total, 51.8% of isolates were from male patients; and 10.1%, 40.7% and 49.2% of isolates were from patients aged ≤ 17 , 18–64 and ≥ 65 years. The most common Gram-negative pathogens received were: *Escherichia coli* (34.9%), *Pseudomonas aeruginosa* (17.5%) and *Klebsiella pneumoniae* (11.6%). An ESBL-producing phenotype was identified in 8.4% of *E. coli* and 5.6% of *K. pneumoniae* isolates. Percent susceptible values for *E. coli* included: 100% for meropenem/vaborbactam and imipenem/relebactam; >99% for ceftazidime/avibactam, meropenem and ceftolozane/tazobactam; 95.6% for piperacillin/tazobactam; and 73.9% for ciprofloxacin. Percent susceptible values for *K. pneumoniae* included: >99% for meropenem/vaborbactam, imipenem/relebactam, ceftazidime/avibactam and meropenem; 97.4% for ceftolozane/tazobactam; 91.9% for piperacillin/tazobactam; and 87.0% for ciprofloxacin. Percent susceptible values for *P. aeruginosa* included: 96.6% for ceftolozane/tazobactam; 92.6% for ceftazidime/avibactam; 92.0% for imipenem/relebactam; 79.9% for piperacillin/tazobactam; ceftazidime 78.1%; 78.0% for meropenem; and 68.6% for ciprofloxacin.

Conclusions: The CANWARD surveillance study has provided 17 years of reference antimicrobial susceptibility testing data.

Introduction

Infections caused by antimicrobial-resistant bacterial pathogens are of worldwide concern.^{1–3} Important Gram-negative pathogens, including ESBL-producing Enterobacterales, and carbapenem-resistant Enterobacterales and *Pseudomonas aeruginosa*, are increasing in prevalence globally.^{4–7} Treatment

options for antimicrobial-resistant organisms can be limited particularly if isolates demonstrate an MDR or XDR phenotype.^{4–7}

Proposed solutions to the global problem of antimicrobial resistance include surveillance, antimicrobial stewardship programs and new agent development.^{2,3} In 2007, we initiated CANWARD, an ongoing, annual, national, Canadian Antimicrobial Resistance Alliance (CARA)/Health Canada partnered surveillance study. CANWARD

serves to assess bacterial pathogens causing infections in patients seeking care in tertiary care medical centres across Canada as well as their *in vitro* susceptibility to commonly tested and recently approved antimicrobial agents.^{8,9} In the current report, we summarize our data for Gram-negative pathogens causing infections in Canadian patients from 2007 to 2023, inclusive, and the *in vitro* susceptibilities (MIC₅₀ and MIC₉₀) of those isolates. It should be stated that since many newer β -lactam/ β -lactamase inhibitor combination agents such as ceftazidime/avibactam, meropenem/vaborbactam, imipenem/relebactam as well as the siderophore cephalosporin cefiderocol are not yet Health Canada approved, or have only very recently obtained Health Canada approval, only limited testing has been performed with these agents. Additionally, we do not report on investigational non-FDA approved β -lactam/ β -lactamase inhibitor combination agents.^{10–13}

Materials and methods

Bacterial isolates

As part of the CANWARD study, tertiary-care medical centres (12 centres in 2007, 10 in 2008, 15 in 2009, 14 in 2010, 15 in 2011, 12 in 2012, 15 in 2013, 13 in 2014, 13 in 2015, 13 in 2016, 14 in 2017, 12 in 2018, 13 in 2019, 10 in 2020, 10 in 2021, 9 in 2022 and 8 in 2023) representing 8 of 10 provinces in Canada submitted pathogens isolated from patients attending emergency rooms, medical and surgical wards, ICUs and hospital clinics.^{8,9} The sites were geographically distributed in a population-based fashion. From 2007 to 2023, each study site was asked to submit clinically significant isolates from bloodstream, respiratory, wound and urinary tract infections as defined by their individual hospital criteria (consecutive, one per patient, per infection site) from inpatients and outpatients attending their hospital. By year, each centre was asked to provide: 2007–200 respiratory, 50 wound and 100 urine isolates, and 30 bloodstream isolates/month; 2008–150 respiratory, 50 wound and 100 urine isolates, and 20 bloodstream isolates/month; 2009–10–100 respiratory, 50 wound and 50 urine isolates, and 15 bloodstream isolates/month (January–November); and 2011–23–100 respiratory, 25 wound and 25 urine isolates, and 10 bloodstream isolates/month (January to October). Surveillance swabs, eye, ear, nose and throat swabs were excluded. Anaerobic bacteria and fungi were also excluded from the primary CANWARD objectives, except for yeast from bloodstream infections. Yeast from bloodstream infections was included in two components of CANWARD: (i) the assessment of the epidemiology of causative agents of bloodstream infections in Canadian hospitalized patients and (ii) the *Candida*-specific objective in which antifungal susceptibility testing was performed (data was published previously¹⁴ and is not presented in this manuscript). Isolate identification was performed by the submitting site and confirmed at the reference site as required, based on morphological characteristics (e.g. Gram stain, colonial morphology, haemolysis patterns) and antimicrobial susceptibility patterns.^{8,9} Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media and stocked in skim milk at -80°C until antimicrobial susceptibility testing was performed.^{8,9} Isolate viability using these methods was >99%. Patient demographics collected with the isolates included biological sex, age, hospital location and specimen source. From 2007 to 2023, annual total numbers of Gram-negative isolates collected were 4215 in 2007, 2750 in 2008, 2965 in 2009, 2735 in 2010, 2249 in 2011, 1549 in 2012, 2007 in 2013, 1727 in 2014, 1797 in 2015, 1836 in 2016, 2071 in 2017, 1763 in 2018, 1904 in 2019, 1452 in 2020, 1486 in 2021, 1450 in 2022 and 1213 in 2023 (1, 2). The focus of this report was the 34 155 Gram-negative pathogens collected by CANWARD from 2007 to 2023. The decline in isolates per year from 2007 to 2011 is mostly related

to a decrease in the number of isolates requested from each participating institution and not extensive changes to the annual collection protocol.

The CANWARD study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Antimicrobial susceptibility testing

Following two subcultures from frozen stock, the *in vitro* activity of antimicrobial agents was determined by the reference broth microdilution method in accordance with CLSI guidelines.¹⁵ Antimicrobial agents were either obtained as laboratory-grade powders from their respective manufacturers or purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Stock solutions were prepared and dilutions made as described by CLSI.¹⁵ MICs were determined using 96-well custom-designed microtitre plates made in-house by our laboratory. Quality control followed CLSI guidelines.¹⁵ MICs were interpreted according to CLSI breakpoints¹⁶ except for tigecycline where FDA MIC breakpoints (<https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria>) were used and for ceftobiprole, where MICs were interpreted according to breakpoints established by Health Canada: Enterobacteriaceae, ≤ 1 mg/L susceptible, 2 mg/L intermediate and ≥ 4 mg/L resistant. Isolates selected for antimicrobial susceptibility testing included the top 20 pathogens and a variety of less common Gram-negative bacilli. Development of a centralized database for the CANWARD study results was created by IHMA (Schaumburg, IL, USA).

Identification of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates

Potential ESBL-producing isolates of *Escherichia coli* and *Klebsiella pneumoniae* were screened for by first identifying isolates with a ceftriaxone and/or ceftazidime MIC of ≥ 1 mg/L and then confirming ESBL production using the CLSI double disc diffusion method, as previously described.^{9,17}

Results

Patient demographics and specimen types

A total of 34 155 Gram-negative isolates were collected between 2007 and 2023; 17 684 (51.8%) of the isolates were from males and 16 468 (48.2%) were from females (biological sex was not indicated for three isolates). By specimen source, 39.4% ($n = 13\,440$) of isolates were from respiratory sources, 37.1% ($n = 12\,688$) were from blood, 17.7% ($n = 6037$) were from urine and 5.8% ($n = 1990$) were from wounds. By patient age group, 3459 (10.1%), 13 898 (40.7%) and 16 795 (49.2%) isolates were from patients aged ≤ 17 years, 18–64 years and ≥ 65 years, respectively (patient age was not indicated for three isolates). In regard to hospital location, 10 635 (31.1%), 8153 (23.9%), 6476 (19.0%), 6323 (18.5%) and 2565 (7.5%) isolates were from patients in medical wards, emergency rooms, ICUs, clinics and surgical wards, respectively (hospital location not indicated for three isolates).

The geographical locations of the referring centres were as follows: 5457 (16.0%) from British Columbia/Alberta, 5707 (16.7%) from Manitoba/Saskatchewan, 9661 (28.3%) from Ontario, 8972 (26.2%) from Quebec and 4358 (12.8%) from New Brunswick/Nova Scotia.

Most common organisms isolated

In total, 61 066 Gram-positive and Gram-negative isolates were isolated by CANWARD from 2007 to 2023. The 20 most common organisms isolated ($n = 55160$, 90.3% of isolates) included

31 128 (56.4%) Gram-negative bacilli and 23 469 (42.5%) Gram-positive cocci. No significant changes occurred over the study period (2007–23) in the proportions of individual Gram-negative bacilli and Gram-positive cocci causing infections (bacteraemia, respiratory, urinary or wound). For example, *E. coli* was consistently the most common pathogen causing urinary tract infections each year.

Of all 34 155 Gram-negative bacilli isolated by CANWARD from 2007 to 2023, the 10 most common accounted 89.9% ($n = 30\,691$) of isolates (Table 1). *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Haemophilus influenzae* and *Enterobacter cloacae* were the most common Gram-negative organisms (Table 1).

Antimicrobial activity against Gram-negative bacilli

The *in vitro* activity of various antimicrobials against frequently isolated Gram-negative bacilli is displayed in Table 2. For *E. coli*,

Table 1. The 10 most frequent Gram-negative bacterial^a pathogens isolated from common patient specimens in Canadian hospital laboratories from 2007 to 2023

Rank	Organism	No. of isolates	% of total isolates
1	<i>Escherichia coli</i>	11 932	34.9
2	<i>Pseudomonas aeruginosa</i>	5961	17.5
3	<i>Klebsiella pneumoniae</i>	3973	11.6
4	<i>Haemophilus influenzae</i>	2351	6.9
5	<i>Enterobacter cloacae</i>	1659	4.9
6	<i>Stenotrophomonas maltophilia</i>	1210	3.5
7	<i>Klebsiella oxytoca</i>	1060	3.1
8	<i>Serratia marcescens</i>	1003	2.9
9	<i>Proteus mirabilis</i>	957	2.8
10	<i>Moraxella catarrhalis</i>	585	1.7
Other ^b		3464	10.1
Total		34 155	

Organisms isolated from blood, respiratory, urine and wound specimens.

^aList also contains *Candida* spp. collected as part of the CANWARD blood-stream infection objective. The *Candida* spp. collected specifically for the *Candida* objective are not included.¹⁴

^bOther: *Achromobacter* spp., *Acinetobacter* spp., *Aeromonas* spp., *Aggregatibacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Bordetella* spp., *Branhamella* spp., *Burkholderia* spp., *Campylobacter* spp., *Capnocytophaga* spp., *Cardiobacterium* spp., *Cedecea* spp., *Chryseobacterium* spp., *Citrobacter* spp., *Comamonas* spp., *Cronobacter* spp., *Cupriavidus* spp., *Delftia* spp., *Eikenella* spp., *Elizabethkingia* spp., *Empedobacter* spp., *Enterobacter* spp., *Escherichia* spp., *Ewingella* spp., *Flavobacterium* spp., *Haemophilus* spp., *Hafnia* spp., *Klebsiella* spp., *Cluyvera* spp., *Ledercia* spp., *Legionella* spp., *Lelliottia* spp., *Moraxella* spp., *Morganella* spp., *Myroides* spp., *Neisseria* spp., *Ochrobactrum* spp., *Oligella* spp., *Pantoea* spp., *Paracoccus* spp., *Pasteurella* spp., *Phenylobacterium* spp., *Pluralibacter* spp., *Proteus* spp., *Providencia* spp., *Pseudocitrobacter* spp., *Pseudomonas* spp., *Psychrobacter* spp., *Rahnella* spp., *Ralstonia* spp., *Raoultella* spp., *Rhizobium* spp., *Roseomonas* spp., *Salmonella* spp., *Serratia* spp., *Shigella* spp., *Sphingobacterium* spp., *Sphingomonas* spp., *Stenotrophomonas* spp., *Vibrio* spp. and *Yersinia* spp.

percent resistant values were >20% for trimethoprim/sulfamethoxazole, ciprofloxacin and levofloxacin. The most active agents against *E. coli* (percent susceptible values >99%) were ceftazidime/avibactam, ceftolozane/tazobactam, ertapenem, imipenem/relebactam, meropenem, meropenem/vaborbactam, plazomicin and tigecycline. For *K. pneumoniae*, percent susceptible values were >99% for ceftazidime/avibactam, imipenem/relebactam, meropenem and plazomicin.

Percent susceptible values against ESBL-producing *E. coli* were 99.9% for meropenem, 99.6% for ertapenem, 95.6% for piperacillin/tazobactam and <50% for fluoroquinolones and trimethoprim/sulfamethoxazole (Table 2). Percent susceptible values against ESBL-producing *K. pneumoniae* were 95.5% for meropenem, 89.9% for ertapenem, 52.0% for piperacillin/tazobactam and <50% for fluoroquinolones and trimethoprim/sulfamethoxazole.

For *E. cloacae*, >99% of isolates were susceptible to ceftazidime/avibactam, plazomicin and meropenem (Table 2). All isolates of *P. mirabilis* isolates were susceptible to ceftazidime/avibactam and meropenem. For *K. oxytoca*, >99% of isolates were susceptible to ceftazidime/avibactam, ertapenem, imipenem/relebactam, meropenem and plazomicin. For *Serratia marcescens*, >99% of isolates were susceptible to ceftazidime/avibactam and meropenem. For *Klebsiella aerogenes*, >99% of isolates were susceptible to amikacin, ceftazidime/avibactam, gentamicin, meropenem and plazomicin. For *Citrobacter freundii*, >99% of isolates were susceptible to ceftazidime/avibactam, meropenem and plazomicin.

Ceftolozane-tazobactam, which in Canada is primarily used to treat infections caused by MDR *P. aeruginosa* was the most active (96.6% susceptible) agent tested against *P. aeruginosa* while percent susceptible values were <80% for cefepime, ceftazidime, fluoroquinolones, meropenem and piperacillin/tazobactam (Table 2). Percent susceptible values were >97% for all agents tested against *H. influenzae* except for ampicillin (80.0%), clarithromycin (87.7%) and trimethoprim/sulfamethoxazole (77.9%). Greater than 97% of *Stenotrophomonas maltophilia* isolates were susceptible to trimethoprim/sulfamethoxazole. For *Acinetobacter baumannii*, percent susceptible values were >95% for amikacin and imipenem/relebactam, while only 1.9% of strains were resistant to colistin (Table 2).

Discussion

The CANWARD study is the only national, ongoing, prospective, CARA/Health Canada partnered surveillance study assessing antimicrobial activity against pathogens received from patients accessing care in Canadian hospitals (hospital clinics, emergency rooms, medical and surgical wards, and ICUs).^{8,9} A total of 61 066 organisms were collected between 2007 and 2023, inclusive, of which 34 155 were Gram-negative organisms (Table 1). As an *in vitro* susceptibility study, the evaluation of antimicrobial activity was based solely upon population MIC values and the interpretation of those values as susceptible, intermediate or resistant by established criteria.¹⁶ Agent potency, which depends upon an agent's pharmacokinetics, along with *in vitro* susceptibility (i.e. pharmacodynamics) and dosing also require consideration as clinically relevant measures when viewing MIC values and their interpretation.^{5,6}

Table 2. Antimicrobial susceptibility testing results for the most common Gram-negative bacilli isolated from common patient specimens in Canadian hospital laboratories from 2007 to 2023

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% Susceptible	% Intermediate	% Resistant	MIC ₅₀	MIC ₉₀	MIC range
<i>Escherichia coli</i> (11 932)						
Amikacin	95.0	3.9	1.1	≤2	4	≤2->64
Amoxicillin/clavulanate	79.8	13.2	7.0	4	16	≤0.06->32
Cefazolin	67.6	12.0	20.4	2	128	≤0.5->128
Cefepime	92.5	2.7	4.8	≤1	≤1	≤1->32
Cefoxitin	90.9	5.1	4.0	4	8	≤0.06->32
Ceftazidime	91.1	1.4	7.5	≤0.5	4	≤0.5->32
Ceftazidime/avibactam	99.9		0.1	≤0.25	≤0.25	≤0.25->16
Ceftobiprole ^b	91.2	0.2	8.6	≤1	≤1	≤1->16
Ceftolozane/tazobactam	99.1	0.2	0.7	0.25	0.5	≤0.12->64
Ceftriaxone	89.4	0.3	10.3	≤1	4	≤1->64
Ciprofloxacin	73.9	1.7	24.4	≤0.06	>16	≤0.06->16
Colistin		99.6	0.4	0.25	0.5	≤0.06->16
Ertapenem	99.6	0.2	0.2	≤0.06	≤0.06	≤0.06->32
Fosfomycin	98.8	0.8	0.4	≤1	4	≤1->512
Gentamicin	90.0	0.5	9.5	≤0.5	4	≤0.5->32
Imipenem/relebactam	100			0.25	0.25	≤0.03-0.5
Levofloxacin	76.7	1.6	21.7	≤0.06	16	≤0.06->32
Meropenem	99.9		0.1	≤0.12	≤0.12	≤0.12->32
Meropenem/vaborbactam	100			≤0.06	≤0.06	≤0.06-0.5
Moxifloxacin				≤0.06	>16	≤0.06->16
Nitrofurantoin	97.1	1.8	1.1	16	32	≤0.5->512
Piperacillin/tazobactam	95.6	1.6	2.8	2	4	≤1->512
Plazomicin	99.3	0.6	0.1	0.5	1	≤0.03->64
Tigecycline ^c	99.9	0.1		0.25	0.5	≤0.03-4
Trimethoprim/sulfamethoxazole	72.7		27.3	≤0.12	>8	≤0.12->8
<i>Escherichia coli</i> , ESBL-positive (1007)						
Amikacin	76.1	17.8	6.1	≤2	8	≤2-64
Amoxicillin/clavulanate	45.3	35.8	18.9	16	32	1->32
Cefazolin		0.1	99.9	>128	>128	4->128
Cefepime	21.0	27.6	51.4	16	>32	≤1->32
Cefoxitin	77.1	14.3	8.6	8	16	0.5->32
Ceftazidime	25.4	11.7	62.9	16	>32	≤0.5->32
Ceftazidime/avibactam	99.6		0.4	≤0.25	0.5	≤0.25->16
Ceftobiprole ^b	2.0	1.3	96.7	>16	>16	≤1->16
Ceftolozane/tazobactam	96.0	1.1	2.9	0.5	1	≤0.12->64
Ceftriaxone	1.2	0.9	97.9	>64	>64	≤1->64
Ciprofloxacin	12.7	1.3	86.0	>16	>16	≤0.06->16
Colistin		99.7	0.3	0.5	1	≤0.06-8
Ertapenem	97.5	1.0	1.5	≤0.06	0.12	≤0.06->32
Fosfomycin	96.7	1.9	1.4	≤1	4	≤1->512
Gentamicin	64.3	1.0	34.7	1	>32	≤0.5->32
Imipenem/relebactam	100			0.25	0.25	0.06-0.5
Levofloxacin	11.6	1.3	87.1	16	32	≤0.06->32
Meropenem	99.5		0.5	≤0.12	≤0.12	≤0.12->32
Meropenem/vaborbactam	100			≤0.06	≤0.06	≤0.06-0.5
Moxifloxacin				16	>16	≤0.06->16
Nitrofurantoin	92.3	4.5	3.2	16	32	≤1-512
Piperacillin/tazobactam	83.6	9.0	7.4	4	16	≤1->512
Plazomicin	99.5	0.3	0.2	0.5	1	≤0.12-32

Continued

Table 2. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% Susceptible	% Intermediate	% Resistant	MIC ₅₀	MIC ₉₀	MIC range
Tigecycline ^c	99.9	0.1		0.5	1	0.12–4
Trimethoprim/sulfamethoxazole	34.1		65.9	>8	>8	≤0.12–>8
<i>Pseudomonas aeruginosa</i> (5961)						
Amikacin	92.0	3.4	4.6	4	16	≤2–>64
Cefepime	79.4	12.6	8.0	4	16	≤1–>32
Ceftazidime	78.1	7.0	14.9	4	32	≤0.5–>32
Ceftazidime/avibactam	92.6		7.4	2	8	≤0.25–>16
Ceftobiprole				4	16	≤1–>16
Ceftolozane/tazobactam	96.6	1.7	1.7	0.5	2	≤0.12–>64
Ciprofloxacin	68.6	9.0	22.4	0.25	4	≤0.06–>16
Colistin		96.3	3.7	1	2	≤0.06–>16
Fosfomycin				64	256	≤1–>512
Imipenem/relebactam	92.0	4.9	3.1	0.5	2	≤0.03–>32
Levofloxacin	52.9	10.6	36.5	1	16	≤0.06–>32
Meropenem	78.0	6.9	15.1	0.5	8	≤0.12–>32
Meropenem/vaborbactam				0.5	8	≤0.06–>32
Moxifloxacin				2	>16	≤0.06–>16
Nitrofurantoin				>512	>512	≤0.5–>512
Piperacillin/tazobactam	79.9	5.9	14.2	4	128	≤1–>512
Plazomicin				4	16	≤0.12–>64
<i>Klebsiella pneumoniae</i> (3973)						
Amikacin	98.8	0.5	0.7	≤2	≤2	≤2–>64
Amoxicillin/clavulanate	91.2	4.5	4.3	2	8	≤0.06–>32
Cefazolin	81.7	6.1	12.2	1	8	≤0.5–>128
Cefepime	94.4	1.4	4.2	≤1	≤1	≤1–>32
Cefoxitin	90.5	4.2	5.3	4	8	0.12–>32
Ceftazidime	93.7	0.5	5.8	≤0.5	1	≤0.5–>32
Ceftazidime/avibactam	99.8		0.2	≤0.25	0.5	≤0.25–>16
Ceftobiprole ^b	93.2	0.5	6.3	≤1	≤1	≤1–>16
Ceftolozane/tazobactam	97.4	0.4	2.2	0.25	1	≤0.12–>64
Ceftriaxone	93.0	0.4	6.6	≤1	≤1	≤1–>64
Ciprofloxacin	87.0	3.7	9.3	≤0.06	0.5	≤0.06–>16
Colistin		97.8	2.2	0.5	1	≤0.06–>16
Fosfomycin				≤0.06	≤0.06	≤0.06–>32
Ertapenem	89.0	3.8	7.2	32	128	≤1–>512
Gentamicin	96.2	0.2	3.6	≤0.5	≤0.5	≤0.5–>32
Imipenem/relebactam	99.3	0.6	0.1	0.25	0.5	0.06–>32
Levofloxacin	87.5	4.4	8.1	≤0.06	1	≤0.06–>32
Meropenem	99.5	0.1	0.4	≤0.12	≤0.12	≤0.12–>32
Meropenem/vaborbactam	99.6		0.4	≤0.06	≤0.06	≤0.06–>32
Moxifloxacin				0.12	1	≤0.06–>16
Nitrofurantoin	40.5	36.2	23.3	64	128	≤1–>512
Piperacillin/tazobactam	91.9	4.2	3.9	2	8	≤1–>512
Plazomicin	99.8	0.1	0.1	0.25	0.5	≤0.12–>64
Tigecycline ^c	95.7	3.5	0.8	1	2	0.06–>16
Trimethoprim/sulfamethoxazole	88.8		11.2	≤0.12	>8	≤0.12–>8
<i>Klebsiella pneumoniae</i> , ESBL-positive (221)						
Amikacin	84.0	7.7	8.3	≤2	8	≤2–>64
Amoxicillin/clavulanate	27.9	34.4	37.7	16	>32	2–>32
Cefazolin			100	>128	>128	8–>128
Cefepime	17.1	18.0	64.9	32	>32	≤1–>32

Continued

Table 2. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% Susceptible	% Intermediate	% Resistant	MIC ₅₀	MIC ₉₀	MIC range
Cefoxitin	69.1	12.0	18.9	8	>32	2->32
Ceftazidime	17.3	4.7	78.0	>32	>32	≤0.5->32
Ceftazidime/avibactam	99.5		0.5	0.5	1	≤0.25-16
Ceftobiprole ^b	5.5	2.2	92.3	>16	>16	≤1->16
Ceftolozane/tazobactam	75.8	4.4	19.8	1	32	≤0.12->64
Ceftriaxone	5.0	2.7	92.3	>64	>64	≤1->64
Ciprofloxacin	16.3	11.8	71.9	4	>16	≤0.06->16
Colistin		95.9	4.1	0.5	1	0.25->16
Fosfomycin	89.9	2.7	7.4	0.12	1	0.06->32
Ertapenem	80.0	10.0	10.0	32	128	2-256
Gentamicin	56.1	0.5	43.4	≤0.5	>32	≤0.5->32
Imipenem/relebactam	98.5	1.5		0.25	0.5	0.06-2
Levofloxacin	24.1	6.9	69.0	4	>32	≤0.06->32
Meropenem	95.5	0.9	3.6	≤0.12	≤0.12	≤0.12->32
Meropenem/vaborbactam	100			≤0.06	≤0.06	≤0.06
Moxifloxacin				4	>16	≤0.06->16
Nitrofurantoin	20.3	34.1	45.6	64	256	8->512
Piperacillin/tazobactam	52.0	15.9	32.1	8	>512	≤1->512
Plazomicin	98.5	0.7	0.8	0.25	0.5	≤0.12->64
Tigecycline ^c	88.2	9.1	2.7	1	4	0.5-16
Trimethoprim/sulfamethoxazole	15.4		84.6	>8	>8	≤0.12->8
<i>Haemophilus influenzae</i> (2351)						
Amoxicillin/clavulanate	97.3	2.4	0.3	0.5	2	≤0.06->32
Ampicillin	80.0	1.7	18.3	≤0.25	16	≤0.25->128
Cefepime	100			≤0.25	≤0.25	≤0.25-2
Ceftazidime/avibactam				≤0.03	0.06	≤0.03->1
Ceftobiprole				≤0.06	0.12	≤0.06-2
Ceftolozane/tazobactam	98.5		1.5	≤0.12	0.25	≤0.12-4
Ceftriaxone	99.9		0.1	≤0.06	≤0.06	≤0.06->4
Cefuroxime	97.9	1.7	0.4	1	2	≤0.25->16
Ciprofloxacin	100			≤0.015	≤0.015	≤0.015->0.5
Clarithromycin	87.7	10.1	2.2	4	16	≤0.03->32
Doxycycline				0.5	1	≤0.25-16
Ertapenem	99.7		0.3	≤0.03	0.12	≤0.03->4
Imipenem/relebactam	99.5		0.5	1	2	≤0.06->8
Levofloxacin	100			≤0.015	0.03	≤0.015->0.5
Meropenem	99.8		0.2	≤0.06	0.12	≤0.06-2
Moxifloxacin	100			≤0.015	0.03	≤0.015->0.5
Piperacillin/tazobactam	99.7		0.3	≤1	≤1	≤1-2
Trimethoprim/sulfamethoxazole	77.9	4.9	17.2	≤0.12	8	≤0.12->8
<i>Enterobacter cloacae</i> (1659)						
Amikacin	98.7	0.7	0.6	≤2	≤2	≤2->64
Cefepime	93.0	5.0	2.0	≤1	2	≤1->32
Ceftazidime	75.9	1.1	23.0	≤0.5	>32	≤0.5->32
Ceftazidime/avibactam	99.5		0.5	≤0.25	1	≤0.25->16
Ceftobiprole ^b	85.1	3.4	11.5	≤1	4	≤1->16
Ceftolozane/tazobactam	81.8	4.5	13.7	0.5	8	≤0.12->64
Ceftriaxone	71.6	2.1	26.3	≤1	>64	≤1->64
Ciprofloxacin	92.6	1.1	6.3	≤0.06	0.12	≤0.06->16
Colistin		81.2	18.8	0.5	>16	≤0.06->16
Fosfomycin				≤0.06	0.5	≤0.06->32

Continued

Table 2. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% Susceptible	% Intermediate	% Resistant	MIC ₅₀	MIC ₉₀	MIC range
Ertapenem	76.9	6.4	16.7	32	512	≤1->512
Gentamicin	96.9	0.4	2.7	≤0.5	≤0.5	≤0.5->32
Imipenem/relebactam	98.8	0.9	0.3	0.25	0.5	0.06->32
Levofloxacin	92.9	1.2	5.9	≤0.06	0.5	≤0.06-32
Meropenem	99.5	0.3	0.2	≤0.12	≤0.12	≤0.12->32
Moxifloxacin				≤0.06	0.5	≤0.06->16
Nitrofurantoin	36.3	42.5	21.2	64	128	≤1->512
Piperacillin/tazobactam	79.1	4.1	16.8	2	64	≤1->512
Plazomicin	99.6	0.2	0.2	0.5	0.5	≤0.12-64
Tigecycline ^c	95.3	3.1	1.6	0.5	1	0.12-16
Trimethoprim/sulfamethoxazole	91.8		8.2	≤0.12	1	≤0.12->8
<i>Proteus mirabilis</i> (957)						
Amikacin	89.6	7.8	2.6	4	8	≤2-32
Amoxicillin/clavulanate	94.9	1.9	3.2	1	4	0.5->32
Cefazolin	3.3	58.0	38.7	4	16	1->128
Cefepime	99.3	0.1	0.6	≤1	≤1	≤1->32
Cefoxitin	95.8	2.6	1.6	4	8	≤0.06->32
Ceftazidime	98.5	0.4	1.1	≤0.5	≤0.5	≤0.5->32
Ceftazidime/avibactam	100			≤0.25	≤0.25	≤0.25-4
Ceftobiprole				≤1	≤1	≤1->16
Ceftolozane/tazobactam	99.2	0.5	0.3	0.5	1	≤0.12-8
Ceftriaxone	97.6	0.7	1.7	≤1	≤1	≤1->64
Ciprofloxacin	82.4	1.3	16.3	≤0.06	2	≤0.06->16
Ertapenem	99.5	0.3	0.2	≤0.06	≤0.06	≤0.06-4
Fosfomycin				4	128	≤1->512
Gentamicin	92.8	1.1	6.1	1	2	≤0.5->32
Imipenem/relebactam	10.0	25.4	64.6	4	4	0.12-16
Levofloxacin	79.3	3.1	17.6	0.12	2	≤0.06->32
Meropenem	100			≤0.12	≤0.12	≤0.12-1
Moxifloxacin				0.5	16	≤0.06->16
Piperacillin/tazobactam	99.6	0.2	0.2	≤1	≤1	≤0.12-64
Plazomicin	41.5	48.3	10.2	4	8	0.25-32
Trimethoprim/sulfamethoxazole	82.3		17.7	≤0.12	>8	≤0.12->8
<i>Klebsiella oxytoca</i> (1060)						
Amikacin	98.6	1.1	0.3	≤2	≤2	≤2->64
Amoxicillin/clavulanate	87.7	5.0	7.3	4	16	0.5->32
Cefazolin	21.2	19.6	59.2	8	>128	≤0.5->128
Cefepime	97.1	1.6	1.3	≤1	≤1	≤1->32
Cefoxitin	94.9	3.2	1.9	2	8	0.5->32
Ceftazidime	97.6	0.3	2.1	≤0.5	1	≤0.5->32
Ceftazidime/avibactam	99.9		0.1	≤0.25	0.5	≤0.25-16
Ceftobiprole ^b	85.7	2.1	12.2	≤1	16	≤1->16
Ceftolozane/tazobactam	98.4	1.3	0.3	0.25	1	≤0.12-64
Ceftriaxone	89.9	1.3	8.8	≤1	2	≤1->64
Ciprofloxacin	96.1	0.8	3.1	≤0.06	≤0.06	≤0.06->16
Colistin		98.1	1.9	0.5	1	≤0.06->16
Ertapenem	99.7	0.1	0.2	≤0.06	≤0.06	≤0.06-32
Fosfomycin				16	128	2->512
Gentamicin	97.6	0.1	2.3	≤0.5	1	≤0.5->32
Imipenem/relebactam	100			0.25	0.5	≤0.03-1
Levofloxacin	94.9	1.8	3.3	≤0.06	0.25	≤0.06->32

Continued

Table 2. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% Susceptible	% Intermediate	% Resistant	MIC ₅₀	MIC ₉₀	MIC range
Meropenem	99.9	0.1		≤0.12	≤0.12	≤0.12–2
Moxifloxacin				≤0.06	0.25	≤0.06–>16
Nitrofurantoin	85.1	12.2	2.7	32	64	≤1–>512
Piperacillin/tazobactam	88.3	0.8	10.9	2	64	≤1–>512
Plazomicin	100			0.5	1	≤0.12–2
Tigecycline ^c	98.7	1.1	0.2	0.5	1	0.06–8
Trimethoprim/sulfamethoxazole	94.6		5.4	≤0.12	0.25	≤0.12–>8
<i>Stenotrophomonas maltophilia</i> (1210)						
Ceftazidime				>32	>32	≤0.5–>32
Ciprofloxacin				4	16	≤0.06–>16
Colistin				8	>16	0.12–>16
Levofloxacin	66.8	12.9	20.3	2	8	≤0.06–>32
Moxifloxacin				1	4	≤0.06–>16
Tigecycline				2	4	0.12–>16
Trimethoprim/sulfamethoxazole	97.9		2.1	0.25	1	≤0.12–>8
<i>Serratia marcescens</i> (1003)						
Amikacin	96.1	2.3	1.6	≤2	4	≤2–>64
Cefepime	98.8	0.8	0.4	≤1	≤1	≤1–>32
Ceftazidime	98.5	0.4	1.1	≤0.5	1	≤0.5–>32
Ceftazidime/avibactam	99.1		0.9	≤0.25	1	≤0.25–>16
Ceftobiprole				≤1	≤1	≤1–>16
Ceftolozane/tazobactam	96.5	1.4	2.1	1	2	≤0.12–64
Ceftriaxone	92.0	2.5	5.5	≤1	≤1	≤1–>64
Ciprofloxacin	82.3	3.6	14.1	≤0.06	1	≤0.06–>16
Ertapenem	98.0	1.0	1.0	≤0.06	0.12	≤0.06–>32
Fosfomycin				16	32	≤1–64
Gentamicin	96.1	0.9	3.0	≤0.5	1	≤0.5–>32
Imipenem/relebactam	76.7	21.6	1.7	1	2	0.25–>32
Levofloxacin	82.2	5.5	12.3	0.12	2	≤0.06–16
Meropenem	99.5	0.3	0.2	≤0.12	≤0.12	≤0.12–8
Moxifloxacin				0.25	2	≤0.06–>16
Nitrofurantoin		0.7	99.3	256	>512	64–>512
Piperacillin/tazobactam	94.8	2.1	3.1	2	4	≤1–256
Plazomicin	97.1	2.1	0.8	1	2	≤0.12–8
Tigecycline ^c	81.7	15.6	2.7	2	4	0.06–>16
Trimethoprim/sulfamethoxazole	96.0		4.0	0.5	1	≤0.12–>8
<i>Klebsiella aerogenes</i> (437)						
Amikacin	99.4	0.6		≤2	≤2	≤2–8
Cefepime	98.0	0.7	1.3	≤1	≤1	≤1–>32
Ceftazidime	73.3	2.3	24.4	≤0.5	>32	≤0.5–>32
Ceftazidime/avibactam	99.1		0.9	≤0.25	0.5	≤0.25–16
Ceftobiprole ^b	98.5		1.5	≤1	≤1	≤1–>16
Ceftolozane/tazobactam	85.9	8.4	5.7	0.5	4	≤0.12–64
Ceftriaxone	72.1	0.9	27.0	≤1	32	≤1–>64
Ciprofloxacin	92.8	2.3	4.9	≤0.06	0.25	≤0.06–>16
Colistin		98.0	2.0	0.5	1	0.12–>16
Ertapenem	93.2	4.4	2.4	0.12	0.5	≤0.06–>32
Fosfomycin				32	64	8–64
Gentamicin	99.8		0.2	≤0.5	≤0.5	≤0.5–32
Imipenem/relebactam	97.3	1.6	1.1	0.25	1	0.06–16
Levofloxacin	92.2	1.1	6.7	≤0.06	0.5	≤0.06–16

Continued

Table 2. Continued

Organism (no. of isolates) Antimicrobial agent ^a	MIC interpretation			MIC (mg/L)		
	% Susceptible	% Intermediate	% Resistant	MIC ₅₀	MIC ₉₀	MIC range
Meropenem	99.3	0.2	0.5	≤0.12	≤0.12	≤0.12->32
Moxifloxacin				0.12	1	≤0.06-16
Nitrofurantoin	18.1	51.0	30.9	64	128	8->512
Piperacillin/tazobactam	73.4	9.6	17.0	4	32	≤1-512
Plazomicin	100			0.5	1	≤0.12-2
Tigecycline ^c	96.3	2.8	0.9	1	2	0.06-8
Trimethoprim/sulfamethoxazole	98.1		1.9	≤0.12	0.5	≤0.12->8
<i>Citrobacter freundii</i> (260)						
Amikacin	97.3	2.7		≤2	≤2	≤2-8
Cefepime	95.3	3.4	1.3	≤1	≤1	≤1->32
Ceftazidime	79.2	0.9	19.9	≤0.5	>32	≤0.5->32
Ceftazidime/avibactam	99.5		0.5	≤0.25	1	≤0.25-16
Ceftobiprole ^b	87.9	3.3	8.8	≤1	2	≤1->16
Ceftolozane/tazobactam	83.7	4.1	12.2	0.25	8	≤0.12->64
Ceftriaxone	77.7	1.5	20.8	≤1	64	≤1->64
Ciprofloxacin	86.5	2.3	11.2	≤0.06	1	≤0.06->16
Colistin		99.1	0.9	0.5	0.5	0.12-8
Ertapenem	96.2	2.1	1.7	≤0.03	0.25	≤0.03-32
Fosfomycin				≤1	2	≤1-2
Gentamicin	90.3	0.4	9.3	≤0.5	2	≤0.5->32
Imipenem/relebactam	98.9	1.1		0.25	0.5	0.12-2
Levofloxacin	88.4	2.3	9.3	≤0.06	1	≤0.06->32
Meropenem	99.6	0.4		≤0.12	≤0.12	≤0.12-2
Moxifloxacin				0.12	4	≤0.06->16
Nitrofurantoin	93.4	4.0	2.6	16	32	4->512
Piperacillin/tazobactam	80.3	4.6	15.1	2	64	≤1->512
Plazomicin	100			0.5	1	≤0.12-2
Tigecycline ^c	95.4	4.2	0.4	0.5	1	0.25-8
Trimethoprim/sulfamethoxazole	82.6		17.4	≤0.12	>8	≤0.12->8
<i>Acinetobacter baumannii</i> (274)						
Amikacin	95.6		4.4	≤2	4	≤2->64
Cefepime	83.7	6.1	10.2	4	32	≤1->32
Ceftazidime	78.0	10.8	11.2	8	32	≤0.5->32
Ceftazidime/avibactam				8	>16	≤0.25->16
Ceftolozane/tazobactam				1	8	≤0.12->64
Ceftriaxone	41.6	47.8	10.6	16	64	≤1->64
Ciprofloxacin	92.3	1.5	6.2	0.25	1	≤0.06-32
Colistin		98.1	1.9	1	2	0.25->16
Gentamicin	92.4	1.8	5.8	≤0.5	2	≤0.5->32
Imipenem/relebactam	95.4		4.6	0.25	0.5	0.06->32
Levofloxacin	92.2		7.8	0.12	1	≤0.06->16
Meropenem	94.2	1.8	4.0	0.5	2	≤0.12->32
Moxifloxacin				≤0.06	0.5	≤0.06->16
Piperacillin/tazobactam	79.2	10.2	10.6	4	128	≤1->512
Plazomicin				1	4	0.25->64
Tigecycline				0.5	1	0.12->16
Trimethoprim/sulfamethoxazole	91.6		8.4	≤0.12	2	≤0.12->8

^aOrganism-antimicrobial combinations for which intrinsic resistance is known¹⁶ have been excluded from the table.

^bHealth Canada approved breakpoints were used to interpret ceftobiprole MICs.

^cFDA approved breakpoints were used to interpret tigecycline MICs.

The most active agents against the Gram-negative bacilli tested by the CANWARD study were the newer β -lactam/ β -lactamase inhibitor combination agents (ceftazidime/avibactam, imipenem/relebactam, meropenem/vaborbactam) and plazomicin as well as the established agents amikacin, cefepime, ceftobiprole, ceftolozane/tazobactam, ertapenem (excluding *P. aeruginosa*), meropenem and tigecycline (excluding *P. aeruginosa*) (Table 2). Percent susceptible values approaching 100% for the newer β -lactam/ β -lactamase inhibitor combination agents versus Gram-negative bacilli, including various resistance phenotypes as well as *P. aeruginosa* has been previously documented.^{10,11,17–23}

In this study, amikacin was very active against *E. coli* (including ESBL-producing isolates) and other Enterobacterales (Table 2). Against *P. aeruginosa* and *A. baumannii*, amikacin was one of the most active agents tested. The excellent *in vitro* activity of amikacin and other aminoglycosides against both Enterobacterales and non-fermenters isolated from patients in hospitals, including those in ICUs, is well established. The reduced usage (selective pressure) of aminoglycosides in favour of other less toxic antimicrobial classes (e.g. β -lactams, fluoroquinolones) over the last three decades has resulted in the maintenance of aminoglycoside activity in the setting of increasing fluoroquinolone resistance.^{18,19} Thus, amikacin represents a potential option for the treatment of infections caused by Gram-negative bacilli resistant to other less toxic agents. Plazomicin, a new aminoglycoside was active against *E. coli* (including 99.5% of ESBL-producing isolates) and other Enterobacterales tested (Table 2) including gentamicin-resistant isolates (data not shown). As previously documented, this agent holds promise for the treatment of ESBL, carbapenem-resistant and MDR Enterobacterales.^{19,20}

In this study, we report that cefepime, ertapenem, meropenem and piperacillin/tazobactam were very active against Enterobacterales. However, only ertapenem and meropenem maintained their activity against ESBL-producing isolates due to the presence of multiple β -lactamases in these isolates. Against *P. aeruginosa*, resistance rates for piperacillin/tazobactam, meropenem and cefepime were less than ~15%. Previous investigators have reported the ongoing activity of these agents against Gram-negative bacilli isolated from hospitalized patients.^{21–23} Ceftolozane-tazobactam is a broad-spectrum cephalosporin with activity against Enterobacterales, including ESBL-producing isolates (Table 2). In this study, we report that ceftolozane-tazobactam was the most active agent tested against *P. aeruginosa* (96.6% susceptibility). We and others have reported that ceftolozane-tazobactam demonstrated excellent *in vitro* activity against resistant Enterobacterales as well as MDR and XDR *P. aeruginosa* isolates.^{24–26} Ceftobiprole is a broad-spectrum cephalosporin with activity versus both Gram-negative and Gram-positive organisms.^{27–29} In this study, we report that its activity against Enterobacterales is greater than ceftriaxone (Table 2) but was inactive against ESBL-producing isolates, which was expected. Ceftobiprole's activity against *P. aeruginosa* is similar to cefepime and ceftazidime (Table 2).

Tigecycline demonstrated excellent *in vitro* activity versus *E. coli* (including ESBL-producing isolates) and was also active against other Enterobacterales including *K. pneumoniae*, *E. cloacae*, *S. marcescens* and *K. oxytoca* (Table 2). These data are

consistent with recent studies showing the excellent *in vitro* activity of tigecycline versus Gram-negative bacilli, including MDR isolates.^{19,24} Tigecycline displayed good activity against *S. maltophilia* and *A. baumannii*, organisms frequently resistant to other antimicrobial classes (Table 2).³⁰ Fosfomycin was highly active *in vitro* against *E. coli* (98.8% susceptible), including ESBL-producing isolates (96.7% susceptible). Fosfomycin's activity versus *E. coli* was consistent with earlier reports.^{31,32}

There are limitations associated with the CANWARD surveillance study including that we cannot be certain that all isolates from clinical specimens represented active infection. In the CANWARD surveillance study, participating medical centres committed to study criteria that encouraged the submission of clinically significant specimens from patients with a presumed infectious disease; however, this interpretation cannot be rigorously controlled by the coordinating site. Another limitation is that we do not have admission date data for each patient/clinical specimen, thus we are not able to provide a more accurate description of community versus healthcare onset. Finally, an important limitation is that antimicrobial susceptibility testing was not performed for all commercially available antimicrobial agents due to the lack of space on the custom-designed susceptibility panels utilized. It is recognized that data on antimicrobials such as cefotaxime, imipenem, tobramycin and others would be beneficial as different hospital formularies stock these and other antimicrobials not tested in this study.

In conclusion, *E. coli*, *P. aeruginosa*, *Klebsiella* spp., *H. influenzae*, *E. cloacae* and *S. maltophilia* represent the most common Gram-negative bacterial pathogens isolated by laboratories in Canadian hospitals. ESBL-producing *E. coli* and *K. pneumoniae* increased significantly over the study period, while carbapenem-resistant *E. coli* and *K. pneumoniae* remained at less than 1% for the entire study period. Percent susceptible values for *E. coli* isolates were highest for meropenem/vaborbactam and imipenem/relebactam, 100%; ceftazidime/avibactam, tigecycline and meropenem, 99.9%; ertapenem, 99.6%; ceftolozane/tazobactam, 99.1%; and piperacillin/tazobactam, 95.6%. Percent susceptible values for *P. aeruginosa* isolates were highest for ceftolozane/tazobactam, 96.6%; ceftazidime/avibactam, 92.6%; and amikacin and imipenem/relebactam, 92.0%.

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

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Transparency declarations

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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.

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Characterization of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: 17 years of the CANWARD study (2007–23)

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Objectives: This study reviewed the patient demographic parameters, molecular characteristics and *in vitro* antimicrobial susceptibility testing results for MRSA isolates infecting inpatients and outpatients presenting for care to tertiary-care Canadian hospitals between 2007 and 2023.

Methods: DNA sequencing was used to generate *spa* types. Pantone-Valentine leukocidin (PVL) genes were detected by PCR. Broth microdilution antimicrobial susceptibility testing (CLSI M7, 12th edition, 2024) was performed with MICs interpreted by CLSI M100 breakpoints (34th edition, 2024) when available.

Results: In total, 2697 MRSA were identified among 12734 *Staphylococcus aureus* isolates submitted to the CANWARD study between 2007 and 2023. The annual proportion of MRSA decreased significantly from 2007 (26.1%) to 2017 (16.0%) and then increased to 24.2% in 2023 ($P < 0.0001$). From 2007 to 2023, community-associated (CA)-MRSA *spa* types increased from 20.8% to 75.0% ($P < 0.0001$), while hospital-associated (HA)-MRSA decreased from 79.2% to 25.0% ($P < 0.0001$). The predominant MRSA *spa* types identified among all isolates were t002 (36.3%), an HA-MRSA genotype, and t008 (24.5%), a CA-MRSA genotype. PVL was detected in 36.0% of all MRSA isolates (76.3% of CA-MRSA; 2.7% of HA-MRSA). Percent susceptible values for all MRSA isolates were $\geq 99\%$ for ceftobiprole, dalbavancin, daptomycin, linezolid, nitrofurantoin and vancomycin. Notable differences in percent susceptible values were identified for clindamycin (HA-MRSA, 38.9%; CA-MRSA, 86.9%) ($P < 0.0001$) trimethoprim/sulfamethoxazole (HA-MRSA, 91.4%; CA-MRSA, 98.8%) ($P < 0.0001$) and doxycycline (HA-MRSA, 96.6%; CA-MRSA, 98.8%) ($P = 0.0003$).

Conclusions: The changing epidemiology of MRSA in Canadian hospitals warrants continued national surveillance efforts as a resource to support therapeutic guidelines and infection control and prevention programmes.

Introduction

Staphylococcus aureus is a medically important, virulent human pathogen that can cause a multitude of serious infections. Methicillin resistance in *S. aureus* is conferred by penicillin-binding protein-2a (*mecA* gene), a transpeptidase that catalyses cell wall crosslinking in the presence of almost all β -lactam agents with the exception of two newer cephalosporins, ceftobiprole and cef- taroline. Outbreaks of MRSA were initially described in the early 1960s shortly after the introduction of methicillin into clinical use. Since that time, MRSA has spread worldwide and presents infection control and prevention programme challenges and therapeutic issues in both hospital (hospital-associated [HA]-MRSA) and community (community-associated [CA]-MRSA) settings.

HA-MRSA is defined by infection >48 h following hospitalization or within 12 months of exposure to healthcare.¹ HA-MRSA is correlated with severe, invasive infections, multidrug resistance, well-established risk factors, serious morbidity and mortality.¹ CA-MRSA is defined by infection in the absence of healthcare exposure and is most often associated with skin and soft tissue in young, otherwise healthy persons, although invasive disease, including bacteraemia associated with sepsis and necrotizing pneumonia can occur.¹ Many CA-MRSA carry Pantone-Valentine leukocidin (PVL) genes that generate enhanced virulence.² CA-MRSA are generally resistant to fewer non- β -lactam antimicrobial classes than are HA-MRSA.¹

In the current study, we reviewed the patient demographic parameters, molecular characteristics and *in vitro* antimicrobial

susceptibility testing results for MRSA isolates infecting inpatients and outpatients presenting for care to tertiary-care Canadian hospitals over a 17-year period from 2007 to 2023. This study expands upon earlier reports on MRSA from the CANWARD study.^{3,4}

Materials and methods

Bacterial isolates

Between 2007 and 2023, 12 734 *S. aureus* isolates were submitted to the CANWARD study, an annual, ongoing national surveillance study assessing bacterial pathogen prevalence and antimicrobial resistance in Canadian hospitals. CANWARD is comprised of tertiary-care medical centres that are geographically distributed in a population-based fashion including eight of the 10 Canadian provinces. Each centre submitted isolates from inpatients and outpatients attending hospital clinics, emergency rooms, medical/surgical wards or intensive care units. Centres were requested to submit defined 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 blood-stream infections. Centre participation varied slightly from year-to-year as did the numbers of isolates requested by infection source.⁵

The CANWARD study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Antimicrobial susceptibility testing

The *in vitro* activity of ceftaxime and other relevant antimicrobial agents was determined by broth microdilution following CLSI guidelines.⁶ MICs were interpreted as susceptible, intermediate or resistant using breakpoints established by the CLSI⁷ when available. For tigecycline, MIC interpretation was based on the US FDA package insert label (susceptible breakpoint ≤ 0.5 mg/L; <https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria>). Ceftobiprole MICs were interpreted using EUCAST breakpoints (susceptible, ≤ 2 mg/L; resistant, >2 mg/L).⁸

MRSA and MSSA isolates were submitted by centres to CANWARD with their methicillin phenotype indicated. Following broth microdilution testing with ceftaxime,⁶ discrepant MRSA and MSSA isolates were sometimes identified. These rare discrepant isolates were re-tested by broth microdilution. In addition, CLSI reference ceftaxime disk diffusion testing⁹ was performed and isolates were assessed for growth on MRSA Select chromogenic media (Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) to resolve phenotype discrepancies. Ceftobiprole-resistant isolates and isolates with not susceptible phenotypes for dalbavancin, daptomycin and vancomycin were confirmed by broth microdilution re-testing given their accepted infrequency.

Molecular typing

From 2007 to 2021, all isolates with an MRSA phenotype were confirmed as MRSA by real-time PCR for *mecA* and species-specific *nuc* genes.¹⁰ Simultaneous detection of the PVL-encoding *lukF-PV* and *lukS-PV* genes was also performed as part of this triplex assay.¹⁰ MRSA isolates were then characterized by sequence-based typing of the repeat region of the staphylococcal protein A (*spa*) gene.¹¹ Sequences were analysed and assigned *spa* types according to the Ridom *spa* server (spaServer.ridom.de; Ridom GmbH).¹² In 2022 and 2023, WGS was used to obtain the aforementioned genetic data. 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 on an internal Galaxy platform (<https://github.com/tseemann/shovill>). StarAMR v0.9.1¹³ was used to detect antimicrobial resistance genes using the ResFinder database v2022-05-24.¹⁴ ABRicate v1.0.1¹⁵ was used to detect PVL using the virulence factor database (VFDB).¹⁶ WGS data can be found on NCBI under BioProject PRJNA1225611.

A high degree of concordance between *spa* types and established Canadian (and USA) epidemic clones has been documented.¹² For the purpose of this study, CA-MRSA and HA-MRSA were defined genotypically based upon their *spa* type and the assignment of that *spa* type to an established Canadian (and USA) epidemic clone.¹² Isolates with *spa* types assigned to PFGE types CMRSA1 (USA600), CMRSA2 (USA100/800), CMRSA3/6, CMRSA4 (USA200), CMRSA5 (USA500), CMRSA8 or CMRSA9 were categorized as HA-MRSA.¹⁷ Isolates with *spa* types identified as PFGE types CMRSA7 (USA400) or CMRSA10 (USA300) were classified as CA-MRSA.¹⁷ All other isolates, such as those with infrequent *spa* types or isolates without an equivalent Canadian epidemic type (such as the USA700, USA1000 and USA1100 strains) were also considered CA-MRSA.

Statistical analysis

Fisher's exact two-tailed test was used to identify statistically significant differences in demographic characteristics, antimicrobial susceptibilities and changes in antimicrobial susceptibility over time for CA-MRSA and HA-MRSA. The Cochran-Armitage test was used to identify statistically significant linear trends in patient demographics, MRSA proportion, *spa* type distribution and PVL status. For all statistical analysis, isolates were defined as either susceptible or non-susceptible to each antimicrobial agent. Isolates with intermediate (where applicable) susceptibility were included as part of the non-susceptible group. Variables included year of study, patient age group (<18 , 18–65, >65 years), biological sex (male, female), patient location (outpatient [emergency room and clinic/office] or inpatient [ICU, medical/surgical ward]) and specimen type (blood, respiratory wound or urine). For CA-MRSA and HA-MRSA and each antimicrobial agent, an initial Cochran-Armitage test was performed to identify organism-antimicrobial agent combinations demonstrating an overall trend (increasing or decreasing) in the proportion of non-susceptible isolates with a *P* value ≤ 0.1 . Identified combinations were then subjected to a second-degree factorial multivariate logistic regression analysis using available demographic variables to isolate the effect of time on antimicrobial susceptibility from changes in demographic variables that may have occurred in the dataset as well as second-degree interactions among the variables. Initially, a stepwise regression model was created including all the variables and second-degree interactions. In order to exclude variables without significant impact on the model, variables were excluded from the final model in such a way to minimize the Bayesian Information Criterion. Finally, a regression model was created including only the variables and interactions with a significant impact on resistance to an antimicrobial. Statistical analysis was performed using JMP pro version 18 software by SAS (Cary, NC, USA). A *P* value of ≤ 0.05 was considered statistically significant in all comparisons.

Results

MRSA in Canadian hospitals between 2007 and 2023

Of the 12 734 *S. aureus* isolates collected during the 17-year study period, 21.2% ($n=2697$) were MRSA. In total, 38.6% ($n=1042$) of all MRSA were isolated from respiratory tract infections, 34.4% ($n=929$) were from bloodstream infections, 24.8% ($n=668$) were from wound infections and 2.2% ($n=58$) were from urinary tract infections. Overall, the annual proportion of *S. aureus* isolates that were MRSA decreased from 26.0%

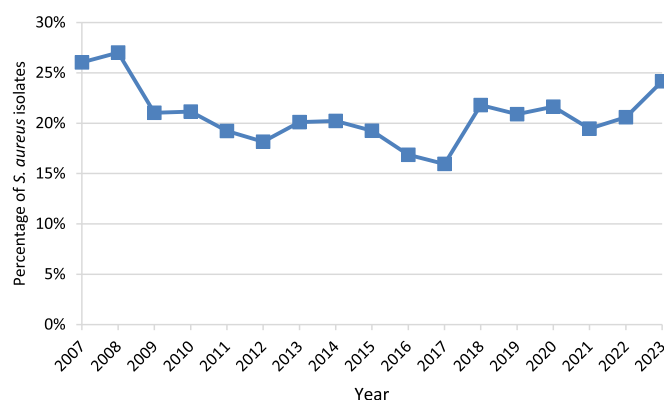


Figure 1. Annual proportion of *S. aureus* isolates identified as MRSA in Canadian hospitals.

(385/1478) in 2007 to 16.0% (115/721) in 2017 before increasing to 24.2% (88/364) in 2023 ($P < 0.0001$) (Figure 1).

Molecular characterization of CA-MRSA and HA-MRSA

Based upon *spa* type, the 2697 MRSA isolates were comprised of 54.8% ($n=1477$) HA-MRSA genotypes and 45.2% ($n=1220$) CA-MRSA genotypes. Figure 2 indicates the annual proportions of MRSA isolates with CA-MRSA and HA-MRSA genotypes. Between 2007 and 2023, CA-MRSA increased significantly from 20.8% (80/385) of MRSA isolates to 75.0% (66/88, $P < 0.0001$). Concurrently, a significant reduction in HA-MRSA from 79.2% (305/385) of MRSA isolates to 25.0% (22/88) was observed during the same period ($P < 0.0001$).

Overall, t008 was the most common CA-MRSA genotype identified in every year from 2007 to 2023; accounting for 24.3% ($n=656$) of all MRSA and 53.8% of all CA-MRSA (Figure 3a). The annual percentage t008 increased significantly over time, rising from 12.0% of MRSA in 2007 to 27.2% of MRSA in 2023 ($P=0.0041$). t128 and t019 were the next most frequently identified *spa* types among CA-MRSA. t128 was identified in every year ($<10\%$ of MRSA in each year) except 2023 and t019 was sporadically identified from 2008 to 2019 but became increasingly common from 2020 to 2023 (as did *spa* types other than t008 and t128). t019 accounted for 12.5% of MRSA in 2023. Other *spa* types, including less common or newly identified *spa* types, increased from 4.4% of MRSA isolates in 2007 to 35.2% in 2023 ($P < 0.0001$).

The most common HA-MRSA genotype identified in every year from 2007 to 2022 was t002 (Figure 3b). It accounted for 36.1% ($n=974$) of all MRSA and 65.9% of all HA-MRSA. The annual percentage of t002 decreased significantly from 53.8% of MRSA in 2007 to 5.7% of MRSA in 2023 ($P=0.026$). In 2023, both t1081 and t002 each accounted for 5.7% of MRSA genotypes. HA-MRSA *spa* types other than t002, t037 and t1081 remained relatively consistent from 2007 to 2023, accounting for approximately 2%–15% of MRSA genotypes per year.

Demographic comparison of CA-MRSA and HA-MRSA

A comparison of the demographic characteristics of patients with infections attributed to CA-MRSA versus HA-MRSA is presented in

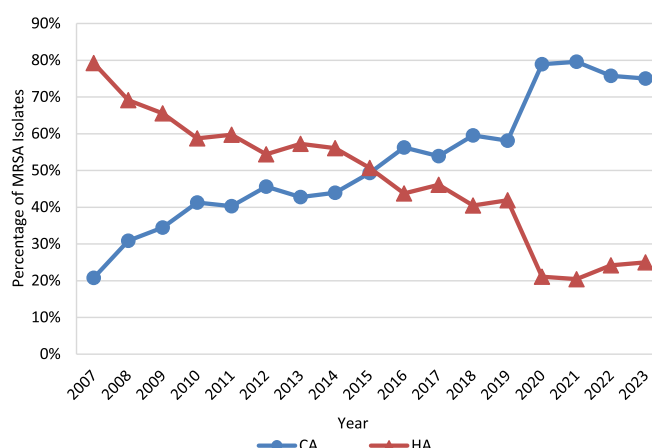


Figure 2. Annual proportion of CA-MRSA and HA-MRSA genotypes in Canadian hospitals.

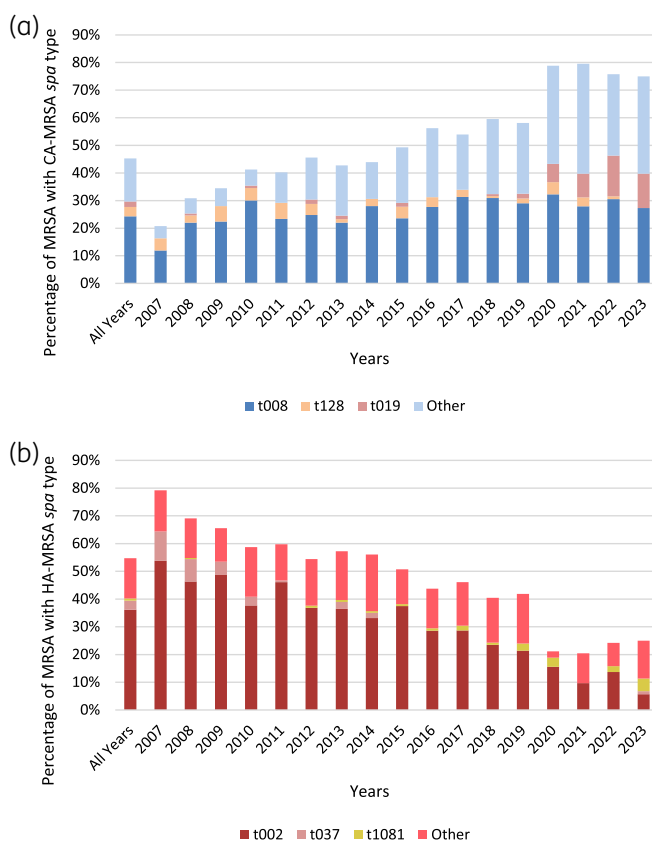


Figure 3. Annual proportion of MRSA isolates with (a) CA-MRSA and (b) HA-MRSA *spa* types.

Table 1. Male patients accounted for 60.4% of CA-MRSA and 58.7% of HA-MRSA. The majority of CA-MRSA infections (68.6%) occurred in patients aged 18–64 years, whereas most of HA-MRSA infections (57.1%) were observed in patients aged ≥ 65 years. The median and mean ages of patients with

Table 1. Demographic characteristics of patients with infections caused by CA-MRSA and HA-MRSA genotypes in Canadian hospitals from 2007 to 2023

Characteristic	CA-MRSA (n=1220)	HA-MRSA (n=1477)	P ^a
Biological sex, n (%) ^b			0.24
male	737 (60.4)	867 (58.7)	
female	482 (39.5)	610 (41.3)	
Mean age, years	44.3	63.9	
Median age, years (range)	46 (1–97)	68 (1–105)	
Age group, years, n (%)			
≤17	152 (12.5)	52 (3.5)	<0.0001
18–64	837 (68.6)	581 (39.3)	<0.0001
≥65	231 (18.9)	844 (57.1)	<0.0001
Region, n (%)			
western Canada	692 (56.7)	447 (30.3)	<0.0001
Ontario	379 (31.1)	504 (34.1)	0.0034
Quebec	80 (6.6)	398 (26.9)	<0.0001
maritimes	69 (5.7)	128 (8.7)	0.0029
Patient location, n (%)			
emergency room	338 (27.7)	214 (14.5)	<0.0001
clinic/office	181 (14.8)	199 (13.5)	0.91
ICU	247 (20.2)	285 (19.3)	0.059
medical/surgical ward	454 (37.2)	779 (52.7)	<0.0001
Infection site, n (%)			
bloodstream	423 (34.7)	506 (34.3)	0.8
respiratory tract	370 (30.3)	672 (45.5)	<0.0001
wound	419 (34.3)	249 (16.9)	<0.0001
urinary tract	8 (0.7)	50 (3.4)	<0.0001

^aP value determined using Fisher’s exact test.

^bBiological sex not available for one isolate.

CA-MRSA and HA-MRSA infection were 46 and 44.3 years, and 68 and 63.9 years, respectively. CA-MRSA were more likely than HA-MRSA to have been isolated in western Canada (56.7% versus 30.3%, $P<0.0001$), whereas HA-MRSA were more frequently isolated than CA-MRSA in Ontario (34.1% versus 31.1%, $P<0.0034$), Quebec (26.9% versus 6.6%, $P<0.0001$) and the maritime provinces (8.7% versus 5.7%, $P=0.0029$).

HA-MRSA (52.7%) and CA-MRSA (37.2%) were both most commonly recovered from patients on medical/surgical wards than other clinical settings. The proportion of CA-MRSA isolates recovered from emergency room patients decreased from 44.7% to 24.2% between 2007 and 2023 ($P=0.0056$). This trend was accompanied by an increase in the proportion of CA-MRSA from patients admitted to medical/surgical wards (23.8% in 2007 to 49.9% in 2023, $P=0.013$).

Bloodstream (34.7%), wound (34.3%) and respiratory tract (30.3%) infections each accounted for approximately one-third of CA-MRSA isolates. The proportion of CA-MRSA from respiratory sources increased from 17.1% in 2007 to 40.1% in 2023 ($P=0.0028$). HA-MRSA was most commonly recovered from respiratory tract (45.5%) and bloodstream (34.2%) infections than other infection sites. The proportion of HA-MRSA from respiratory sources increased significantly from 36.1% in 2007 to 72.7% in

2023 ($P=0.0011$). Concurrently, over the same time period, the proportion of HA-MRSA from bloodstream infections decreased from 45.0% to 18.2% ($P=0.0145$).

PVL genes were detected in 36.0% ($n=970$) of all MRSA isolates. By genotype, 76.3% (931/1220) of CA-MRSA were PVL-positive compared to 2.6% (39/1477) of HA-MRSA (Table 2). Between 2007 and 2023, the PVL-positive rate among HA-MRSA isolates increased from 0.3% to 9.1% ($P=0.005$). In contrast, the proportion of PVL-positive CA-MRSA decreased from 92.5% to 68.2% during the same time period ($P=0.0002$). No significant changes in PVL status were observed among the dominant *spa* types for both CA-MRSA and HA-MRSA (Figure 3). When compared with PVL-negative isolates, PVL-positive strains were more likely to be recovered from patients admitted to a medical/surgical ward ($P<0.0001$) or presenting to an emergency room ($P<0.0001$) (data not shown) than from patients in other locations. Additionally, PVL-positive isolates were more frequently associated with wound infections ($P<0.0001$) than other infection types (data not shown).

Antimicrobial susceptibilities of CA-MRSA and HA-MRSA

Percent susceptible values for all MRSA isolates were $\geq 99\%$ for ceftobiprole, dalbavancin, daptomycin, linezolid, nitrofurantoin and vancomycin (Table 3). The modal vancomycin MIC for all MRSA isolates was 1 mg/L and remained unchanged over time. No vancomycin-resistant isolates were detected, but an intermediate MIC interpretation (4 mg/L) was observed in two isolates, both of which were HA-MRSA, including one with *spa* type t002. One isolate that was not susceptible to dalbavancin (MIC, 0.5 mg/L) was also identified and confirmed; it was a CA-MRSA t019 *spa* type. The antimicrobial susceptibility testing profile for isolates of the emerging CA-MRSA *spa* type t019 ($n=34$) was not significantly different from the profile for t008 isolates, the most common CA-MRSA *spa* type ($n=320$). Ceftobiprole resistance (MIC, 4 mg/L) was confirmed in two isolates of HA-MRSA. Less than 25% of all MRSA isolates were susceptible to clarithromycin, ciprofloxacin, levofloxacin and moxifloxacin. Percent susceptible values for clindamycin (HA-MRSA, 38.9%; CA-MRSA, 86.9%) ($P<0.0001$), doxycycline (HA-MRSA, 96.6%; CA-MRSA, 98.8%) ($P=0.0003$) and trimethoprim/sulfamethoxazole (HA-MRSA, 91.4%; CA-MRSA, 98.8%) ($P<0.0001$) were significantly lower for HA-MRSA than CA-MRSA.

Over time, resistance to clarithromycin, clindamycin and fluoroquinolones declined significantly ($P<0.0001$) (data not shown). Clarithromycin resistance was highest in Quebec, more frequent in isolates from patients aged ≥ 65 years and more common in HA-MRSA than CA-MRSA ($P<0.0001$). Similarly, clindamycin resistance was associated with older adults and more common in HA-MRSA than CA-MRSA ($P<0.0001$). Fluoroquinolone resistance was higher in eastern Canada, predominantly observed in isolates from patients aged ≥ 65 years and more common in HA-MRSA than CA-MRSA ($P<0.0001$). Fluoroquinolone resistance was also elevated in medical/surgical wards compared to intensive care units ($P=0.0005$) and higher in outpatient isolates compared to isolates from hospitalized patients ($P<0.0001$). Additionally, fluoroquinolone resistance was more frequent in urinary and respiratory tract infection isolates than in wound or bloodstream isolates ($P=0.0001$). Resistance to trimethoprim/

	Study year														Total	P ^a		
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020			2021	2022
PVL gene, n (%)																		
HA-MRSA																		
positive	1 (0.3)	1 (0.5)	0 (0.0)	4 (3.1)	0 (0.0)	1 (1.5)	9 (9.9)	2 (2.3)	0 (0.0)	3 (6.1)	1 (1.9)	3 (5.5)	5 (10.2)	2 (10.5)	3 (15.8)	2 (8.7)	2 (9.1)	39 (2.7)
negative	304 (99.7)	187 (99.5)	152 (100)	127 (96.9)	92 (100)	67 (98.5)	82 (90.1)	86 (97.7)	73 (100)	46 (93.9)	52 (98.1)	52 (94.5)	44 (89.8)	17 (89.5)	16 (84.2)	21 (91.3)	20 (90.9)	1438 (97.3)
CA-MRSA																		
positive	74 (92.5)	66 (78.6)	68 (85.0)	78 (84.8)	52 (83.9)	39 (68.4)	46 (67.6)	54 (78.3)	50 (70.4)	42 (66.7)	43 (69.4)	61 (75.3)	47 (69.1)	59 (83.0)	52 (70.3)	55 (76.4)	45 (68.2)	931 (76.3)
negative	6 (7.5)	18 (21.4)	12 (15.0)	14 (15.2)	10 (16.1)	18 (31.6)	22 (32.4)	15 (21.7)	21 (29.6)	21 (33.3)	19 (30.6)	20 (24.7)	21 (30.9)	12 (17)	22 (29.7)	17 (23.6)	21 (31.8)	289 (23.7)

sulfamethoxazole was higher in western Canada than other regions of Canada ($P<0.0001$); no other significant trends were identified for trimethoprim/sulfamethoxazole.

MRSA is a common HA pathogen² and is also an important pathogen in the community. Over time, CA-MRSA has accounted for an increasing proportion of MRSA isolates in hospitals and long-term care facilities in Canada¹⁸ and worldwide.^{19–22} In the current study, the prevalence of CA-MRSA among all MRSA in Canada increased from 20.8% of isolates in 2007 to 75.0% in 2023 (Figure 2). This rise coincided with both a stepwise decrease in the annual proportion of *S. aureus* isolates that were MRSA from 26.0% in 2007 to 16.0% in 2017 followed by a stepwise increase to 24.2% in 2023 (Figure 1). The observed increase in MRSA prevalence beginning in 2018 is concerning given previous decreases from 2007 to 2017. Explanations for this observation are likely multifactorial but may include reduced healthcare spending on hospital infection control and prevention and antimicrobial stewardship programmes, the emergence of other priorities (e.g. SARS-CoV-2 pandemic) as well as the admission of sicker patients to hospital, longer hospital and ICU stays, greater numbers of invasive clinical procedures, an aging patient population and significantly higher MRSA colonization rates among the elderly than among the general population.^{2,22}

CA-MRSA is more susceptible to many non- β -lactam antimicrobial agents (clarithromycin, clindamycin, doxycycline, tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin and moxifloxacin) than are HA-MRSA in Canada (Table 3). In the current study, we also observed percent susceptible values for clarithromycin, clindamycin and fluoroquinolones decreased significantly over time from 2007 to 2023. This finding is in concordance with a report from the SENTRY surveillance program that collected consecutive, clinically significant isolates of

Table 3. Antimicrobial susceptibility of CA-MRSA and HA-MRSA genotypes in Canadian hospitals from 2007 to 2023

Organism (no. tested)/antimicrobial agent	MIC (mg/L)			MIC interpretation		
	MIC ₅₀	MIC ₉₀	MIC range	% Susceptible	% Intermediate	% Resistant
MRSA (n = 2697)						
ceftobiprole	≤1	2	≤1–4	99.9	–	0.1
ciprofloxacin	>16	>16	0.25 to >16	22.2	0.5	77.3
clarithromycin	>16	>16	0.25 to >16	18.7	0.6	80.7
clindamycin	0.25	>8	0.25 to >8	60.6	0.1	39.3
dalbavancin	0.06	0.06	≤0.03–0.5	99.9	–	–
daptomycin	0.25	0.5	0.06–4	99.9	–	–
doxycycline	≤0.12	1	≤0.12–16	97.8	1.0	1.2
levofloxacin	>32	>32	0.12 to >32	14.1	–	85.9
linezolid	2	2	≤0.12–16	100	–	–
moxifloxacin	8	>16	≤0.06 to >16	19.1	4	76.9
nitrofurantoin	16	16	8–128	99.7	0.1	0.2
tigecycline	0.25	0.5	0.03–2	98.2	–	–
trimethoprim/sulfamethoxazole	≤0.12	0.25	≤0.12 to >8	94.7	–	5.3
vancomycin	1	1	≤0.12–4	99.9	0.1	0
HA-MRSA (n = 1477)						
ceftobiprole	≤1	2	≤1–4	99.9	–	0.1
ciprofloxacin	>16	>16	0.25 to >16	8.6	0.3	91.1
clarithromycin	>16	>16	0.25 to >16	8.2	0.5	91.3
clindamycin	>8	>8	0.25 to >8	38.9	–	61.1
dalbavancin	0.06	0.06	≤0.03–0.25	100	–	–
daptomycin	0.25	0.5	0.06–4	99.9	–	–
doxycycline	≤0.12	2	≤0.12–16	96.6	1.0	2.4
levofloxacin	>32	>32	0.12 to >32	2.9	–	97.1
linezolid	2	4	≤0.12–4	100	–	–
moxifloxacin	8	>16	≤0.06 to >16	6.7	0.5	92.8
nitrofurantoin	16	16	8–128	99.5	0.2	0.3
tigecycline	0.25	0.5	0.03–2	97.5	–	–
trimethoprim/sulfamethoxazole	≤0.12	0.5	≤0.12 to >8	91.4	–	8.6
vancomycin	1	1	≤0.12–4	99.9	0.1	0
CA-MRSA (n = 1220)						
ceftobiprole	≤1	2	≤1–2	100	–	0
ciprofloxacin	16	>16	0.25 to >16	38.6	0.8	60.6
clarithromycin	>16	>16	0.25 to >16	31.4	0.7	67.9
clindamycin	0.25	>8	0.25 to >8	86.9	0.1	13.0
dalbavancin	0.06	0.12	≤0.03–0.5	99.9	–	–
daptomycin	0.25	0.5	0.12–2	99.8	–	–
doxycycline	≤0.12	0.5	≤0.12–16	98.8	1.0	0.2
levofloxacin	4	8	0.12 to 32	63.1	–	36.9
linezolid	2	2	0.5 to >16	99.9	–	0.1
moxifloxacin	2	2	≤0.06 to >16	38.1	9.2	52.7
nitrofurantoin	16	16	8–128	99.9	–	0.1
tigecycline	0.25	0.5	0.03–2	99.1	–	–
trimethoprim/sulfamethoxazole	≤0.12	≤0.12	≤0.12 to >8	98.8	–	1.2
vancomycin	1	1	0.5–2	100	0	0

bacterial pathogens (including *S. aureus*) in North America, Europe, Latin America and the Asia-Pacific region from 2007 to 2016.²⁶ The SENTRY surveillance program also reported lower percent susceptible values for HA-MRSA compared with CA-MRSA for clindamycin, doxycycline and trimethoprim/sulfamethoxazole,²⁶ similar to our study (Table 3).

Vancomycin remains the most common parenteral treatment for MRSA infections.²⁷ In the current study, its activity remained stable over time and only two isolates with an intermediate MIC interpretation (both MICs 4 mg/L) were identified from 2007 to 2023 (Table 3). The modal vancomycin MICs for all MRSA isolates were 1 mg/L in all study years of the CANWARD

study (2007–23) and we did not observe vancomycin MIC creep, confirming the findings of earlier investigators.²⁸ In our study, we also identified and confirmed one isolate (CA-MRSA) that were not susceptible to dalbavancin (isolate MIC of 0.5 mg/L and corresponding vancomycin MIC of 1 mg/L) and two isolates (both HA-MRSA) that were not susceptible to ceftobiprole (both isolate MICs were 4 mg/L). In Canada, dalbavancin is primarily used alone to treat acute bacterial skin and skin structure infections²⁹ and ceftobiprole to treat severe MRSA infections particularly in the case of failing initial antimicrobial therapy.³⁰ Ceftobiprole's utility is further enhanced by the addition of other agents such as vancomycin and daptomycin for synergistic activity for persistent infections.³⁰ The need for judicious use and targeted application of newer agents like dalbavancin and ceftobiprole is crucial to preserve the effectiveness of these last-resort therapies.

Limitations of the CANWARD study relevant to reporting MRSA data have been previously described and remain relevant.⁴ Importantly, we cannot guarantee that the present study accurately reflects the true proportion of community- and healthcare-associated MRSA cases in Canadian hospitals. In addition, there is growing evidence that traditional molecular definitions can no longer consistently distinguish between CA-MRSA and HA-MRSA strains.³¹ Our data may also be confounded by minor inconsistencies in the annual number of contributing sites and changes in the number of isolates, and their associated specimen sources, collected each year.⁵ Despite this variability, our data describe MRSA as a proportion of *S. aureus*, and as such we believe that the observed trends represent true changes in the burden of MRSA in Canada as well as the distribution of MRSA genotypes.

In conclusion, the changing epidemiology of MRSA in Canadian hospitals warrants continued national surveillance efforts, including genomic surveillance, as a resource to support therapeutic guidelines and infection control and prevention programmes.

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Disclaimer

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Comparison of antimicrobial resistance and serotype patterns in *Streptococcus pneumoniae* from blood cultures and respiratory specimens in Canadian hospitals from the CANWARD study (2007–23)

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Objectives: To compare the antimicrobial resistance and serotype patterns in *Streptococcus pneumoniae* collected from blood cultures and respiratory specimens in Canada from 2007 to 2023.

Methods: *S. pneumoniae* isolates were submitted from Canadian hospitals as part of the ongoing national surveillance study, CANWARD. Antimicrobial susceptibility testing was performed by the CLSI broth microdilution method. Serotypes were determined using WGS and/or the Quellung method.

Results: Of the 3111 *S. pneumoniae* isolates collected, 1103 (35.5%) were from blood cultures and 2008 (64.5%) were from respiratory specimens. The most common serotypes overall were 3, 19A, 22F and 11A. Serotypes 4, 5, 7F, 8, 12F, 14, 19A and 22F were more frequently identified ($P \leq 0.01$) among blood culture isolates, while 6C, 7C, 11A, 19F, 21, 23A, 23B, 23F, 31, 34, 35B, 35F and non-typeable strains were more commonly recognized ($P \leq 0.03$) among respiratory isolates. Susceptibility rates were significantly lower in respiratory isolates than blood culture isolates for penicillin, ceftriaxone, clarithromycin, clindamycin and doxycycline ($P \leq 0.03$). Overall, 8.3% of isolates were MDR/XDR; rates increased over the course of the study ($P = 0.004$). MDR/XDR rates were higher in respiratory isolates (10.5%) than in blood culture isolates (5.8%) ($P < 0.0001$). Serotypes 19A (40.6%) and 15A (27.6%) were predominant in the MDR/XDR isolates.

Conclusions: *S. pneumoniae* from respiratory specimens demonstrated lower antimicrobial susceptibilities and higher MDR rates compared with isolates from blood cultures. Significant differences in the serotypes isolated from blood culture and respiratory specimens were observed. A greater proportion of isolates from blood cultures were serotypes covered by current pneumococcal vaccines.

Introduction

Streptococcus pneumoniae has a significant impact on public health worldwide as the causative agent of invasive pneumococcal disease (IPD) and a common cause of respiratory infections, including community-acquired pneumonia (CAP) and acute otitis media. An estimated 3.7 million episodes of severe pneumococcal disease occurred worldwide in children in 2015.¹ The annual incidence rates of IPD in Canada were consistently between 9.1 and 10.9/100 000 population for 2009–19, with a potentially COVID public health measure-impacted rate of 5.9/100 000 reported for 2020.² IPD rates were highest

for children <1 year and 1–4 years of age as well as individuals older than 60 years.² *S. pneumoniae* is the leading cause of CAP, being identified in a recent study as the causative agent of CAP in 33–50% of cases that had an established aetiology.³ Mortality rates of 8.3% were recently reported for hospitalized adults with IPD and noninvasive all-cause pneumonia in the USA.⁴

S. pneumoniae infections frequently require antibiotic therapy. Due to the high worldwide prevalence of penicillin-non-susceptible *S. pneumoniae*, it has been recognized as a priority pathogen by the WHO for new antibiotic development.⁵ Globally, *S. pneumoniae* was the fourth leading pathogen contributing to the burden of

antimicrobial resistance in 2019 contributing to >250 000 deaths associated with antimicrobial resistance.⁶

Vaccines are effective at reducing pneumococcal disease. There are two types of pneumococcal vaccines available: the 23-valent polysaccharide vaccine PNEUMOVAX®23 (PPSV23) and the pneumococcal conjugate vaccines (PCVs). PPSV23 includes 23 pneumococcal serotypes and has been available since 1983 but is only effective for adults.⁷ The conjugate vaccines are effective for children and adults. The introduction of the first pneumococcal conjugate vaccine, Prevnar®, a 7-valent pneumococcal vaccine (PCV7), into routine infant immunization programs in the early 2000s resulted in significant reductions of IPD.⁸ A 13-valent pneumococcal conjugate vaccine, Prevnar®13 (PCV13), replaced PCV7 in routine infant immunization programs largely in response to the significant rise in the incidence of serotype 19A, which was frequently MDR.⁹ Currently, VAXNEUVANCE™, a 15-valent pneumococcal conjugate vaccine (PCV15), and PREVNAR20™, a 20-valent pneumococcal conjugate vaccine (PCV20), are recommended for children and adults in Canada.¹⁰ In 2024, CAPVAXIVE®, a 21-valent conjugate vaccine with different serotype coverage from the sequentially developed previous conjugate vaccines was recommended for use in adult pneumococcal immunization programs in Canada.¹¹ PPSV23 had been recommended for use in adults following a dose of PCV15 as an alternative to vaccination with PCV20;¹⁰ however, vaccination with PCV20 or PCV21 are now preferred.¹¹

The purpose of this study was to compare the antimicrobial resistance and serotype patterns in *S. pneumoniae* collected from blood cultures and respiratory specimens in Canada from 2007 to 2023 as part of the CANWARD study. This study expands upon an earlier report on *S. pneumoniae* from the CANWARD study.¹²

Materials and methods

Bacterial isolates

Between January 2007 and December 2023, 3111 *S. pneumoniae* isolates from blood and respiratory cultures were collected by the annual CANWARD surveillance program. CANWARD is an ongoing national Public Health Agency of Canada-PHAC/Canadian Antimicrobial Resistance Alliance partnered surveillance study evaluating pathogen prevalence and antimicrobial resistance in bacterial pathogens isolated from patients attending tertiary care hospitals across Canada.¹³ Participating hospital sites submitted their first 100 respiratory isolates and the first 10 blood isolates per month, for 10 months, each study year. Isolates were shipped to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada) where the *S. pneumoniae* identifications were confirmed by MALDI-TOF mass spectrometry (Bruker Daltonics, Billerica, MA, USA) and bile solubility, as required, and isolates were stocked for further testing.

The CANWARD surveillance study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Antimicrobial susceptibility testing

As previously described,¹³ antimicrobial susceptibility testing was performed by broth microdilution using in-house prepared 96-well microtitre panels in accordance with CLSI methods.¹⁴ MICs were interpreted based on CLSI breakpoints.¹⁵ Here 2939 isolates had complete antimicrobial susceptibility testing profiles for the MDR analysis. MDR was defined as resistance to three or more classes of antimicrobials, represented by

penicillin, clarithromycin, clindamycin, doxycycline, levofloxacin, trimethoprim/sulfamethoxazole and vancomycin. For the MDR analysis, penicillin resistance was defined using the CLSI breakpoint for oral penicillin V (MIC ≥ 2 mg/L). Isolates resistant to five or more of the antimicrobial classes included in the MDR definition were considered XDR.¹⁶

Serotyping

Isolates collected from 2007 to 2022 were serotyped by the Quellung reaction,¹⁷ with pool, group, type and factor commercial antisera (SSI Diagnostica; Statens Serum Institut, Copenhagen, Denmark). From late 2022 onwards, *S. pneumoniae* were sequenced using the Illumina platform and serotypes were identified directly from whole-genome data using the WADE pipeline (<https://github.com/phac-nml/wade>). Isolates that were non-typeable by WGS were confirmed by Quellung reaction. Serotypes 15B and 15C were grouped as 15B/C due to the reported reversible switching between serotypes.^{18,19}

Statistical analysis

Differences in antimicrobial susceptibility rates, MDR/XDR rates, serotypes and patient demographics between *S. pneumoniae* isolates from respiratory specimens and blood cultures were assessed for significance ($P \leq 0.05$) using a two-tailed Fisher's exact test. Changes in serotype proportion, antimicrobial susceptibility and MDR rates throughout the study were assessed for statistical significance ($P \leq 0.05$) using the Cochran-Armitage test for trend. Based on the test limitations, the Cochran-Armitage test for trend analysis could not be performed on portions of the datasets with low annual isolate counts (counts of <5 in >20% of data points) for a particular parameter.

Results

Of the 3111 *S. pneumoniae* isolates collected as part of CANWARD 2007–23, 1103 (35.5%) were obtained from blood cultures while 2008 (64.5%) were from respiratory specimens. A comparison of the demographic characteristics of patients from whom *S. pneumoniae* was isolated from blood cultures and respiratory cultures is presented in Table 1. Male patients accounted for 55.5% of blood culture isolates and 61.7% of respiratory isolates ($P=0.0009$). Blood culture isolates were collected at a higher rate than respiratory isolates from children 0 to <2 years of age ($P=0.0003$). A significantly greater number of blood culture isolates than respiratory isolates were received from sites in central Canada ($P=0.0003$). A significantly greater number of respiratory isolates compared with blood culture isolates were received from sites in eastern Canada ($P<0.0001$).

There were 57 serotypes identified in the *S. pneumoniae* collected as part of the CANWARD study. Five serotypes were uniquely observed with single isolates from blood culture specimens (9A, 9L, 11F, 12A, 25F). Ten serotypes were observed only for respiratory isolates: 10B, 10F, 11D, 18B, 28F, 41F (each, $n=1$), 6D, 17A (each, $n=2$), 37 ($n=3$) and 35C ($n=4$). Figure 1 displays the proportion of *S. pneumoniae* isolated from blood and respiratory isolates for serotypes of which >30 isolates were identified in the entire dataset. Overall, the most commonly observed serotypes were 3 ($n=275$, 8.8%), 19A ($n=248$, 8.0%), 22F ($n=189$, 6.1%), 11A ($n=181$, 5.8%), 23A ($n=138$, 4.4%), 15B/C ($n=133$, 4.3%), 6C ($n=119$, 3.8%), 15A ($n=115$, 3.7%), 19F ($n=111$, 3.6%) and 9N ($n=109$, 3.5%). The 10 most common serotypes isolated from blood cultures (representing 59.4% of isolates, $n=655$) in order of descending frequency were 19A, 3, 22F,

Table 1. Demographic characteristics of patients with *S. pneumoniae* collected from blood and respiratory specimens as part of the CANWARD study (2007–23)

Characteristic	Blood culture isolates (n=1103)	Respiratory isolates (n=2008)	P ^a
Biological sex, n (%)			0.0009
male	612 (55.5)	1238 (61.7)	
female	491 (44.5)	770 (38.3)	
Age group, years, n (%)			
0–<2	69 (6.3)	68 (3.4)	0.0003
2–<6	71 (6.4)	97 (4.8)	NS ^b
6–<18	51 (4.6)	95 (4.7)	NS ^b
18–<50	287 (26.0)	502 (25.0)	NS ^b
50–<65	274 (24.8)	543 (27.0)	NS ^b
≥65	351 (31.8)	703 (35.0)	NS ^b
Region, n (%)			
western Canada ^c	459 (41.6)	801 (39.9)	NS ^b
central Canada ^d	553 (50.1)	870 (43.3)	0.0003
eastern Canada ^e	91 (8.3)	337 (16.8)	<0.0001

^aP value determined using Fisher's exact test^bNS, not statistically significant^cWestern Canada includes the provinces of British Columbia, Alberta, Saskatchewan and Manitoba^dCentral Canada includes the provinces of Ontario and Quebec^eEastern Canada includes the provinces of New Brunswick and Nova Scotia

4, 7F, 12F, 8, 9N, 11A and 15B/C. Comparatively, the 10 most common serotypes isolated from respiratory specimens (representing 58.1% of isolates, $n=1166$) in order of descending frequency were 3, 11A, 19A, 23A, 22F, 15B/C, 6C, 19F, 15A, 23B and 35B (serotypes rank ordered as 10 and 11 listed as both $n=79$). The top 10 serotypes by specimen source are presented visually in Figure S1 (available as [Supplementary data](#) at JAC Online).

The annual serotype distribution of blood and respiratory isolates and the overall comparison of serotypes by specimen source are detailed in Table S1. Serotypes that were identified significantly more frequently for blood culture isolates than for respiratory isolates included: 4, 5, 7F, 8, 12F, 14, 19A and 22F. Serotypes that were identified significantly more frequently for respiratory isolates than for blood culture isolates included: 6C, 7C, 11A, 19F, 21, 23A, 23B, 23F, 31, 34, 35B, 35F and non-typeable isolates. Few serotypes were identified in sufficient annual numbers to allow for trend analysis. Of those with sufficient datapoints, an increasing trend in prevalence of serotype 9N was observed $P<0.0001$ overall (blood and respiratory isolates combined) while no significant trends were observed for serotype 3, 11A, 22F or 23A.

The percentages of *S. pneumoniae* isolates (stratified as all, blood culture and respiratory), from the entire CANWARD study (2007–23) and the most recent study year (2023), that had a serotype covered by PCV13, PCV15, PCV20, PCV21 and PPSV23 are presented in Table 2. The highest percentage of isolates covered by the vaccines by specimen type was observed for PPSV23

and blood culture isolates (77.8% for all years and 85.7% in 2023, respectively). The percentage of isolates with serotypes included in PCV21 was significantly greater than PPSV23 overall and for respiratory isolates ($P<0.0001$) over the course of the study although no significant differences were observed for the 2023-year subset. The percentage of isolates with serotypes included in PPSV23 was significantly greater than PCV20 overall ($P<0.0001$), for blood culture isolates ($P=0.001$) and for respiratory isolates ($P=0.009$). Similarly, the percentage of isolates with serotypes included in PCV20 was significantly greater than PCV15 ($P<0.0001$) and PCV15 included a significantly greater number of isolates than PCV13 ($P<0.0001$) for all specimen sources (all, blood and respiratory). There was no significant difference in the percentage of isolates with vaccine-included serotypes between the most recent study year (2023) and the entire study dataset (2007–23) for any of the vaccines.

The antimicrobial susceptibilities of *S. pneumoniae* isolates from blood cultures and respiratory specimens, overall and by year, are presented in Table 3. Susceptibility rates were significantly lower in *S. pneumoniae* isolates from respiratory specimens compared with blood culture isolates for penicillin (oral and meningitis breakpoints, $P<0.0001$; non-meningitis breakpoints, $P=0.03$), ceftriaxone (meningitis breakpoint, $P=0.006$), clarithromycin ($P=0.02$), clindamycin ($P=0.0003$) and doxycycline ($P<0.0001$). A trend of decreasing ceftriaxone susceptibility (meningitis breakpoint) was observed overall over the course of the study, from 98.8% in 2007 to 90.9% in 2023 ($P<0.0001$). Decreasing susceptibility trends were also observed for clarithromycin overall and specifically for the blood culture isolates ($P=0.01$ and $P=0.007$, respectively), with susceptibility rates decreasing overall from 80.7% in 2007 to 69.1% in 2023 and 87.3% in 2007 to 71.4% in 2023 for the blood culture isolates. Similarly, decreasing susceptibility trends were observed for doxycycline overall and specifically for respiratory isolates ($P=0.04$), with susceptibility rates decreasing overall from 88.8% in 2007 to 83.6% in 2023 and 87.4% in 2007 to 82.4% in 2023 for respiratory isolates. No other significant trends were observed for other antimicrobials and specimen sources for which the trend analysis could be performed [penicillin (IV, non-meningitis overall), clindamycin (overall and respiratory isolates) and trimethoprim-sulfamethoxazole (all sources)].

The overall and annual rates of MDR and/or XDR in *S. pneumoniae* isolates from blood and respiratory specimens are listed in Table 4. Overall, 8.6% of the isolates were MDR/XDR. MDR/XDR rates in the respiratory isolates (10.0%) were significantly higher than the rates in blood culture isolates (5.8%) ($P<0.0001$). The subsets of MDR (7.5% versus 4.6%, $P=0.002$) and XDR (2.6% versus 1.3%, $P=0.02$) were identified at significantly higher rates in respiratory isolates compared with blood culture isolates. Trend analysis demonstrated significant increases in MDR/XDR *S. pneumoniae* from 2007 to 2023 for all isolates ($P=0.004$) and for the respiratory ($P=0.03$) subset. The same trend was observed for the MDR subset of all *S. pneumoniae* ($P=0.005$).

MDR and/or XDR was observed in 26 serotypes of the *S. pneumoniae* isolates overall (17 in blood culture isolates and 22 in respiratory isolates). The serotype distribution of MDR/XDR *S. pneumoniae* isolates in the CANWARD study (2007–23), overall and by specimen source, are displayed in Figure 2. The

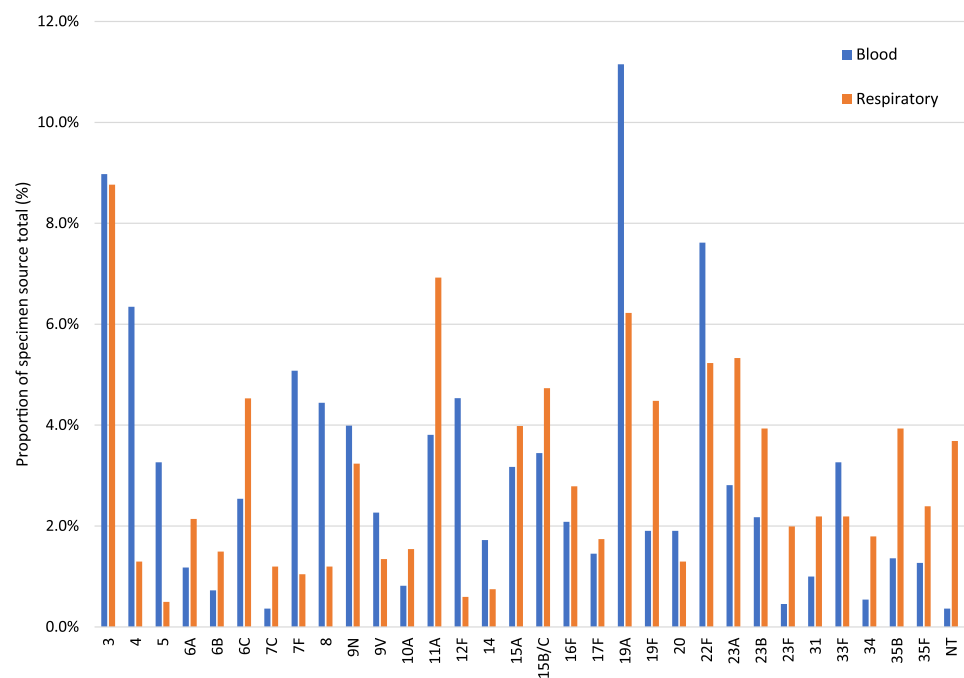


Figure 1. Proportion of *S. pneumoniae* isolated from blood and respiratory specimens by serotype ($n > 30$) in the CANWARD study, 2007–23. Serotypes for which <30 isolates were collected between 2007 and 2023 include: 6D (2; 0 Blood, 2 Respiratory), 7C (28; 4 Blood, 24 Respiratory), 9A (1; 1 Blood, 0 Respiratory), 9L (1; 1 Blood, 0 Respiratory), 10B (1; 0 Blood, 1 Respiratory), 10F (1; 0 Blood, 1 Respiratory), 11B (5; 1 Blood, 4 Respiratory), 11D (1; 0 Blood, 1 Respiratory), 11F (1; 1 Blood, 0 Respiratory), 12A (1; 1 Blood, 0 Respiratory), 13 (14; 5 Blood, 9 Respiratory), 17A (2; 0 Blood, 2 Respiratory), 18B (1; 0 Blood, 1 Respiratory), 18C (25; 7 Blood, 18 Respiratory), 21 (30; 2 Blood, 28 Respiratory), 22A (3; 1 Blood, 2 Respiratory), 25F (1; 1 Blood, 0 Respiratory), 28A (16; 4 Blood, 12 Respiratory), 28F (1; 0 Blood, 1 Respiratory), 29 (5; 2 Blood, 3 Respiratory), 33A (5; 4 Blood, 1 Respiratory), 35A (9; 2 Blood, 7 Respiratory), 35C (4; 0 Blood, 4 Respiratory), 37 (3; 0 Blood, 3 Respiratory), 38 (21; 11 Blood, 10 Respiratory) and 41F (1; 0 Blood, 1 Respiratory).

Table 2. Proportion of *S. pneumoniae* isolates collected from blood and respiratory specimens as part of the CANWARD study with serotypes contained in the pneumococcal conjugate vaccines

Percentage of isolates by specimen source/year(s)					
All		Blood culture isolates		Respiratory isolates	
	2007–23	2023	2007–23	2023	2007–23
Vaccine ($n=3111$)	($n=55$)	($n=1103$)	($n=21$)	($n=2008$)	($n=34$)
PCV13	35.5	40.0	43.7	57.1	30.9
PCV15	44.1	41.8	54.6	61.9	38.3
PCV20	59.8	67.3	71.6	85.7	53.3
PCV21	73.2	69.1	74.9	52.4	72.3
PPSV23	64.7	69.1	77.8	90.5	57.5

predominate serotypes among the MDR/XDR isolates were 19A ($n=78$, 40.6%) and 15A ($n=53$, 27.6%). Serotype 9V was identified more frequently among MDR/XDR blood culture isolates compared with respiratory isolates (13.3% versus 2.6%, $P=0.003$). Serotypes 19F and non-typeable isolates were identified more frequently among MDR/XDR respiratory isolates compared with blood culture isolates (10.9% versus 0%, $P=0.005$ and

9.9% versus 0%, $P=0.009$, respectively). The percent of MDR/XDR *S. pneumoniae* that were serotypes covered by PCV13, PCV15, PCV20, PCV21 and PPSV23 overall were 53.2%, 56.0%, 58.7%, 65.5% and 60.0%, respectively. For the blood/respiratory isolates, the percent of isolates with serotypes covered by PCV13, PCV15, PCV20, PCV21 and PPSV23 were 61.7%/50.5%, 65.0%/53.1%, 70.0%/55.2%, 76.6%/62.0% and 71.7%/56.3%, respectively. For both the blood culture and respiratory isolates, the most common resistance pattern/serotype combinations were MDR 15A resistant to clarithromycin, clindamycin and doxycycline and XDR 19A resistant to clarithromycin, clindamycin, doxycycline, penicillin and trimethoprim/sulfamethoxazole, as detailed in Table S2.

Discussion

S. pneumoniae causes significant morbidity and mortality in the very young, elderly and immunocompromised patients as the causative agent of IPD and a leading cause of respiratory infections.^{1–4} The purpose of this study was to compare the demographics, serotypes and antimicrobial susceptibility rates of *S. pneumoniae* isolates collected from blood cultures and respiratory specimens of patients in Canadian hospitals between 2007 and 2023. A review of the demographic parameters demonstrated that respiratory isolates were collected significantly more frequently from male patients than from female patients.

Table 3. Annual antimicrobial susceptibility testing results for *S. pneumoniae* collected from blood and respiratory specimens as part of the CANWARD study (2007–23)

CANWARD study year	% susceptible by specimen source (overall/blood/respiratory)									
	Penicillin (oral, penicillin V and IV, meningitis) (n = 2954)	Penicillin (IV, non-meningitis) (n = 2954)	Ceftriaxone (meningitis) (n = 2989)	Ceftriaxone (non-meningitis) (n = 2989)	Clarithromycin (n = 2987)	Clindamycin (n = 2987)	Doxycycline (n = 2986)	Levofloxacin (n = 2987)	SXT ^a (n = 2997)	Vancomycin (n = 2981)
2007	79.8/86.0/76.7	97.7/99.1/97.0	98.8/99.5/98.4	99.7/100/99.5	80.7/87.3/77.3	94.1/96.7/92.8	88.8/91.5/87.4	99.4/100/99.1	86.3/88.4/85.2	100/100/100
2008	80.8/83.3/80.0	98.8/99.1/98.7	97.0/99.1/96.4	99.2/100/99	80.2/86.0/78.5	91.3/93.0/90.8	85.3/89.4/84.1	98.6/100/98.2	82.5/85.5/81.7	100/100/100
2009	88.7/9.7/86.3	100/100/100	97.5/99.1/96.4	99/100/97.9	83.7/88.9/77.9	96.6/98.1/94.7	88.2/91.7/84.2	99.5/99.1/100	91.1/96.3/85.3	100/100/100
2010	84.5/83.5/85.6	97.7/98.2/97.1	98.1/98.2/98.1	99.1/98.2/100	76.9/73.1/80.8	94.3/94.4/94.2	90.6/90.7/90.4	99.1/99.1/99.0	79.7/78.7/80.8	100/100/100
2011	83.5/91.5/79.7	97.8/98.3/97.6	94.5/96.6/93.5	98.4/100/97.6	76.9/76.3/77.2	89.0/89.8/88.6	84.6/88.1/82.9	98.9/98.3/99.2	85.2/91.5/82.1	100/100/100
2012	89.0/92.0/88.0	97.0/92.0/98.7	94.9/93.8/95.5	100/100/100	76.5/77.1/76.1	90.4/91.7/89.8	86.0/91.7/83.0	98.5/97.9/98.9	89.0/89.6/88.6	100/100/100
2013	84.5/92.5/78.2	98.3/100/97.0	96.1/98.8/94.1	98.9/100/98.0	72.9/68.8/76.2	92.8/96.3/90.1	84.0/87.5/91.2	97.1/98.7/96.0	83.9/84.8/83.2	100/100/100
2014	87.1/90.5/85.7	99.3/100/99.0	94.6/97.6/93.3	100/100/100	77.4/83.3/75.0	92.5/95.2/91.3	85.6/95.2/81.7	100/100/100	82.1/82.9/81.7	100/100/100
2015	79.7/95.3/72.2	100/100/100	98.5/100/97.8	100/100/100	66.2/65.1/66.7	91.7/95.3/90.0	82.7/88.4/80.0	100/100/100	83.5/81.4/84.4	100/100/100
2016	85.5/91.7/82.7	99.1/100/98.8	99.1/100/98.8	100/100/100	76.1/77.8/75.3	92.3/97.2/90.1	89.7/94.4/87.7	100/100/100	88.9/91.7/87.7	100/100/100
2017	83.3/87.0/80.9	100/100/100	95.6/97.8/94.1	100/100/100	79.8/82.6/77.9	90.4/91.3/89.7	88.6/91.3/86.8	100/100/100	85.1/82.6/86.8	100/100/100
2018	81.5/90.5/76.6	100/100/100	94.1/95.2/93.5	100/100/100	79.0/83.3/76.6	90.8/97.6/87.0	80.7/88.1/76.6	99.2/100/98.7	89.9/88.1/90.9	100/100/100
2019	77.5/82.9/73.3	95.0/97.1/93.3	91.3/91.4/91.1	96.3/97.1/95.6	77.5/71.4/82.2	88.8/82.9/93.3	86.3/80.0/91.1	100/100/100	77.5/77.1/77.8	100/100/100
2020	83.8/90.0/80.0	98.8/100/98.0	95.0/93.3/96.0	100/100/100	71.3/76.7/68.0	87.5/90.0/86.0	80.0/86.7/76.0	100/100/100	88.8/90.0/88.0	100/100/100
2021	80.6/85.7/79.3	94.4/100/93.1	86.1/85.7/86.2	100/100/100	77.8/85.7/75.9	91.7/100/89.7	77.8/85.7/75.9	100/100/100	75.0/85.7/72.4	100/100/100
2022	85.1/87.5/82.6	100/100/100	93.6/95.8/91.3	100/100/100	87.2/87.5/87.0	97.9/100/95.7	91.5/95.8/87.0	100/100/100	80.9/79.2/82.6	100/100/100
2023	87.3/95.2/82.4	98.2/100/97.1	90.9/95.2/88.2	100/100/100	69.1/71.4/67.6	92.7/100/88.2	83.6/85.7/82.4	100/100/100	81.8/81.0/82.4	100/100/100
2007–23	82.8/88.1/79.9	98.4/99.1/98.1	96.6/97.8/95.9	99.4/99.7/99.2	78.1/80.5/76.7	92.4/94.8/91.1	86.4/90.1/84.4	99.2/99.5/99.0	84.8/83.9/86.5	100/100/100
P ^b (2007–23)	NA ^c /NA ^c /NA ^c	NS ^d /NA ^c /NA ^c	< 0.0001 ^f /NA ^c /NA ^c	NA ^c /NA ^c /NA ^c	0.01 ^f /0.007 ^f /NS ^d	NS ^d /NA ^c /NS ^d	0.04 ^f /fNA ^c /0.04 ^f	NA ^c /NA ^c /NA ^c	NS ^d /NS ^d /NS ^d	NA ^c /NA ^c /NA ^c
P ^b (Blood versus Resp.)	<0.0001	0.03	0.006	NS	0.02	0.0003	<0.0001	NS	NS	NS

^aSXT, trimethoprim/sulfamethoxazole.^bCalculated using the Cochran–Armitage test for trends.^cNA, not available; isolate counts were <5 in >20% of data points, therefore, the Cochran–Armitage test for trend analysis could not be performed.^dNS, not statistically significant.^eSignificance of the difference between total study susceptibility rates (2007–23) of blood culture isolates and respiratory isolates calculated using Fisher's exact test.^ff, decreasing trend in susceptibility rate.

Previous studies have shown higher pneumococcal carriage rates in men than women,²⁰ which may result in a higher frequency of respiratory infections. The current study also demonstrated that significantly more *S. pneumoniae* isolates were collected from blood cultures than respiratory specimens in children <2 years of age. The high burden of IPD among young children has been well documented.^{1,2,8}

Notable differences in the serotypes identified from blood culture and respiratory isolates were observed in this study. Serotypes 4, 7F, 8, 19A and 22F were predominant (included in the top 10) from blood cultures and isolated significantly more frequently from that specimen source than from respiratory isolates. These serotypes have been reported to have high invasive disease potential in studies comparing serotypes associated with nasopharyngeal carriage and those associated with IPD in the USA^{21,22} and Portugal.²³ However, serotypes 19A and 22F were also predominant serotypes identified from respiratory specimens in our study. Similarly, serotype 3 is considered a serotype with high invasive potential but was identified among the top 10 serotypes for both the blood culture and respiratory isolates. Sá-Leão et al. reported heterogeneous disease potential for some serotypes, including serotypes 3, 19A and 22F, with specific clones demonstrating increased invasive potential,²³ which may partially explain why a number of serotypes were associated with both specimen sources in our study. Serotypes 6C, 19F, 23A and 35B were predominant (included in the top 10) among respiratory isolates and were detected more frequently from that specimen source than from blood culture isolates in our study. These serotypes have been reported to have a lower invasive disease potential and to be associated with nasopharyngeal carriage.^{21–23} Serotypes 11A and 15B/C are associated with nasopharyngeal carriage but were isolated among the top 10 serotypes from both blood culture and respiratory isolates, although notably, serotype 11A was significantly more common among the respiratory isolates. Heterogeneous invasive potential has also been reported for serotype 11A.²³

Although few serotypes were observed in sufficient annual frequencies to allow for trend analysis, the proportion of isolates with serotype 9N was found to increase over the course of the study (2007–23) while no trends were observed for serotypes 3, 11A, 22F and 23F. The increase in serotype 9N has been previously reported in Canadian IPD isolates.²⁴ Notably, serotype 9N was one of the most common non-PCV13 serotypes reported from adults with IPD in the WHO-commissioned Pneumococcal Serotype Replacement and Distribution Estimation (PSERENADE) project.²⁵ PPSV23 and the newly available PCV21 provide coverage of serotype 9N; it will be important to monitor if use of PCV21 reverses this trend in future years. The continued predominance of serotype 3, despite its inclusion in all conjugate vaccine formulations, has been noted worldwide^{25,26} and is postulated to be due to lower vaccine effectiveness for serotype 3.²⁷ While serotype 22F is included in PCV15, PCV20 and PCV21, these conjugate vaccines have only been recently introduced in Canada so we would not anticipate that they would have yet impacted the serotype distribution of circulating *S. pneumoniae* strains.

Antimicrobial susceptibility rates for penicillin, ceftriaxone, clarithromycin, clindamycin and doxycycline were significantly lower in respiratory isolates compared with blood culture isolates. Similarly, penicillin, macrolide and tetracycline resistance

rates were found to be higher in non-IPD isolates than IPD isolates in a multicentre evaluation of *S. pneumoniae* from adults in the USA.⁴ While *S. pneumoniae* usually only transiently infects a patient during a case of IPD, pneumococcal carriage in the nasopharynx is often long term. A longer duration of nasopharyngeal carriage of *S. pneumoniae* provides a greater risk of antibiotic exposure, resulting in a fitness advantage to maintaining resistance mechanisms,²⁸ and supports the observations presented herein. The susceptibility rates of the *S. pneumoniae* isolated from respiratory specimens in our study were comparable to the rates described for Canadian isolates by the Canadian Respiratory Organism Surveillance Study (CROSS)²⁹ conducted a decade earlier (1997–2002), except for a notable increase in the resistance rates for clarithromycin, clindamycin and doxycycline in the contemporary dataset. The susceptibility rates of the invasive *S. pneumoniae* isolates in this study were similar to those reported for IPD isolates collected between 2011 and 2020 in Canada³⁰ with slightly greater susceptibility rates observed in the CANWARD study for penicillin (IV, meningitis), clarithromycin and clindamycin. The overall decreasing trends in clarithromycin and doxycycline susceptibility rates observed in this study are partially attributable to the predominant 15A and 19A MDR isolates, which most commonly display resistance to both clarithromycin and doxycycline. The association of serotypes 12F, 15A, 19A and 22F with macrolide resistance has been previously discussed.¹² MDR rates were significantly higher in respiratory isolates than in blood culture isolates. The Mohanty et al. study from the USA reported similar MDR rates, 5.0% for IPD and 12.3% for non-IPD isolates,⁴ to those reported in this study, 5.8% for blood culture and 10.0% for respiratory isolates. An increasing trend of MDR/XDR was observed throughout the study years (2007–23) for isolates from all sources and specifically for the respiratory isolates. The MDR rates for respiratory isolates of *S. pneumoniae* have been significantly increasing for many years as this trend was observed in the earlier Canadian study (CROSS) starting in 1997.²⁹

Overall and for both specimen sources, the most common MDR serotypes were 15A and 19A. Differences by specimen source among the MDR isolates were noted for other serotypes with serotypes 19F and non-typeable MDR isolates being exclusively identified in respiratory isolates and MDR serotype 9V being more commonly identified in blood culture isolates. The emergence of MDR 15A and 19A has been consistently described in international studies.^{24,31,32}

A greater proportion of blood culture isolates had serotypes covered by vaccines compared with respiratory isolates. This observation was expected as this study covered a time period in which multiple vaccines were developed and implemented as part of public health programs to address the predominantly circulating serotypes causing IPD. The significant increases in the proportion of isolates targeted by the higher-valency conjugate vaccines, compared with PCV13, observed in this study were comparable with those reported globally as part of the PSERENADE project.²⁵ The proportion of MDR isolates with serotypes contained in PCV13 and PCV15 was greater than the vaccines' coverage of serotypes in all of the *S. pneumoniae* isolates. Conversely, PCV20, PCV21 and PPSV23's coverage of serotypes for all *S. pneumoniae* isolates in the study was greater than the proportion of MDR isolates with serotypes included in those vaccines. The additional serotypes

Table 4. Annual rates and trend analysis of MDR and XDR *S. pneumoniae* collected from blood and respiratory specimens as part of the CANWARD study (2007–23)

Isolate Source/MDR Phenotype	Percentage MDR or XDR by year ^a																	P ^b	% 2007–23
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023		
All																			
MDR/XDR	6.5	9.5	3.4	7.1	11.5	10.1	7.8	10.4	8.3	7.7	8.8	11.8	12.5	16.3	13.9	2.1	14.5	0.004†	8.6
MDR	5.5	7.2	3.0	3.8	7.1	6.1	5.0	6.9	6.8	7.7	6.1	10.1	8.8	15.0	8.3	2.1	12.7	0.005†	6.5
XDR	0.9	2.4	0.5	3.3	4.4	4.0	2.8	3.5	1.5	0	2.6	1.7	3.8	1.3	5.6	0.0	1.8	NA ^c	2.1
Blood																			
MDR/XDR	3.8	7.1	2.8	5.6	10.2	8.3	2.5	4.9	4.7	0	8.7	7.1	17.1	13.3	14.3	4.2	9.5	NA ^c	5.8
MDR	3.3	6.2	2.8	2.8	6.8	0	1.3	4.9	2.3	0	6.5	7.1	14.3	13.3	14.3	4.2	9.5	NA ^c	4.6
XDR	0.5	0.9	0	2.8	3.4	8.3	1.3	0	2.3	0	2.2	0	2.9	0	0	0	0	NA ^c	1.3
Respiratory																			
MDR/XDR	7.8	10.3	4.2	8.7	12.2	10.7	11.9	12.6	10.0	11.1	8.8	14.3	8.9	18.0	13.8	0	17.6	0.03†	10.0
MDR	6.6	7.4	3.2	4.8	7.3	8.0	7.9	7.8	8.9	11.1	5.9	11.7	4.4	16.0	6.9	0	14.7	NS ^d	7.5
XDR	1.2	2.8	1.1	3.8	4.9	2.7	4.0	4.9	1.1	0	2.9	2.6	4.4	2.0	6.9	0	2.9	NA ^c	2.6

^aA total of 2939 isolates had complete antimicrobial susceptibility testing profiles for the MDR analysis. The number of isolates per year was 635 in 2007 (212 Blood; 423 Respiratory), 503 in 2008 (113 Blood; 390 Respiratory), 203 in 2009 (108 Blood; 95 Respiratory), 212 in 2010 (108 Blood; 104 Respiratory), 182 in 2011 (59 Blood; 123 Respiratory), 99 in 2012 (24 Blood; 75 Respiratory), 180 in 2013 (79 Blood; 101 Respiratory), 144 in 2014 (41 Blood; 103 Respiratory), 133 in 2015 (43 Blood; 90 Respiratory), 117 in 2016 (36 Blood; 81 Respiratory), 114 in 2017 (36 Blood; 81 Respiratory), 119 in 2018 (42 Blood; 77 Respiratory), 80 in 2019 (35 Blood; 45 Respiratory), 80 in 2020 (30 Blood; 50 Respiratory), 36 in 2021 (7 Blood; 29 Respiratory), 47 in 2022 (24 Blood; 23 Respiratory) and 55 in 2023 (21 Blood; 34 Respiratory).

^bP < 0.05 indicates statistical significance in annual percentage of MDR/XDR and MDR rates according to the Cochran–Armitage test for trend.

^cNA, not available; isolate counts were < 5 in > 20% of data points, therefore, the Cochran–Armitage test for trend analysis could not be performed.

^dNS, not statistically significant.

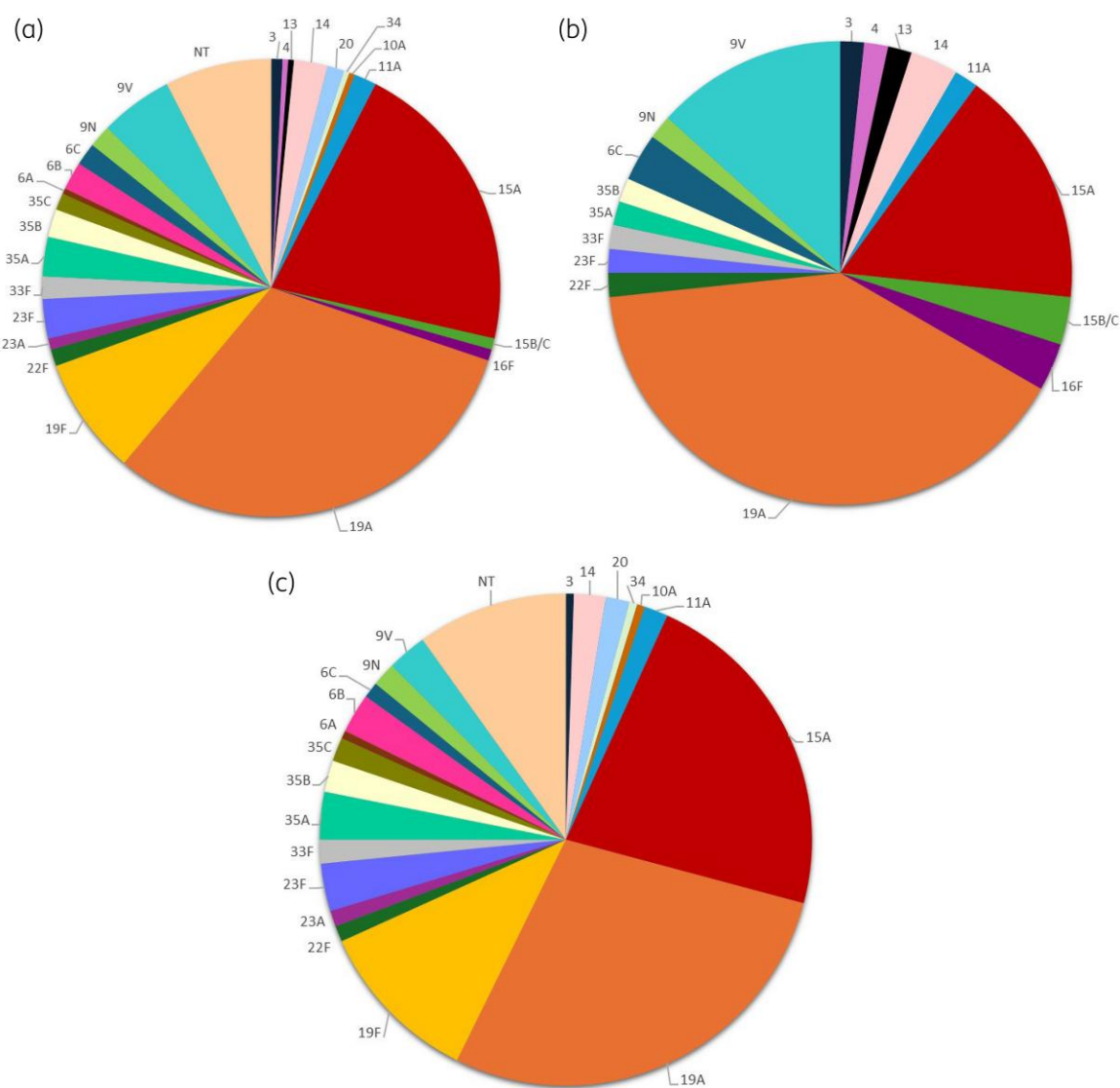


Figure 2. Serotype distribution of MDR/XDR *S. pneumoniae* isolates in the CANWARD study, 2007–23. a) All MDR/XDR isolates ($n=252$), b) MDR/XDR isolates from blood culture isolates ($n=60$), c) MDR/XDR isolates from respiratory isolates ($n=192$).

included in PCV20 and PPSV23, compared with PCV15, were common ($>15\%$) in the overall dataset but uncommon ($<5\%$) among the MDR isolates. While PCV21 does not provide coverage for some of the common MDR isolates, notably those with serotype 19F and 9V, it is the only currently available vaccine that provides coverage for serotype 15A, which is predominant among the MDR isolates. The inclusion of 15A in PCV21 is a significant development in the fight against antimicrobial resistance, particular MDR, in *S. pneumoniae*.

The previously described limitations of the CANWARD study in regards to evaluating antimicrobial resistance and serotype patterns in *S. pneumoniae* remain relevant.¹² Notably, the study only collects the first 100 respiratory isolates each year and the first 10 blood isolates per month (January–October), which may not fully capture seasonal variation of pneumococcal disease and may be impacted by regional IPD outbreaks. The identity and annual number of contributing sites has varied slightly throughout the

study, which may impact the distribution of pneumococcal isolates submitted. However, replacement participating sites were specifically selected with an intent to maintain the geographical representation of the study.

Overall, this study highlighted numerous differences between Canadian isolates of *S. pneumoniae* from blood cultures and respiratory specimens. The noted differences between specimen sources and observed trends in serotype prevalence, decreased antimicrobial susceptibilities and increasing MDR rates support both ongoing pneumococcal surveillance as well as further development of pneumococcal vaccines.

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

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Transparency declarations

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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

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

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Increasing rates of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in Canadian Hospitals: 17 years of the CANWARD study, 2007–23

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Objectives: ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* are major causes of infections, including urinary tract, abdominal, nosocomial pneumonia, diabetic foot and bloodstream infections. This study examines trends in their prevalence, genotypes and antibiotic susceptibility and identifies at-risk populations.

Methods: Between 2007 and 2023, bacterial isolates were collected from sentinel laboratories (CANWARD study). Identification, antimicrobial susceptibility testing and WGS were performed. Trends in incidence, resistance patterns, beta-lactamase genotypes and demographic associations were analysed using multifactorial regression and trend analysis.

Results: Among 11931 *E. coli* and 3973 *K. pneumoniae* isolates, 8.4% and 5.6%, respectively, were ESBL-producers, with prevalence increasing from 2007–08 to 2021–23 (*E. coli*: 3.6% to 11.8%; *K. pneumoniae*: 2.2% to 8.0%; $P < 0.001$). Susceptibility among ESBL *E. coli* declined for amoxicillin-clavulanate (78.7% to 28.6%) but increased for gentamicin (43.5% to 70.3%) ($P < 0.001$). MDR among ESBL-positive *E. coli* declined (82.4% to 63.4%; $P < 0.001$) but remained stable among ESBL-positive *K. pneumoniae*. ESBL *E. coli* was associated with inpatients, respiratory specimens and males, while *K. pneumoniae* was linked to inpatient status and regional variations. Genotypic analysis showed increasing *bla*_{CTX-M-15} in *K. pneumoniae* and *bla*_{CTX-M-27} in *E. coli*. *bla*_{OXA-1} and *bla*_{TEM-1} were associated with MDR and first-generation β -lactam/ β -lactamase inhibitor resistance.

Conclusion: ESBL *E. coli* and *K. pneumoniae* have increased in prevalence, with evolving resistance patterns. While MDR in *E. coli* has declined, resistance to key antimicrobials, including sulphonamides, fluoroquinolones and first-generation β -lactam/ β -lactamase inhibitors remains high. Resistance rates varied significantly by region of Canada and by inpatient status, sex and specimen type.

Introduction

Escherichia coli and *Klebsiella pneumoniae* represent leading causes of urinary tract infection (UTI), deep-seeded abdominal infections, complicated skin and soft tissue infections, bloodstream infections and nosocomial pneumonia.^{1,2} ESBL-producing *E. coli* and *K. pneumoniae* have significantly increased in prevalence globally and now account for a significant proportion of *E. coli* and *K. pneumoniae* clinical isolates, ranging between 14.8% and 53.5% of *E. coli* and 8.0% and 38.3% of *K. pneumoniae* with particularly

high proportions in the global South.² The rapid global spread of these ESBL-producing organisms is partly attributable to the successful uropathogenic ST131 *E. coli* clone which accounts for a significant proportion of isolates globally,^{3–5} as well as similarly successful ESBL-producing *K. pneumoniae* clones ST11 and ST147.^{4,6}

Further complicating the increase in prevalence of ESBL-producing *E. coli* and *K. pneumoniae*, increasing MDR has been reported in numerous surveillance studies of ESBL-producing organisms.^{4,5,7,8} Along with resistance to cephalosporins,

ESBL-producing Enterobacteriaceae frequently demonstrate resistance to commonly used oral agents such as fluoroquinolones, sulphonamides and furans, significantly limiting non-parenteral options for treatment of infections, including relatively innocuous syndromes such as lower UTIs.⁹ In turn, this has resulted in greater reliance on broader spectrum agents such as carbapenems and β -lactam/ β -lactamase inhibitor combinations, risking further selection of increasingly resistant organisms. Indeed, recent reports have highlighted resistance to piperacillin-tazobactam in ESBL-producing *E. coli* and *K. pneumoniae* driven by acquisition of first-generation inhibitor-resistant β -lactamases such as OXA-1¹⁰ and CMY-2.¹¹

The purpose of this study was to describe the epidemiology of ESBL-producing *E. coli* and *K. pneumoniae* over time, describe patterns of resistance and MDR, identify patient factors associated with recovery of an ESBL-producing organism and to analyse trends in ESBL genotypes over time.

Materials and methods

Bacterial isolates

E. coli and *K. pneumoniae* isolates were collected as part of the CANWARD study taking place between January 2007 and December 2023. CANWARD is an ongoing national Public Health Agency of Canada-PHAC/Canadian Antimicrobial Resistance Alliance (CARA) partnered surveillance study evaluating the activity of antimicrobial agents against bacterial isolates submitted by clinical laboratories from patients in tertiary care hospitals across Canada. Additional details on the CANWARD methods are available in this supplement.^{add reference} demographic data collected included: ward type [outpatient (emergency rooms and outpatient clinics) or inpatient (surgical wards, medical wards or ICU)], specimen type [urine, wound/abscess (including abdominal abscesses), bloodstream or respiratory], age, biological sex, geographic region [British Columbia, Central provinces (Alberta, Saskatchewan and Manitoba), Ontario, Quebec and Atlantic Provinces (New Brunswick and Nova Scotia)]. All isolates were transferred to the CANWARD central laboratory (Health Sciences Centre, Winnipeg, Canada) where identification was confirmed by colonial appearance, spot testing and/or MALDI-TOF mass spectrometry (Bruker Daltonics, Billerica, MA, USA) as required. The study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing and quality control was performed by broth microdilution using 96-well panels in accordance with CLSI M100 and M07 standards in place at the time of testing.^{12,13} The antibiotics included in this study were amoxicillin-clavulanate, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, ceftriaxone, ciprofloxacin, doxycycline, ertapenem, meropenem, nitrofurantoin, piperacillin-tazobactam, tobramycin and trimethoprim-sulfamethoxazole. The CLSI M100 Standards, Thirty-Fourth Edition was used for all interpretation of susceptibility results.¹² *E. coli* and *K. pneumoniae* with ceftriaxone and/or ceftazidime MIC of ≥ 1 mg/L were considered potential ESBL-producers. ESBL-production was confirmed using the CLSI confirmatory disc test.¹² In brief, suspect isolates were tested using the Kirby-Bauer disc method against ceftriaxone, ceftriaxone-clavulanate, ceftazidime and ceftazidime-clavulanate. Any isolate where the clavulanate-containing disc increased the zone diameter of the parent antimicrobial by ≥ 5 mm was considered an ESBL-producer. MDR was defined as any ESBL-producer with additional resistance to two or more of the following in accordance with Canadian recommendations for laboratory interpretation of multiple or extensive drug

resistance⁹: gentamicin, piperacillin-tazobactam, meropenem, ciprofloxacin and trimethoprim-sulfamethoxazole.

Whole-genome sequencing

All phenotypic ESBL-producing isolates were subjected to WGS for analysis of beta-lactamase genes using the IlluminaMiSeq platform. 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 NextSeqTM platform. Paired-end, 151 bp indexed reads were generated using the Illumina NextSeqTM platform. FastQC v.0.11.9 (<https://github.com/s-andrews/FastQC>) was used to assess quality control metrics for Illumina reads. Contigs were assembled using Shovill v.1.1.0 on an internal Galaxy platform (<https://github.com/tseemann/shovill>). Staramr v.0.10.0¹⁴ was used to detect antimicrobial resistance genes using the ResFinder database v.2022-05-24.¹⁵

Statistical analysis

For the purpose of statistical analysis, isolates were categorized as either susceptible or non-susceptible based on the 2024 CLSI breakpoint. Intermediate susceptibility was included with the non-susceptible group. Year of isolation (combined in the following blocks for statistical analysis: 2007–08, 2009–11, 2012–14, 2015–17, 2018–20 and 2021–23), biological sex, ward type (in- or outpatient), region [British Columbia, Prairies (Alberta, Manitoba and Saskatchewan), Ontario, Quebec and Atlantic (Nova Scotia and New Brunswick)], specimen type and age (<18, 18–64 and >64 years) were included as explanatory variables for confirmed ESBL-production, resistance to the tested antibiotics and MDR in a second-degree multifactorial step-wise logistic regression model. Potentially explanatory variables were included or excluded from the final nominal logistic regression model on the basis of minimum Bayesian information criterion (BIC). To evaluate the genetic relationships between specific β -lactamase genes present in the isolates and multi-drug resistance, a second-degree multifactorial step-wise logistic regression model was created using the presence or absence of the most common β -lactamases genes (present in at least 30 isolates per species in the study): *bla*_{CTX-M-15}, *bla*_{OXA-1}, *bla*_{TEM-1}, *bla*_{CTX-M-27}, *bla*_{CTX-M-14}, *bla*_{SHV-11}) as explanatory variables for multi-drug resistance. Last, to evaluate trends in the most common β -lactamase genes over time, the Cochran-Armitage test of trend was used. All statistical analysis was performed using JMP Pro v.18.0.2.

Results

Over the study period, 11931 *E. coli* and 3973 *K. pneumoniae* were submitted for analysis (Table 1). Of these, 1007 (8.4%) *E. coli* and 221 (5.6%) *K. pneumoniae* had an ESBL phenotype by double disc test. The proportion of ESBL-positive isolates significantly increased between 2007–08 and 2021–23 from 3.6% to 11.8% for *E. coli* and from 2.2% to 8.0% for *K. pneumoniae* (both $P < 0.001$), independent of other measured variables. Table 1 described the susceptibility and trends over time for ESBL-producing *E. coli* and *K. pneumoniae*. Overall, the most active agents against the ESBL-producing *E. coli* and *K. pneumoniae* were ceftazidime-avibactam and meropenem (susceptibility $\geq 99.5\%$). Ceftolozane-tazobactam was also very active against ESBL-producing *E. coli* (susceptibility 96.0%), but less so versus *K. pneumoniae* (susceptibility 75.8%). Among confirmed ESBL-producing *E. coli*, significant changes in antibiotic susceptibility also took place during the study period (Table 1).

Table 1. ESBL proportion and susceptibility of ESBL-positive *E. coli* and *K. pneumoniae* isolates to select antimicrobials of in the CANWARD study over time

Organism, antimicrobial	Percentage susceptible (N)							P value
	All years	2007–08	2009–11	2012–14	2015–17	2018–20	2021–23	
<i>Escherichia coli</i>								
Percentage ESBL	8.4 (1007/11931)	3.6 (125/3457)	4.2 (154/3645)	8.6 (202/2356)	10.4 (255/2443)	12.7 (277/2178)	11.8 (215/1825)	<0.001
Amoxicillin-clavulanate	45.3 (428/945)	78.7 (85/108)	48.0 (59/123)	43.3 (74/171)	44.2 (92/208)	42.5 (68/160)	28.6 (50/175)	<0.001
Ceftazidime	24.4 (242/954)	30.9 (17/53)	39.0 (48/123)	31.0 (53/171)	30.8 (64/208)	15.8 (35/222)	14.3 (25/175)	<0.001
Ceftazidime-avibactam	99.6 (834/837)	NA	100 (123/123)	99.4 (170/171)	100 (208/208)	98.8 (158/160)	100 (175/175)	NS
Ceftolozane-tazobactam	96.0 (789/822)	NA	100 (46/46)	95.9 (164/171)	98.1 (204/208)	94.5 (210/222)	94.3 (165/175)	NS
Ceftriaxone	1.2 (12/1007)	1.9 (2/108)	0.8 (1/123)	4.1 (7/171)	1.0 (2/208)	0.0 (0/222)	0.0 (0/175)	NS
Ciprofloxacin	12.7 (128/1007)	12.0 (13/108)	6.5 (8/123)	11.7 (20/171)	9.6 (20/208)	16.7 (37/222)	17.1 (30/175)	NS
Doxycycline	34.7 (323/930)	21.3 (23/108)	34.8 (16/77)	31.0 (53/171)	33.6 (70/208)	35.1 (78/222)	47.4 (83/175)	<0.001
Ertapenem	97.5 (980/1005)	99.1 (105/106)	95.9 (118/123)	97.7 (167/171)	98.6 (205/208)	96.8 (215/222)	97.1 (170/175)	<0.001
Gentamicin	64.4 (648/1007)	43.5 (47/108)	52.8 (65/123)	60.8 (104/171)	71.6 (149/208)	72.1 (160/222)	70.3 (123/175)	<0.001
Meropenem	99.5 (1002/1007)	100 (108/108)	100 (123/123)	99.4 (170/171)	100 (208/208)	99.1 (220/222)	98.9 (173/175)	NS
Nitrofurantoin	92.5 (925/1000)	93.4 (99/106)	87.8 (108/123)	95.2 (158/166)	94.2 (196/208)	92.3 (205/222)	90.9 (159/175)	NS
Piperacillin-tazobactam	83.6 (842/1007)	76.9 (83/108)	87.0 (107/123)	87.1 (149/171)	81.3 (169/208)	82.0 (182/222)	86.9 (152/175)	NS
TMP-SMX	34.1 (343/1007)	28.7 (31/108)	30.9 (38/123)	35.7 (61/171)	31.3 (65/208)	33.8 (75/222)	41.7 (73/175)	NS
MDR	74.7 (752/1007)	82.4 (89/108)	82.1 (101/123)	77.8 (133/177)	76.9 (160/208)	71.2 (258/222)	63.4 (111/175)	<0.001
<i>Klebsiella pneumoniae</i>								
Percentage ESBL	5.6 (221/3973)	2.2 (17/769)	3.5 (31/890)	5.3 (31/582)	7.5 (47/625)	9.1 (55/608)	8.0 (40/499)	<0.001
Amoxicillin-clavulanate	27.8 (57/205)	84.6 (11/13)	32.3 (10/31)	41.9 (13/31)	19.1 (9/47)	20.9 (9/43)	12.5 (5/40)	<0.001
Ceftazidime	17.3 (37/214)	40.0 (4/10)	25.8 (8/31)	25.8 (8/31)	17.0 (8/47)	10.9 (6/55)	7.5 (3/40)	0.002
Ceftazidime-avibactam	99.5 (191/192)	NA	100 (31/31)	100 (31/31)	100 (47/47)	100 (43/43)	97.5 (39/40)	NS
Ceftolozane-tazobactam	75.8 (138/182)	NA	88.9 (8/9)	80.6 (25/31)	68.1 (32/47)	74.5 (41/55)	80.0 (32/40)	NS
Ceftriaxone	5.0 (11/221)	23.5 (4/17)	9.7 (3/31)	3.2 (1/31)	6.4 (3/47)	0.0 (0/55)	0.0 (0/40)	0.001
Ciprofloxacin	16.3 (36/221)	23.5 (4/17)	16.1 (5/31)	29.0 (9/31)	4.3 (2/47)	12.7 (7/55)	22.5 (9/40)	NS
Doxycycline	29.1 (53/182)	NA	22.2 (2/9)	25.8 (8/31)	29.8 (14/47)	27.3 (10/55)	35.0 (14/40)	NS
Ertapenem	89.9 (195/217)	100 (13/13)	96.8 (30/31)	87.1 (27/31)	83.0 (39/47)	87.3 (48/55)	95.0 (38/40)	NS
Gentamicin	56.1 (97/221)	41.1 (7/17)	54.8 (17/31)	51.6 (16/31)	48.9 (23/47)	65.5 (36/55)	62.5 (25/40)	NS
Meropenem	95.5 (211/221)	100 (17/17)	100 (30/30)	93.5 (29/31)	95.7 (45/47)	89.1 (49/55)	100 (39/39)	NS
Nitrofurantoin	20.3 (32/158)	7.7 (1/13)	N/A	N/A	29.9 (14/47)	18.2 (10/55)	15.0 (6/40)	NS
Piperacillin-tazobactam	52.0 (115/221)	47.1 (8/17)	48.4 (15/31)	54.8 (17/31)	36.2 (17/47)	52.7 (29/55)	72.5 (29/40)	NS
TMP-SMX	15.4 (34/221)	29.4 (5/17)	32.3 (10/31)	6.5 (2/31)	6.4 (3/47)	18.2 (10/55)	10.0 (4/40)	NS
MDR	85.1 (188/221)	82.4 (14/17)	80.6 (25/31)	87.1 (27/31)	93.6 (44/47)	85.5 (47/55)	80.0 (32/40)	NS

NA, not available. NS, not significant.

Table 2. Demographic and specimen factors associated with isolation of an ESBL-producing *E. coli* and *K. pneumoniae*

Organism, demographic parameter	Percentage ESBL (n/N)	P value (univariate)	Odds ratio (95%CI) (multivariate model)
<i>Escherichia coli</i>			
Patient type			
Inpatient	11.6 (655/5643)	<0.001	1.66 (1.44–1.91)
Outpatient	5.6 (352/6287)		
Specimen type			
Urine	6.3 (247/3953)	<0.001	Respiratory versus others; 1.99 (1.63–2.42) Wound/abscess and blood versus urine; 1.24 (1.00–1.55)
Wound/abscess	11.6 (49/422)		
Lower respiratory tract	16.8 (198/1177)		
Blood	8.0 (513/6379)		
Biological sex			
Male	10.4 (509/4874)	<0.001	1.27 (1.11–1.46)
Female	7.1 (498/7057)		
Age group, years			
<18	4.3 (46/1067)	<0.001	>18 versus others; 2.53 (1.88–3.50)
18–64	8.9 (435/4877)		
≥65	8.8 (526/5987)		
Region			
British Columbia	9.7 (115/1188)	<0.001	Ontario, BC and Prairies versus others; 2.33 (2.00–2.72) Ontario versus BC and Prairies; 1.42 (1.21–1.66)
Prairies (Alberta, Saskatchewan, Manitoba)	8.8 (247/2823)		
Ontario	12.8 (411/3209)		
Quebec	4.9 (156/3171)		
Atlantic (Nova Scotia, New Brunswick)	5.1 (78/1540)		
<i>Klebsiella pneumoniae</i>			
Patient type			
Inpatient	7.0 (174/2480)	<0.001	2.16 (1.55–3.02)
Outpatient	3.2 (47/1493)		
Specimen type			
Urine	5.3 (40/754)	NS	a
Wound/abscess	8.1 (14/173)		
Lower respiratory tract	6.1 (60/986)		
Blood	5.2 (107/2060)		
Biological sex			
Male	6.2 (134/2166)	0.0603	a
Female	4.8 (87/1807)		
Age group, years			
<18	4.8 (14/292)	NS	a
18–64	6.3 (107/1707)		
≥65	4.1 (100/1974)		
Region			
British Columbia	6.4 (26/406)	<0.001	Ontario and BC vs. others; 2.32 (1.76–3.07)
Prairies (Alberta, Saskatchewan, Manitoba)	5.0 (45/906)		
Ontario	9.2 (108/1179)		
Quebec	3.2 (32/1015)		
Atlantic (Nova Scotia, New Brunswick)	2.1 (10/467)		

NS, not significant.

ªVariable excluded from the model on the basis of minimum BIC.

Table 3. Trends in detection of common (detected in >30 isolates) β -lactamase genes in ESBL-producing *E. coli* and *K. pneumoniae* over 17 years

Organism	Percentage of ESBL-positive isolates with gene							P value
	All years	2007–08	2009–11	2012–14	2015–17	2018–20	2021–23	
<i>Escherichia coli</i>	(n=872)	(n=108)	(n=123)	(n=171)	(n=208)	(n=222)	(n=175)	
CTX-M-15	59.0	59.3	66.7	63.7	56.7	52.7	59.4	NS
OXA-1	36.4	45.4	51.2	40.9	37.5	27.9	25.7	<0.001
TEM-1	31.1	51.9	35.8	33.3	21.2	26.6	30.3	<0.001
CTX-M-27	18.0	3.7	8.1	12.3	19.2	28.4	24.6	<0.001
CTX-M-14	11.0	20.4	15.5	12.3	8.2	7.7	8.6	<0.001
<i>Klebsiella pneumoniae</i>	(n=221)	(n=17)	(n=31)	(n=31)	(n=47)	(n=55)	(n=40)	
CTX-M-15	60.2	47.1	41.9	38.7	68.1	63.6	82.5	<0.001
OXA-1	39.4	35.3	22.6	32.3	61.7	40.0	32.5	NS
TEM-1	56.6	52.9	35.5	71.0	68.1	58.2	47.5	NS

NS, not significant.

Amoxicillin-clavulanate susceptibility declined from 78.7% to 28.6% and ceftazidime susceptibility declined from 30.9% to 14.3% (both $P < 0.001$) while gentamicin susceptibility increased from 43.5% to 70.3% and doxycycline susceptibility increased from 21.3% to 47.4% (both $P < 0.001$). The proportion of MDR isolates among ESBL-producing *E. coli* significantly decreased from 82.4% to 63.4% ($P < 0.001$). Among confirmed ESBL-producing *K. pneumoniae*, amoxicillin-clavulanate susceptibility significantly declined from 84.6% (11/13) to 12.5% (5/40) ($P < 0.001$), ceftazidime susceptibility declined from 40.0% to 7.5% ($P = 0.002$) and ceftriaxone susceptibility declined from 23.5% to 0% ($P = 0.001$). The proportion of MDR isolates among ESBL-producing *K. pneumoniae* did not change significantly during the study period. Of note, there was no significant change in susceptibility to ciprofloxacin or trimethoprim-sulfamethoxazole over the study period for either ESBL-producing *E. coli* or *K. pneumoniae*. However, resistance rates remained high with only 12.7% and 34.1% of *E. coli* and 16.3% and 15.4% of *K. pneumoniae* being susceptible to ciprofloxacin and trimethoprim-sulfamethoxazole, respectively. Likewise, no trend in resistance to nitrofurantoin was observed over time, with overall susceptibility remaining high for *E. coli* at 92.5% and low for *K. pneumoniae* at 20.3%.

Several patient variables were found to be independently associated with recovery of a confirmed ESBL-producing *E. coli* or *K. pneumoniae* (Table 2). ESBL-producing *E. coli* were significantly more likely to be recovered from inpatients [OR 1.66 (1.44–1.91)], from respiratory tract specimens [OR 1.99 (1.63–2.42)] compared to other specimens and from wound or abscess specimens compared to urine and blood cultures [OR 1.24 (1.00–1.55)], from biologically male patients [OR 1.27 (1.11–1.46)] and from adult (>18 years) patients compared to paediatric patients [OR 2.53 (1.88–3.50)]. There were also strong regional differences in recovery of ESBL-producing *E. coli*, with significantly higher proportions recovered in Ontario, British Columbia and the Prairies (compared to Quebec and the Atlantic provinces [OR 2.33 (2.00–2.72)] and significantly higher proportions in Ontario compared to BC and the Prairies [OR 1.42 (1.21–1.66)]. Among patient characteristics for *K. pneumoniae*, only inpatient status was associated with a higher proportion of ESBL-producing isolates after multivariate analysis [OR 2.16 (1.55–3.02)]. Regional differences were also

noted among ESBL-producing *K. pneumoniae*, with higher proportions in British Columbia and Ontario compared to other regions [OR 2.32 (1.76–3.07)].

Genotyping of β -lactamase genes revealed significant heterogeneity in the genotypes and number of beta-lactamase genes carried by both *E. coli* and *K. pneumoniae* (Appendices S1–S4, available as [Supplementary data](#) at JAC Online). *bla*_{CTX-M-15} was the most commonly detected ESBL beta-lactamase in both *E. coli* and *K. pneumoniae* and most isolates of both species had multiple beta-lactamases. *K. pneumoniae* generally had a greater variety of beta-lactamases and was more frequently found to carry multiple beta-lactamase genes. In *E. coli*, *bla*_{CTX-M-27} was the second most commonly encountered ESBL beta-lactamase, and most commonly detected in isolates with a single beta-lactamase, 153 of 181 (84.5%) of *bla*_{CTX-M-27} detections were in organisms with a single beta-lactamase (Appendices S1 and S2). In addition, significant trends in the genotypes of both ESBL-producing *E. coli* and *K. pneumoniae* occurred over time (Table 3). In *E. coli*, the most commonly detected gene was *bla*_{CTX-M-15} (59.3% of ESBL-producing strains), which showed no significant trend over time. However, a significant decrease in the proportion of isolates with *bla*_{OXA-1} (from 45.4% to 25.7%, $P < 0.001$), *bla*_{TEM-1} (from 51.9% to 30.3%, $P < 0.001$) and CTX-M-14 (from 20.4% to 8.6%, $P < 0.001$) were noted over the course of the study. Conversely, a significant increase in the detection of *bla*_{CTX-M-27} was noted over time, increasing from 3.7% to 24.6% over the course of the study ($P < 0.001$). In ESBL-producing *K. pneumoniae*, the most commonly detected β -lactamase gene was *bla*_{CTX-M-15}, occurring in 60.2% of isolates. Proportion of isolates with *bla*_{CTX-M-15} increased significantly during the study, from 47.1% to 82.5% ($P < 0.001$). The proportion of isolates with *bla*_{OXA-1} and *bla*_{TEM-1} remained unchanged throughout the study period.

Among ESBL-positive *E. coli* with common (>30 isolates) β -lactamase genes (*bla*_{CTX-M-15}, *bla*_{OXA-1}, *bla*_{TEM-1}, *bla*_{CTX-M-27} and *bla*_{CTX-M-14}), presence of specific β -lactamase genes was associated with MDR (Table 4). Multivariate analysis indicated that the presence of *bla*_{OXA-1} [OR 7.18 (4.62–11.41)] and *bla*_{TEM-1} [OR 2.82 (1.75–4.63)] was associated with MDR. Presence of *bla*_{CTX-M-15} was negatively correlated to MDR [OR 0.54 (0.36–0.80)].

Table 4. β-lactamase genotypes associated with multi-drug resistance in univariate and multivariate models

Organism, ESBL	MDR (% <i>, n/N</i>)	OR (95%CI) (univariate)	OR (95%CI) (multivariate)
<i>Escherichia coli</i>			
CTX-M-15 (% <i>, n/N</i>)			
Present 64.0 (595/930)	79.2 (471/594)	1.41 (1.03–1.93)	0.54 (0.36–0.80)
Absent 36.0 (335/930)	72.8 (244/335)		
OXA-1			
Present 39.5 (367/930)	91.6 (336/367)	5.26 (3.50–7.91)	7.18 (4.62–11.41)
Absent 60.5 (563/930)	67.3 (379/562)		
TEM-1			
Present 33.7 (313/930)	78.9 (247/313)	1.19 (0.86–1.65)	2.82 (1.75–4.63)
Absent 66.3 (617/930)	75.9 (468/617)		
CTX-M-27			
Present 19.5 (181/930)	73.5 (133/181)	0.79 (0.55–1.15)	^a
Absent 80.5 (749/930)	77.7 (582/749)		
CTX-M-14			
Present 11.9 (107/930)	69.4 (77/111)	0.64 (0.42–0.99)	1.47 (0.85–2.54)
Absent 88.1 (819/930)	77.9 (638/819)		
<i>Klebsiella pneumoniae</i>			
CTX-M-15 (% <i>, n/N</i>)			
Present 72.5 (132/182)	87.9 (117/133)	0.63 (0.20–1.98)	^a
Absent 27.5 (50/182)	92.0 (46/50)		
OXA-1			
Present 47.5 (86/182)	97.7 (84/86)	9.69 (2.18–43.14)	8.23 (2.22–53.4)
Absent 52.5 (96/182)	81.3 (78/96)		
TEM-1			
Present 68.7 (125/182)	94.4 (118/125)	4.98 (1.86–13.30)	4.12 (1.53–11.94)
Absent 31.3 (57/182)	77.2 (44/57)		
SHV-11			
Present 25.8 (47/182)	93.6 (44/47)	2.11 (0.59–7.56)	^a
Absent 74.2 (135/182)	87.4 (118/135)		

^aVariable excluded from the model on the basis of minimum BIC.

Likewise, among ESBL-positive *K. pneumoniae* with common (>30 isolates) β-lactamase genes (*bla*_{CTX-M-15}, *bla*_{OXA-1}, *bla*_{TEM-1}, *bla*_{SHV-11}), both *bla*_{OXA-1} [OR 8.23 (2.22–53.4)] and *bla*_{TEM-1} [OR 4.12 (1.53–11.94)] were associated with MDR.

Discussion

A number of important trends in ESBL-producing *E. coli* were identified in this study (Table 1). Overall, the proportion of ESBL-positive isolates increased significantly over time. This mirrors similar observations globally that have been attributed to spread of successful ESBL-positive *E. coli* clones (e.g. ST131) as a cause of infections in humans with potential animal and environmental reservoirs.¹⁶ A significant decline in amoxicillin-clavulanate and ceftazidime susceptibility, and increased susceptibility to doxycycline and gentamicin with a concurrent reduction in MDR isolates was also observed over the study period. Decreasing susceptibility to amoxicillin-clavulanate and ceftazidime reflect changes in predominant β-lactamases in these organisms, specifically an increase in the proportion of isolates expressing *bla*_{CTX-M-27} and a concurrent decline in proportion of isolates with *bla*_{CTX-M-14} (Table 3). While OXA-1 expression and

TEM-1 overexpression have been associated with resistance to first-generation beta-lactamase inhibitor combinations,¹⁰ we nevertheless observed a significant decline in the proportion of *E. coli* isolates with *bla*_{OXA-1} and *bla*_{TEM-1} despite an increase in amoxicillin-clavulanate and stable piperacillin-tazobactam resistance. This suggests that other factors such as impermeability may be affecting resistance to these agents. MDR in *E. coli* was positively associated with the presence of *bla*_{OXA-1} and *bla*_{TEM-1} and negatively associated with *bla*_{CTX-M-15} (Table 4). This is probably due to *bla*_{OXA-1} and *bla*_{TEM-1} being commonly co-located with other resistance determinants on plasmids or other mobile resistance elements, and therefore this association may reflect co-localization of MDR determinants.¹⁷ In addition, isolates expressing OXA-1 are more likely to be MDR due to commonly being additionally resistant to first-generation beta-lactamase inhibitor combinations.¹⁰ It is noteworthy that the emergence of *bla*_{CTX-M-27}-producing *E. coli* over the course of the study was also associated with a decline in MDR, suggesting the possibility that *bla*_{CTX-M-27} is not as commonly co-located with other resistance determinants. This is supported both by the observation that *bla*_{CTX-M-27} was noted to be the only beta-lactamase present in 84.5% of isolates where it was detected, and rarely co-located

with *bla*_{OXA-1} or other beta-lactamases that would broaden resistance to include first-generation beta-lactamase inhibitor combinations, and by the observation that gentamicin resistance (which is included in the MDR definition) is much less commonly observed in isolates with *bla*_{CTX-M-27} compared to other isolates (6.6% versus 43.7%, $P < 0.001$, data not shown).

An important observation in our data is that nearly all ESBL-producing organisms in this study remained susceptible to both meropenem and ceftazidime-avibactam throughout the study period with no evidence of trending resistance (Table 1). This observation suggests that these agents remain good empiric treatment options in infections caused by ESBL-producing bacteria. In particular, among all beta-lactam/beta-lactamase inhibitor combinations we studied, ceftazidime-avibactam had the most reliable activity against ESBL-producing organisms of both species. While it is reassuring that the proportion of resistant isolates was noted to be stable for ciprofloxacin or trimethoprim-sulfamethoxazole, drugs commonly used for treatment of infections caused by *E. coli* and *K. pneumoniae*, only a minority of isolates of both species were susceptible to these agents, limiting their utility as empiric treatments. Conversely, nitrofurantoin remained active against >90% of ESBL *E. coli* with no evidence of increasing resistance over time, indicating it remains a treatment option for uncomplicated UTI. While no trend in nitrofurantoin resistance in *K. pneumoniae* was noted during the study, resistance rates remained high, significantly limiting its clinical utility.

Several demographic factors were strongly associated with isolation of an ESBL-producing *E. coli* from patients (Table 2). Unsurprisingly, inpatients were more than twice as likely to have an ESBL-producing *E. coli* isolated from a sample than outpatients. This is probably related to antimicrobial pressure and previous exposure as well as disease severity in inpatient populations.^{7,18} As in previous studies,^{7,19,20} older age and male sex were associated with higher rates, probably reflecting increased antibiotic exposure and prevalence of more complicated infections such as complicated lower urinary tract infections. Specimen type was also associated with increased proportion of ESBL-producing *E. coli*. Respiratory specimens showed the highest proportion of ESBL-producing *E. coli* while urinary sources had the lowest. This is probably a reflection of the patient populations from which *E. coli* is commonly isolated from these sites. *E. coli* is primarily isolated from the respiratory secretions of patients with hospital-acquired pneumonia/ventilator-associated pneumonia (HAP/VAP), in which resistance is relatively common due to significant hospital exposure.²¹ Likewise, wound infections caused by *E. coli* are more commonly associated with complex wounds, abdominal infections and diabetic foot infections in patients with significant antimicrobial and hospital exposure.²² Unlike these specimen types, *E. coli* is a common cause of both community and hospital onset urinary tract infections and due to the community onset of many UTIs, fewer patients with UTI are expected to have had previous exposure to antimicrobials or other risk factors for MDR infections.²³ Likewise, bloodstream *E. coli* isolates are most probably originating from the urinary tract, and similar proportions of ESBL-producing bacteria would be expected from both these sites.²⁴ Geographic differences, where higher proportions of ESBL-producing *E. coli* are found in Ontario and British Columbia probably stem from larger population centres with significantly higher rates of international arrivals from high-prevalence countries, resulting in earlier and more consistent importation of

ESBL-producing *E. coli*. However, other factors such as overcrowding and socioeconomic disparities also play a role in local spread of ESBL-producing *E. coli* and probably play a role in increased prevalence in these settings as has been shown in large urban settings.²⁵ Interestingly, the province of Quebec had very low proportions of ESBL-producing *E. coli* compared to others regions studied despite being represented primarily by large population centres in this study (e.g. Montreal). The difference between prevalence between neighbouring provinces Quebec and Ontario could be explained by multiple factors including antibiotic utilization, infection control or socioeconomic factors and merits further study of the factors that may be mitigating spread of ESBL-producing *E. coli*.

Similar trends were observed in *K. pneumoniae*. We observed a similar ~4-fold increase in proportion of isolates expressing an ESBL over time during the study (Table 1). As in *E. coli*, the increase in ESBL-producing *K. pneumoniae* over time probably reflects ongoing emergence of successful clones, as has been documented previously.⁴ Susceptibility rates to amoxicillin-clavulanate and ceftriaxone declined significantly over the study period. The decline in ceftriaxone susceptibility is explained by the increasing proportion of *bla*_{CTX-M-15}-positive isolates over time (Table 3). The decrease in amoxicillin-clavulanate susceptibility over time in the absence of increasing proportions of *bla*_{OXA-1} or other first-generation inhibitor-resistant β -lactamases suggests the possibility of other mechanisms of resistance at play, including potential impermeability or porin alterations or over expression of ESBL or non-ESBL β -lactamases. Detection of the narrow-spectrum β -lactamase *bla*_{TEM-1} and *bla*_{OXA-1} was strongly associated with MDR *K. pneumoniae*. This also probably reflects co-localization with other resistance determinants on MDR plasmids in *K. pneumoniae*, as has been demonstrated in plasmid studies of MDR isolates.²⁶

Unlike in *E. coli*, there were few demographic factors associated with the detection of an ESBL-producing *K. pneumoniae*. Only inpatient status and isolation from British Columbia or Ontario were independently associated with detection of an ESBL-producing *K. pneumoniae*. The association with inpatient status is likely driven by selective pressure from antibiotic use which contributes to the risk of infection with antibiotic-resistant pathogens as well as spread of drug-resistant pathogens within hospital facilities as has been shown in multiple studies.^{27,28} Regional differences in ESBL-producing *K. pneumoniae* with higher proportions in larger population centres likely reflect similar epidemiology as *E. coli*, reflecting increased and earlier importation and complex socioeconomic factors associated with overcrowding and spread of enteric organisms.

In conclusion, ESBL-producing *E. coli* and *K. pneumoniae* demonstrated increasing prevalence and evolving resistance patterns over the 17-year study period. In *E. coli*, the proportion of MDR isolates has declined, as have the proportion of isolates with *bla*_{OXA-1}, *bla*_{TEM-1} and *bla*_{CTX-M-14}, concurrently with an increase in isolates with *bla*_{CTX-M-27}. In *K. pneumoniae*, increasing *bla*_{CTX-M-15} prevalence correlated with a sharp decline in ceftriaxone susceptibility. Resistance rates to fluoroquinolones and sulfonamides in both species have been high and stable over the course of the study while resistance rates to carbapenems and ceftazidime-avibactam have been low and stable. Regional and patient factors, particularly inpatient status influenced likelihood of isolating an ESBL-producing organism of either species.

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Transparency declarations

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Disclaimer

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Supplementary data

Appendices S1–S4 are available as [Supplementary data](#) at JAC Online.

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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 Enterobacteriales 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 Enterobacteriales 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).

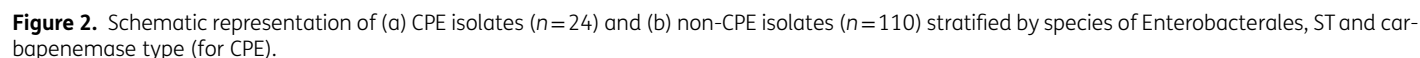
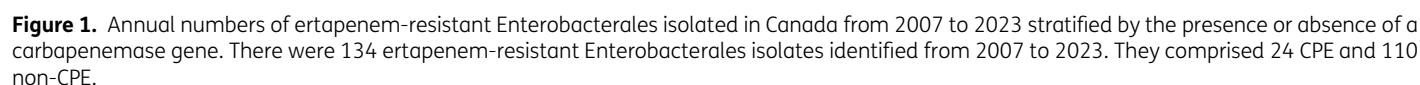
The distribution of carbapenemase genes among CPE isolates, stratified by bacterial species and ST, is shown in Figure 2(a). In total, 28 carbapenemase genes were identified in the 24 CPE isolates: 15 KPC (10 KPC-3, 5 KPC-2), three OXA-48-like (OXA-181, OXA-301 and OXA-48), one NDM (NDM-5) and one SME (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

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

		mg/L		MIC interpretation			
Isolate genotype							
Antimicrobial agent	No. of isolates tested	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.

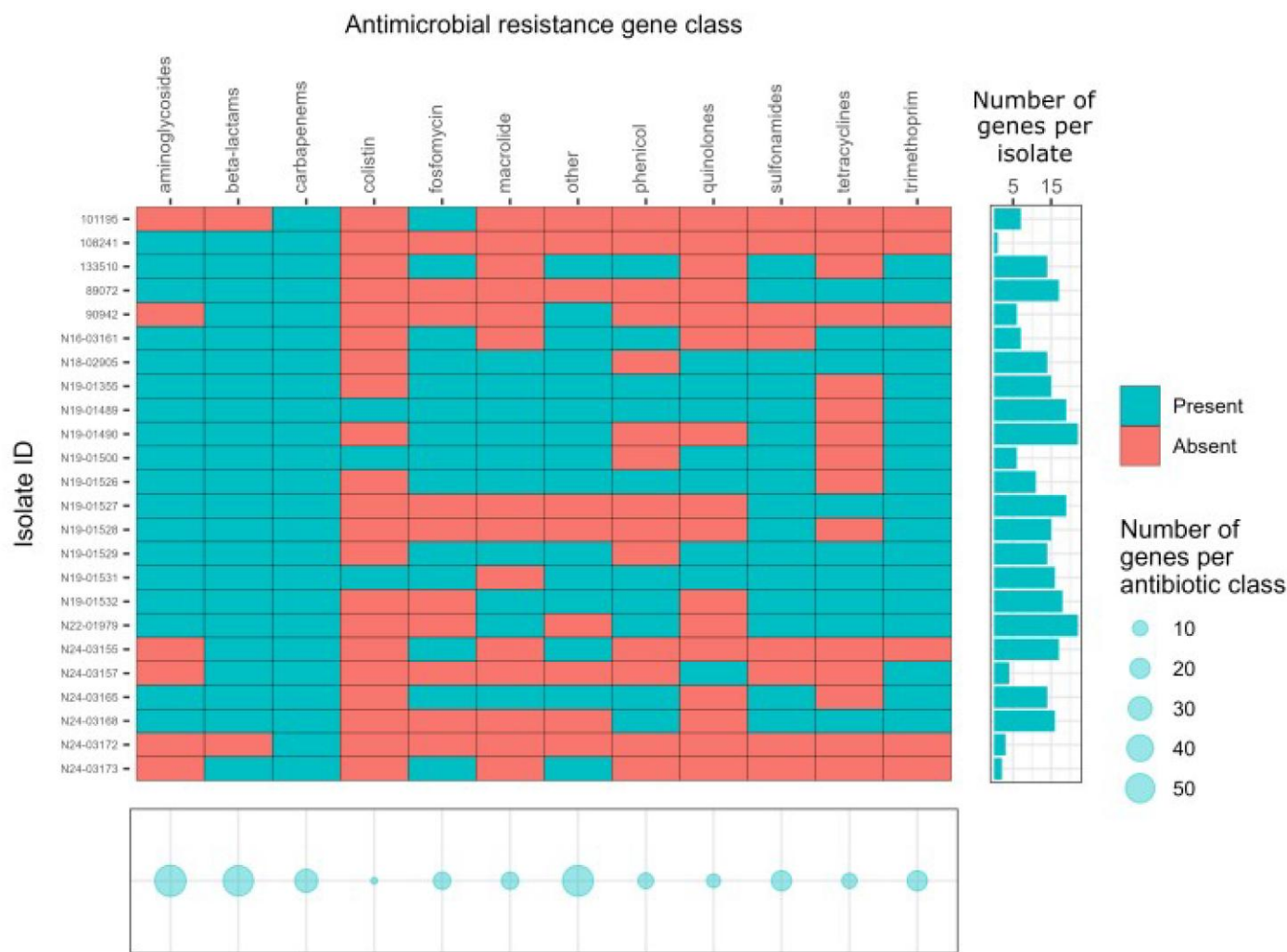


Figure 3. Schematic representation of the distribution of AMR resistance gene category classes for CPE isolates ($n=24$). Multiple genes per category may be present; however, duplicated genes per isolate were not counted.

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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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 or Xediton Pharmaceuticals Inc.

Supplementary data

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

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

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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 β -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.^{1–3} Resistance to carbapenems is of particular concern, has been reported worldwide and is often associated with limited treatment options for infected patients.⁴ The WHO has classified carbapenem-resistant *P. aeruginosa* as a high priority pathogen because it frequently causes difficult-to-treat infections.⁵

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,⁶ alterations of outer membrane porins⁷ or overexpression of chromosomal AmpC.⁸ Resistance can also be in the form of carbapenemase genes acquired through horizontal transfer of mobile genetic elements.⁹ Lastly, the presence of class D β -lactamases have also been shown to contribute to carbapenem resistance.^{10,11} 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.^{9,12,13} High-risk clones are distributed globally; they are frequently MDR, described as highly pathogenic and can cause severe

infections.^{9,14} Thus far in Canada, the incidence of high-risk clones has been very low.¹⁵ 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.¹⁵ 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.¹⁶ 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,¹⁶ 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.¹⁷ Carbapenem-resistant *P. aeruginosa* were defined as resistant to meropenem, imipenem or both. CLSI-resistant breakpoints for meropenem and imipenem was ≥ 8 mg/L.¹⁶

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 v0.10.0¹⁸ was used to detect antimicrobial resistance genes using the ResFinder database v2022-05-24.¹⁹ 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.²⁰ ABRicate v1.0.1²¹ was used to detect appropriate virulence genes using the virulence factor database (VFDB).²² 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 2024-12-18.1²³ was used to identify: point mutations in *oprD* that may lead to a decrease in outer membrane permeability;⁷ mutations in *mexR*, *nalD* and *nalC* that affect the expression of MexAB-OprM efflux pump⁶ and mutations in *ampR*, *ampD* and *dacB* that affect the expression of chromosomal cephalosporinase AmpC.⁸ A previously published list of specific mutations in the aforementioned genes, which have been associated with carbapenem resistance, was used to screen our data.¹³ 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.²⁴ Data were visualized with minimum spanning trees created in SeqSphere or using Microreact.²⁵

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 β -lactam/ β -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.¹⁶ 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.²⁶

All carbapenem-resistant *P. aeruginosa* were screened for potential carbapenem resistance markers, including β -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).^{6,8,13} Carbapenemase genes were only identified in 0.8% of isolates; two isolates carried *bla*_{IMP-26} and one isolate carried *bla*_{GES-5}. 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*_{OXA-50}-like family were detected in 98.1% of isolates. Only one *bla*_{OXA-50}-like variant was found per isolate, with the following distribution, *bla*_{OXA-50} (32.6%; *n*=121), *bla*_{OXA-395} (11.1%; *n*=41), *bla*_{OXA-396} (5.7%; *n*=21), *bla*_{OXA-486} (7.8%; *n*=29), *bla*_{OXA-488} (19.4%; *n*=72) and *bla*_{OXA-494} (21.6%; *n*=80). The remaining 1.9% (*n*=7) carbapenem-resistant *P. aeruginosa* did not match any known variant of the *bla*_{OXA-50}-like family, but showed the closest similarity to *bla*_{OXA-395}, suggesting the presence of a potential novel variant. Other OXA genes identified included *bla*_{OXA-10} (0.8% of isolates, *n*=3), *bla*_{OXA-2} (0.5%, *n*=2) and *bla*_{OXA-5} (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 ₅₀	MIC ₉₀	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 ^a	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 ^b	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

^aNA, not applicable.^bEUCAST 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*_{GES-5}; *n*=1) or ST235 (*bla*_{IMP-26}; *n*=2). All of the top six STs harboured a *bla*_{OXA-50}-like gene. The distribution of other OXA genes were as follows: *bla*_{OXA-2} was found in ST235 (1.0%, *n*=1), *bla*_{OXA-5} in ST17 (1.0%, *n*=1) and *bla*_{OXA-10} 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.²⁷ 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 report¹⁵ and from 3.3% reported by another 2009 national Canadian surveillance study.²⁸ 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.^{15,28,29} Furthermore, it has been suggested that admission to an ICU stay is a risk factor for carbapenemase-producing *P. aeruginosa* infection.²⁹ There were no significant differences in demographic information between the current study and the previously published 2007–16 CANWARD study.¹⁵

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.^{15,30,31} The higher susceptibility to ceftazidime–avibactam (83.3%) observed in Turkey³⁰ 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.¹⁵ Additionally, a recent

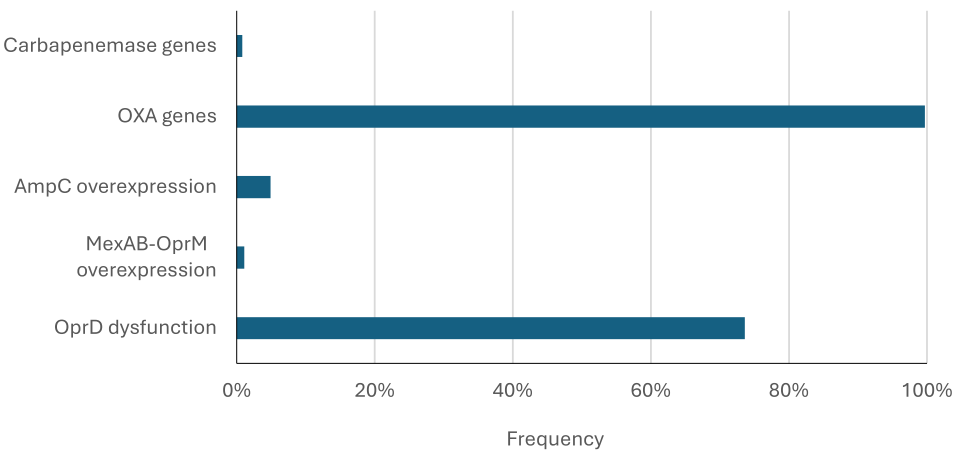


Figure 1. Distribution of possible mechanisms of resistance to carbapenems in 371 carbapenem-resistant *P. aeruginosa* isolated in Canadian hospitals in 2018–23.

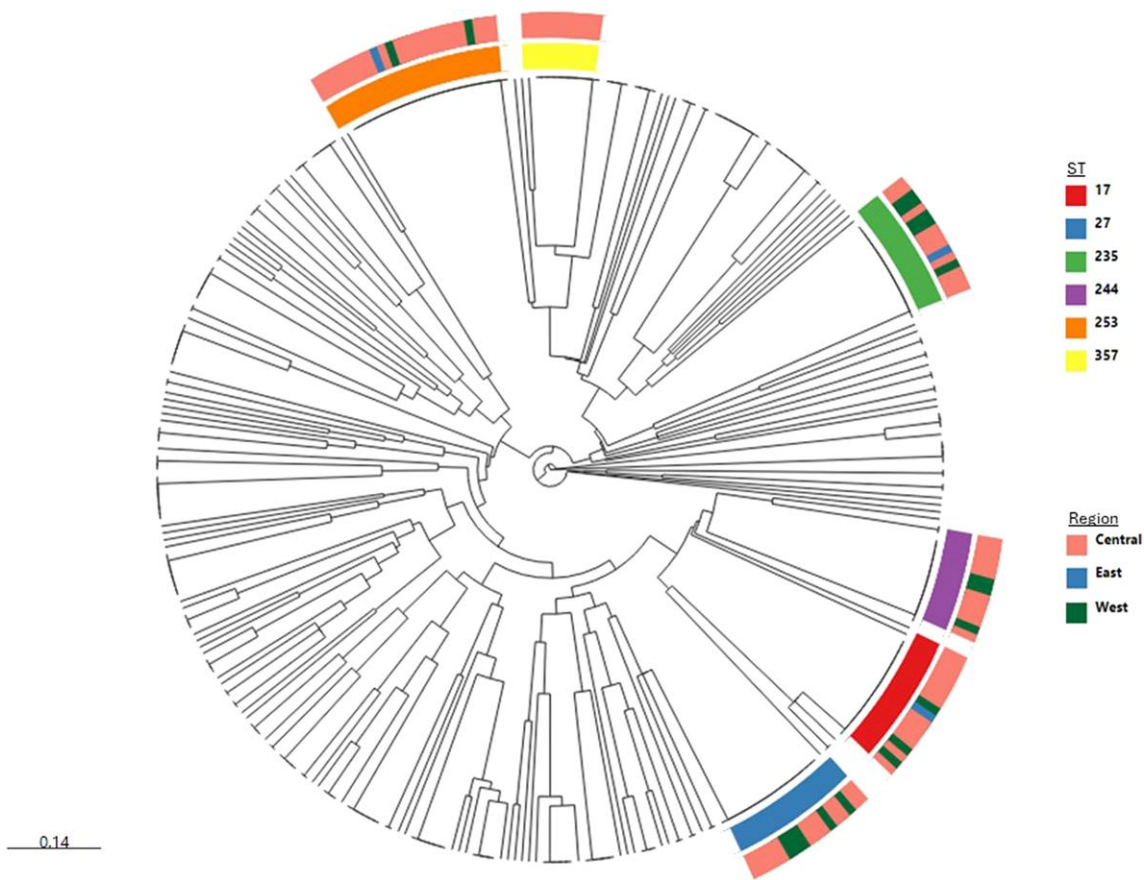


Figure 2. Phylogenetic tree illustrating ST distribution of 371 carbapenem-resistant *P. aeruginosa* isolated in Canadian hospitals in 2018–23. The tree is made up of 151 different STs. The six most common STs are highlighted in the inner ring as ST17 (red, $n=17$), ST27 (blue, $n=17$), ST235 (green, $n=16$), 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.³² 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%).^{31,32} In contrast, meropenem–vaborbactam, which is not yet recommended for treatment of carbapenem-resistant

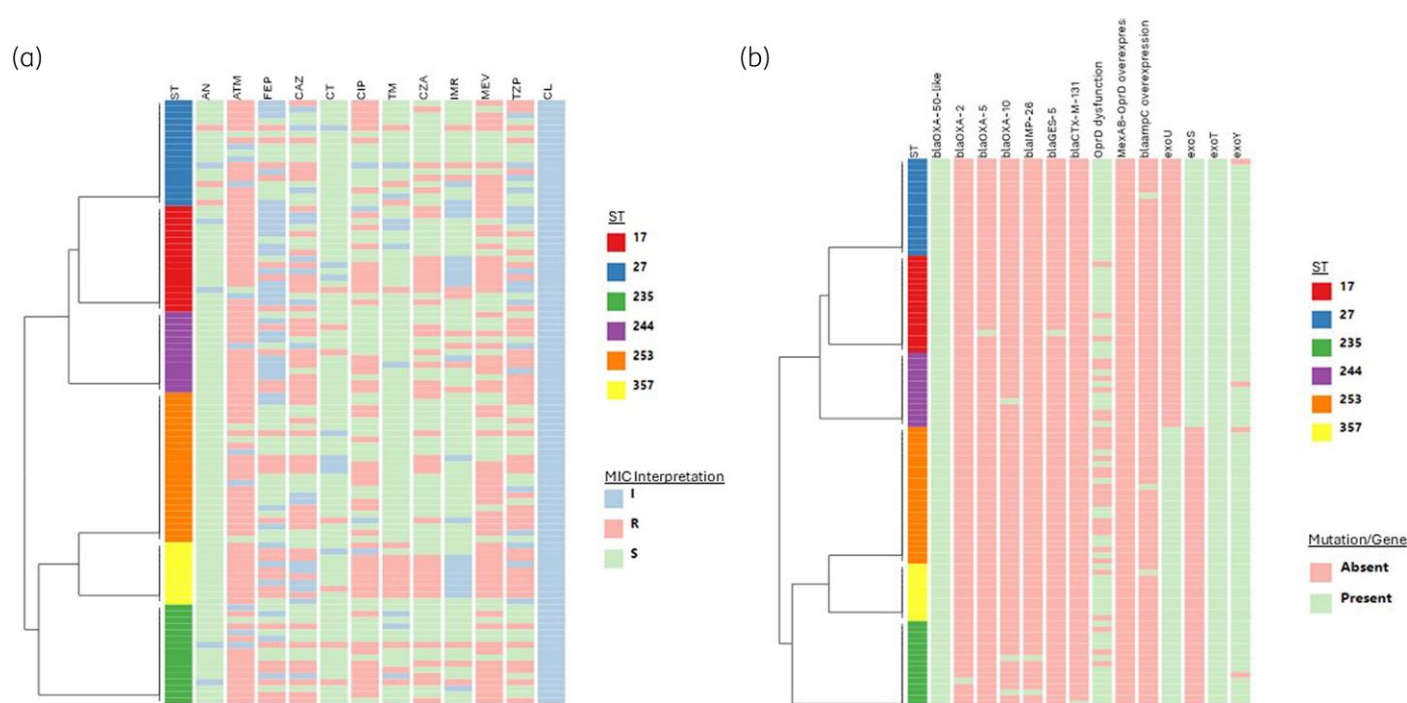


Figure 3. (a) Phylogenetic tree of the six most common STs ($n=97$) stratified by MIC interpretation data for amikacin (AN), aztreonam (ATM), ceftipime (FEP), ceftazidime (CAZ), ceftazidime–avibactam (CZA), ceftolozone–tazobactam (CT), ciprofloxacin (CIP), colistin (CL), imipenem–relebactam (IMR), meropenem (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%).³² 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 β -lactamase, porin inactivation, efflux pump overproduction and AmpC overexpression.^{6,8,13} Previous studies have shown increases in the proportion of *P. aeruginosa* isolates harbouring carbapenemases,^{32,33} 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.¹³ 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.^{15,28} In contrast, the prevalence of carbapenemase genes are significantly different internationally. Israel reported 9.4% of carbapenem-resistant isolates carried a carbapenemase,³²

whereas China and South America reported 32% and 69% carried a carbapenemase gene, respectively.³⁴ In the current study, the majority of carbapenem resistance in *P. aeruginosa* is likely attributable to the presence of intrinsic class D OXA genes³⁵ (98.1%) in combination with inactivation of the porin gene OprD (73.5%). Other rarely described class D β -lactamase genes like bla_{OXA}-2 and bla_{OXA}-10 are also suspected to cause carbapenem resistance particularly in the presence of low outer membrane permeability.^{11,36} 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%).^{13,32} 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.^{13,15} 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.^{9,27,32,37}

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

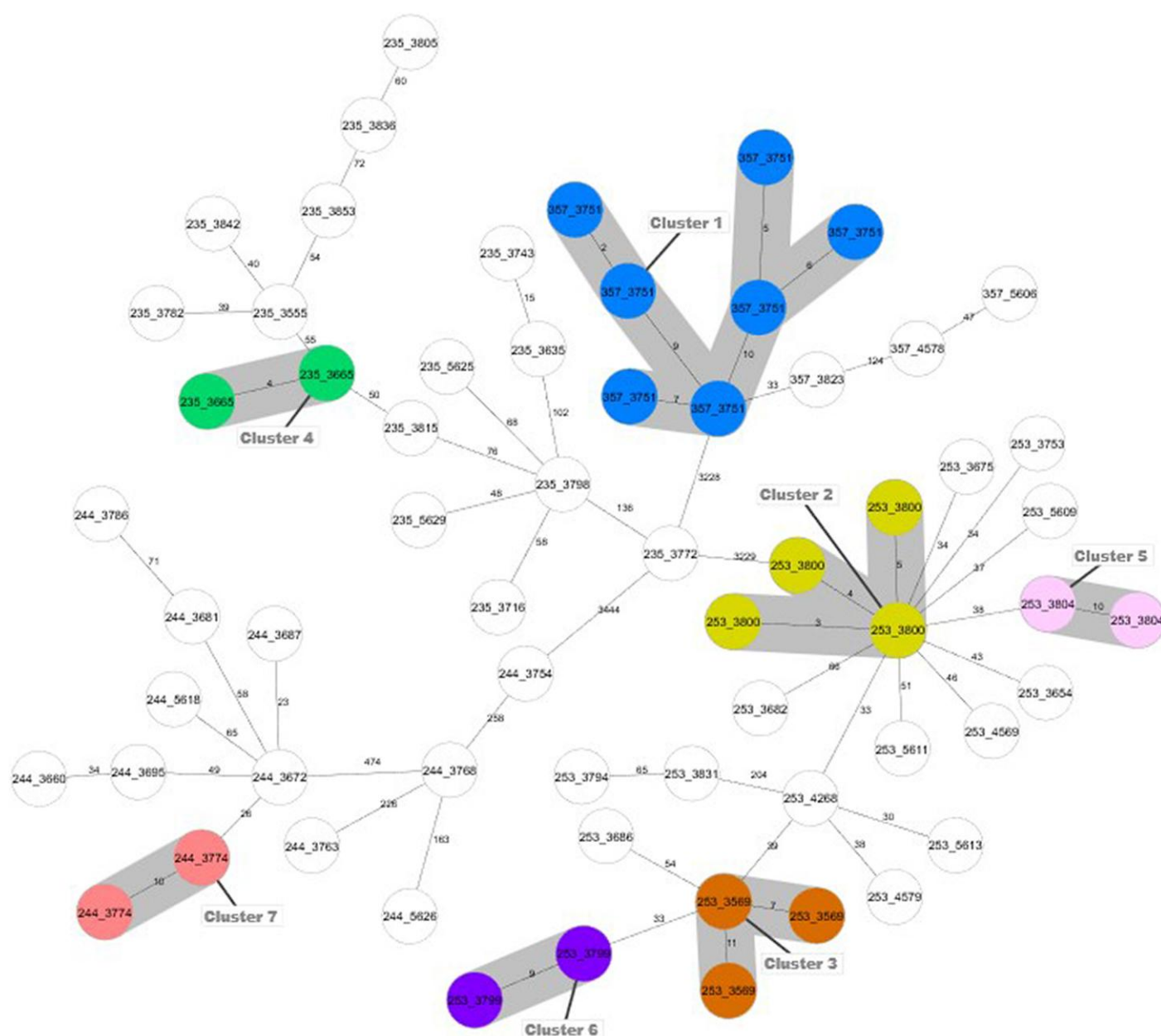


Figure 4. MLST of high-risk clones circulating amongst carbapenem-resistant *P. aeruginosa* ($n=63$). 7 clusters were identified using cgMLST. ST235_CT3665 ($n=2$), ST244_CT3774 ($n=2$), ST253_CT3569 ($n=3$), ST253_CT3799 ($n=2$), ST253_CT3804 ($n=2$), ST253_CT3800 ($n=4$) and ST357_CT3751 ($n=7$). Clusters are shaded in grey and represent isolates that have ≤ 12 allele differences.

amikacin were the most active antimicrobials against ST253 clones. All ST253 isolates tested positive for the *exoU* gene, which encodes the most toxic of the four cytotoxins and has been associated with increased premature mortality.^{27,41} Similarly, Peirano et al.⁴² found ST253 to be associated with the *exoU* gene. This gene encodes a toxin that helps the bacteria damage host cells and cause more severe infections. The presence of *exoU* in ST253 underscores this clone's ability to cause aggressive infections and highlights the importance of monitoring such clones with epidemiological surveillance and infection control measures. In our study, 11/24 (45.8%) of ST253 isolates were found

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 1984.⁴³ 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.⁴³ 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%.¹⁵ 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.⁴¹ 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.^{44,45} Unlike other high-risk clones, ST244 is not always associated with antimicrobial resistance, even though a wide diversity of β -lactamases have been associated with this clone.^{9,44} 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*.⁴⁴ 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.^{9,46} From 2007 to 2016, ST357 accounted for 3.1% of the total isolates,¹⁵ 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*_{IMP} and *bla*_{OXA} variants, this was not observed in our study.^{47,48} 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.^{9,49} 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.^{15,44}

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.

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

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Transparency declarations

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Disclaimer

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Supplementary data

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

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Distribution and antimicrobial susceptibility profile of pathogens recovered from the bloodstream of Canadian patients: results from the CANWARD study (2007–23)

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Objectives: Bloodstream infections are associated with significant morbidity and mortality. The purpose of this study was to determine the most common pathogens causing bacteraemia among Canadian patients and to evaluate their antimicrobial susceptibility profiles.

Methods: Annually from 2007 to 2023, bloodstream isolates from patients admitted to or evaluated at Canadian hospitals were collected by sentinel laboratories (CANWARD surveillance study). Antimicrobial susceptibility testing was performed by broth microdilution.

Results: In total, 26 067 bloodstream isolates were obtained over 17 years of the CANWARD study (14 954 from inpatients, 11 107 from outpatients, no patient location for six isolates). The five most common pathogens were *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis* and *Streptococcus pneumoniae*. There was little variation in pathogen distribution over time but certain pathogens were more common among inpatients (e.g. *Candida albicans*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*) while others were more common among outpatients (e.g. *Streptococcus pyogenes*, *Streptococcus agalactiae*). The proportion of ESBL-producing *E. coli* increased from 4.5% (2007–09) to 12.7% (2019–23). The proportion of ESBL-producing *K. pneumoniae* increased from 2.0% (2007–09) to 7.1% (2018–23). Vancomycin susceptibility among *Enterococcus faecium* decreased from 84.9% (2007–09) to 69.0% (2019–23). In contrast, the proportions of *S. aureus* that were MRSA and *P. aeruginosa* that had a difficult-to-treat resistance phenotype were relatively stable.

Conclusions: There has been little variation in the pathogens commonly causing bacteraemia among Canadian patients over the course of the CANWARD study, but some resistant phenotypes (e.g. ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, VRE) are being encountered more frequently in recent years.

Introduction

Bacteraemia is defined as the presence of viable bacteria in the blood.¹ Bloodstream infections may be classified as either primary (no documented source of infection) or secondary (due to a localized/focal infection), and are associated with significant patient morbidity and mortality.^{1–6} The mortality rate among patients with bacteraemia in several publications has ranged between 14.3% and 33.2%, and is influenced by many variables including the source of infection, the pathogen, patient

comorbidities and the severity of illness.^{2–6} Mortality is particularly high among bacteraemic patients with septic shock.⁷ Multiple studies have demonstrated an increase in mortality when there is a delay in the initiation of appropriate empiric antimicrobial therapy among patients with bacteraemia.^{2,3,8} Of concern, in recent years bloodstream infections due to certain antimicrobial-resistant organisms, including *Enterobacterales* that harbour an ESBL, have been observed with increasing frequency.^{9–12} Antimicrobial resistance, in turn, has been associated with patients receiving inappropriate empiric treatment.²

When patients present to an emergency department with sepsis or develop sepsis while admitted to a hospital, clinicians are faced with the task of selecting appropriate empiric antimicrobial therapy prior to culture results being known. Information used in making therapeutic decisions includes consideration of the likely source of infection (and the most common pathogens causing that infection), severity of illness and patient risk factors for harbouring an antimicrobial-resistant pathogen. Data from national surveillance studies can serve as an additional tool to help define the most likely pathogens a clinician will encounter should the patient prove to be bacteraemic. There has not been a large-scale surveillance study investigating the epidemiology and susceptibility profile of bloodstream isolates recovered from Canadian patients published in recent years. The purpose of this study was to determine the most common pathogens recovered from the bloodstream of patients admitted to or evaluated at Canadian hospitals and to assess whether the pathogen distribution and the susceptibility profile of the most frequently recovered bloodstream pathogens has changed over time.

Materials and methods

Bacterial isolates

Bloodstream isolates were obtained as a part of the CANWARD surveillance study, January 2007 to December 2023. CANWARD is an ongoing national Public Health Agency of Canada-PHAC/Canadian Antimicrobial Resistance Alliance partnered surveillance study evaluating *in vitro* activities of antimicrobial agents against bacterial pathogens isolated by clinical laboratories from patients attending tertiary care hospitals across Canada.¹³ Each isolate included was considered clinically significant by protocols in place at the submitting laboratory. The CANWARD surveillance study sets annual quotas for bloodstream isolates and requires isolates be collected consecutively, one per patient, from both in- and outpatients attending emergency rooms (ERs), hospital clinics, medical/surgical wards and intensive care units each year. All isolates are shipped to the CANWARD coordinating laboratory (Health Sciences Centre, Winnipeg, Canada) where their identities are confirmed by colonial appearance, spot testing,¹⁴ and/or MALDI-TOF mass spectrometry (Bruker Daltonics, Billerica, MA, USA) as required. Tertiary care hospitals in 8 of the 10 Canadian provinces participated in the CANWARD surveillance study, with the number of hospitals varying between 8 and 15 depending on the study year. The CANWARD surveillance study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

Antimicrobial susceptibility testing

Following two subcultures from frozen stock, antimicrobial susceptibility testing was performed by broth microdilution using in-house prepared 96-well microtitre panels, as described by CLSI.¹⁵ MICs were interpreted using current CLSI breakpoints, when available.¹⁶ Putative ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* were identified as isolates with a ceftaxime and/or ceftazidime MIC of ≥ 1 mg/L. ESBL-production was verified using the CLSI phenotypic confirmatory disk test.¹⁵ Difficult-to-treat resistance (DTR) among *Pseudomonas aeruginosa* was defined as isolates testing not susceptible (intermediate or resistant) to all of the following antimicrobials: meropenem, piperacillin-tazobactam, ceftazidime and ciprofloxacin.¹⁷

Statistical analysis

The Cochran–Armitage Test of trend was used to determine whether changes in antimicrobial susceptibility over time were statistically significant. Statistical analysis was performed using JMP Pro version 18.0.2.

Results

In total, 26 067 bloodstream isolates were recovered from Canadian patients as part of the CANWARD Study between 2007 and 2023. Of these isolates, 14 954 (57.4%) were obtained from inpatients (intensive care unit, surgical ward or medical ward) and 11 107 (42.6%) were obtained from outpatients (ER or clinic). The distribution of isolates by specific ward type was as follows: 3823 (14.7%) from patients in an intensive care unit, 2003 (7.7%) from patients on a surgical ward, 9128 (35.0%) from patients on a medical ward, 9485 (36.4%) from patients in an ER and 1622 (6.2%) from clinic patients. The ward type was not available for six isolates. Stratified by age, 2581 isolates (9.9%) were obtained from patients ≤ 17 years old, 11 373 isolates (43.6%) were obtained from patients 18–64 years old, and 12 110 isolates (46.5%) were obtained from patients ≥ 65 years old (no age documented for three patients). Stratified by biological sex, 14 834 isolates (56.9%) were obtained from male patients while 11 231 isolates (43.1%) were obtained from female patients (no biological sex documented for two patients).

The 10 most common pathogens recovered from the bloodstream of patients admitted to or evaluated at Canadian hospitals are listed in Table 1. The five most frequently isolated organisms were *E. coli* (24.5% of isolates), *Staphylococcus aureus* (17.3% of isolates), *K. pneumoniae* (7.9% of isolates), *Staphylococcus epidermidis* (4.7% of isolates) and *Streptococcus pneumoniae* (4.3% of isolates). These pathogens accounted for 58.7% of all bloodstream isolates in CANWARD. The pathogen distribution differed when comparing inpatients with outpatients (Table 1). While *E. coli*, *S. aureus* and *K. pneumoniae* were the pathogens most frequently recovered from both inpatients and outpatients, a higher proportion of bloodstream infections due to *E. coli* was found among outpatients. *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Candida albicans* were more commonly isolated from inpatients, while *S. pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae* were more frequently isolated from outpatients. The pathogen distribution also varied depending on the specific ward type (Table 2). Among inpatients, *C. albicans* was more frequently recovered from patients admitted to an intensive care unit or a surgical ward. In general, *P. aeruginosa* and *E. cloacae* were more frequently isolated from patients in the intensive care unit, a surgical ward, or a medical ward relative to an ER or clinic. Conversely, the top 10 pathogens recovered from ER patients included *S. pyogenes* and *S. agalactiae*, and a relatively higher proportion of *E. coli* and *S. pneumoniae*.

The pathogen distribution stratified by patient age and biological sex is presented in Table 3. The most common bloodstream pathogen among patients ≤ 17 years of age was *S. aureus*, while *E. coli* was most common among patients ≥ 65 years of age. The overall pathogen distribution by biological sex was comparable between males and females, but the proportion of bloodstream infections due to *E. coli* was higher for females. The top 10 bloodstream pathogens among Canadian patients, stratified by study time period, is provided in Table 4. There was some minor variability in pathogen distribution over the course of the study, but *E. coli*, *S. aureus* and *K. pneumoniae*, were consistently the most common pathogens recovered.

Table 1. Top 10 pathogens causing bloodstream infections among patients admitted to or evaluated at Canadian hospitals (CANWARD)

All locations		Inpatient ^a		Outpatient ^b	
Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)
<i>Escherichia coli</i>	6379 (24.5)	<i>Escherichia coli</i>	3055 (20.4)	<i>Escherichia coli</i>	3323 (29.9)
<i>Staphylococcus aureus</i>	4515 (17.3)	<i>Staphylococcus aureus</i>	2609 (17.4)	<i>Staphylococcus aureus</i>	1904 (17.1)
<i>Klebsiella pneumoniae</i>	2060 (7.9)	<i>Klebsiella pneumoniae</i>	1210 (8.1)	<i>Klebsiella pneumoniae</i>	850 (7.7)
<i>Staphylococcus epidermidis</i>	1215 (4.7)	<i>Staphylococcus epidermidis</i>	866 (5.8)	<i>Streptococcus pneumoniae</i>	696 (6.3)
<i>Streptococcus pneumoniae</i>	1108 (4.3)	<i>Enterococcus faecalis</i>	778 (5.2)	<i>Staphylococcus epidermidis</i>	349 (3.1)
<i>Enterococcus faecalis</i>	1091 (4.2)	<i>Pseudomonas aeruginosa</i>	698 (4.7)	<i>Enterococcus faecalis</i>	312 (2.8)
<i>Pseudomonas aeruginosa</i>	1003 (3.8)	<i>Enterococcus faecium</i>	504 (3.4)	<i>Streptococcus pyogenes</i>	306 (2.8)
<i>Enterobacter cloacae</i>	687 (2.6)	<i>Enterobacter cloacae</i>	484 (3.2)	<i>Pseudomonas aeruginosa</i>	305 (2.7)
CoNS ^c	603 (2.3)	<i>Candida albicans</i>	480 (3.2)	<i>Streptococcus agalactiae</i>	255 (2.3)
<i>Enterococcus faecium</i>	602 (2.3)	<i>Streptococcus pneumoniae</i>	412 (2.8)	CoNS	232 (2.1)
Other ^d	6804 (26.1)	Other	3858 (25.8)	Other	2575 (23.2)

^aInpatient = isolates obtained from patients on surgical wards, medical wards and intensive care units.

^bOutpatient = isolates obtained from patients seen in clinics or an emergency room.

^cCoNS, coagulase-negative staphylococci (not speciated).

^dOther: *Abiotrophia* spp., *Achromobacter* spp., *Acinetobacter* spp., *Aerococcus* spp., *Aeromonas* spp., *Aggregatibacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Arcanobacterium* spp., *Bacillus* spp., *Brevibacillus* spp., *Brevibacterium* spp., *Burkholderia* spp., *Campylobacter* spp., *Candida* spp., *Capnocytophaga* spp., *Cardiobacterium* spp., *Cedecea* spp., *Chryseobacterium* spp., *Citrobacter* spp., *Clostridium* spp., *Comamonas* spp., *Corynebacterium* spp., *Cronobacter* spp., *Cryptococcus* spp., *Cupriavidus* spp., *Cutibacterium* spp., *Delftia* spp., *Dermabacter* spp., *Diphtheroids*, *Eikenella* spp., *Elizabethkingia* spp., *Enterobacter* spp., *Enterococcus* spp., *Escherichia* spp., *Flavobacterium* spp., *Gemella* spp., *Gordonia* spp., *Granulicatella* spp., *Haemophilus* spp., *Hafnia* spp., *Jeptogalococcus* spp., *Klebsiella* spp., *Kocuria* spp., *Kytococcus* spp., *Lactobacillus* spp., *Lactococcus* spp., *Leclercia* spp., *Leifsonia* spp., *Lelliottia* spp., *Leuconostoc* spp., *Listeria* spp., *Macrococcus* spp., *Micrococcus* spp., *Moraxella* spp., *Morganella* spp., *Mycobacterium* spp., *Neisseria* spp., *Ochrobactrum* spp., *Paenibacillus* spp., *Pantoea* spp., *Paracoccus* spp., *Pasteurella* spp., *Phenylobacterium* spp., *Pluralibacter* spp., *Proteus* spp., *Providencia* spp., *Pseudomonas* spp., *Ralstonia* spp., *Raoultella* spp., *Rhodococcus* spp., *Roseomonas* spp., *Rothia* spp., *Saccharomyces* spp., *Salmonella* spp., *Serratia* spp., *Shigella* spp., *Sphingobacterium* spp., *Sphingomonas* spp., *Staphylococcus* spp., *Stenotrophomonas* spp., *Stomatococcus* spp., *Streptococcus* spp., *Trichosporon* spp., *Trueperella* spp., *Vibrio* spp., *Weissella* spp., *Yersinia* spp.

Antimicrobial susceptibility for the top 10 bacterial pathogens recovered from the bloodstream of Canadian patients, subdivided by study time period, is presented in Table 5. Coagulase-negative staphylococci (not speciated) were excluded from this table as antimicrobial susceptibility testing was not performed for these. The proportion of *E. coli* isolates that were ESBL-producers increased over the course of the study, from 4.5% (2007–09) to 12.7% (2019–23). There was a corresponding reduction in susceptibility of ceftriaxone over the 17 years of the study and a slight reduction in piperacillin-tazobactam susceptibility. Among *E. coli* isolates, susceptibility to ciprofloxacin, ertapenem and meropenem, remained fairly constant, with ertapenem, meropenem and piperacillin-tazobactam demonstrating the highest susceptibility rates. The proportion of *K. pneumoniae* isolates that were ESBL-producers also increased over time (from 2.0% in 2007–09 to 7.1% in 2018–23), although to a lesser extent than was observed for *E. coli*. Ertapenem, meropenem and gentamicin demonstrated good *in vitro* activity versus *K. pneumoniae* (>95% of isolates testing susceptible) over the course of the study. Susceptibility rates for most antimicrobials were either stable or improved slightly for bloodstream isolates of *P. aeruginosa*. The proportion of isolates with a DTR phenotype varied between 1.9% and 3.3% for most of the study (excluding the 2007–09 time period) with no clear temporal trend.

The proportion of *S. aureus* isolates that were MRSA declined early in the study but has since remained relatively stable at 17.4% to 19.4% (2010–23). Over 97% of *S. aureus* bloodstream isolates remained susceptible to ceftiofloxacin, daptomycin, doxycycline, linezolid, trimethoprim-sulfamethoxazole and vancomycin over the course of the CANWARD Study. The proportion of *S. pneumoniae* isolates that tested susceptible to penicillin (oral breakpoints) varied from 86.1% to 92.7%, without a clear trend to increasing or decreasing susceptibility over time. Irrespective of the study time period, over 98% of bloodstream *S. pneumoniae* isolates tested susceptible to ceftriaxone (non-meningitis breakpoints), levofloxacin and vancomycin. An increase in the proportion of *E. faecium* bloodstream isolates that were vancomycin resistant was observed over the study years, with a corresponding reduction in vancomycin susceptibility from 84.9% (2007–09) to 69.0% (2019–23).

The proportion of isolates with a common antimicrobial-resistant phenotype, stratified by ward type, is presented in Table 6. MRSA, ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, *P. aeruginosa* demonstrating DTR and VRE were all more frequently recovered from inpatients (intensive care unit, surgical ward, or medical ward) in comparison with outpatients (ER or clinic).

Table 2. Top 10 pathogens causing bloodstream infections among patients admitted to or evaluated at Canadian hospitals (CANWARD), stratified by hospital location

Hospital location			Surgical ward			Medical ward			ER			Clinics		
ICU														
Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Number (% of total)
<i>Staphylococcus aureus</i>	592 (15.5)	<i>Escherichia coli</i>	395 (19.7)	<i>Escherichia coli</i>	2103 (23.0)	<i>Escherichia coli</i>	3006 (31.7)	<i>Staphylococcus aureus</i>	318 (19.6)					
<i>Escherichia coli</i>	557 (14.6)	<i>Staphylococcus aureus</i>	372 (18.6)	<i>Staphylococcus aureus</i>	1645 (18.0)	<i>Staphylococcus aureus</i>	1586 (16.7)	<i>Escherichia coli</i>	317 (19.5)					
<i>Staphylococcus epidermidis</i>	327 (8.6)	<i>Klebsiella pneumoniae</i>	180 (9.0)	<i>Klebsiella pneumoniae</i>	755 (8.3)	<i>Klebsiella pneumoniae</i>	740 (7.8)	<i>Klebsiella pneumoniae</i>	110 (6.8)					
<i>Klebsiella pneumoniae</i>	275 (7.2)	<i>Enterococcus faecalis</i>	118 (5.9)	<i>Enterococcus faecalis</i>	445 (4.9)	<i>Streptococcus pneumoniae</i>	643 (6.8)	CoNS	95 (5.9)					
<i>Enterococcus faecalis</i>	215 (5.6)	<i>Staphylococcus epidermidis</i>	103 (5.1)	<i>Staphylococcus epidermidis</i>	436 (4.8)	<i>Streptococcus pyogenes</i>	283 (3.0)	<i>Staphylococcus epidermidis</i>	77 (4.7)					
<i>Pseudomonas aeruginosa</i>	191 (5.0)	<i>Candida albicans</i>	93 (4.6)	<i>Pseudomonas aeruginosa</i>	432 (4.7)	<i>Staphylococcus epidermidis</i>	272 (2.9)	<i>Enterococcus faecalis</i>	63 (3.9)					
<i>Candida albicans</i>	190 (5.0)	<i>Pseudomonas aeruginosa</i>	75 (3.7)	<i>Enterobacter cloacae</i>	283 (3.1)	<i>Enterococcus faecalis</i>	249 (2.6)	<i>Pseudomonas aeruginosa</i>	57 (3.5)					
<i>Enterococcus faecium</i>	162 (4.2)	<i>Enterococcus faecium</i>	68 (3.4)	<i>Enterococcus faecium</i>	274 (3.0)	<i>Pseudomonas aeruginosa</i>	248 (2.6)	<i>Streptococcus pneumoniae</i>	53 (3.3)					
CoNS ^a	157 (4.1)	<i>Enterobacter cloacae</i>	62 (3.1)	<i>Streptococcus pneumoniae</i>	266 (2.9)	<i>Streptococcus agalactiae</i>	221 (2.3)	<i>Enterobacter cloacae</i>	52 (3.2)					
<i>Enterobacter cloacae</i>	139 (3.6)	<i>Klebsiella oxytoca/Raoultella spp.</i>	55 (2.7)	<i>Candida albicans</i>	197 (2.2)	Viridans group streptococci	178 (1.9)	Viridans group streptococci	37 (2.3)					
Other	1018 (26.6)	Other	482 (24.1)	Other	2292 (25.1)	Other	2059 (21.7)	Other	443 (27.3)					

^aCoNS, coagulase-negative staphylococci (not speciated).

Table 3. Top 10 pathogens causing bloodstream infections among patients admitted to or evaluated at Canadian hospitals (CANWARD), stratified by patient age and biological sex

Patient age	18–64 years old				≥65 years old				Biological sex			
	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Pathogen	Number (% of total)	Male	Female	Number (% of total)	Pathogen
≤17 years old												
Staphylococcus aureus	Staphylococcus aureus	350 (13.6)	Staphylococcus aureus	2367 (20.8)	Escherichia coli	3678 (30.4)	Escherichia coli	3027 (20.4)	Escherichia coli	3352 (29.8)		
	Escherichia coli	335 (13.0)	Escherichia coli	2366 (20.8)	Staphylococcus aureus	1798 (14.8)	Staphylococcus aureus	2841 (19.2)	Staphylococcus aureus	1674 (14.9)		
Staphylococcus epidermidis	Staphylococcus epidermidis	328 (12.7)	Klebsiella pneumoniae	891 (7.8)	Klebsiella pneumoniae	1062 (8.8)	Klebsiella pneumoniae	1226 (8.3)	Klebsiella pneumoniae	834 (7.4)		
	Streptococcus pneumoniae	192 (7.4)	Streptococcus pneumoniae	564 (5.0)	Enterococcus faecalis	600 (5.0)	Enterococcus faecalis	731 (4.9)	Staphylococcus epidermidis	534 (4.8)		
Klebsiella pneumoniae	Klebsiella pneumoniae	107 (4.1)	Staphylococcus epidermidis	472 (4.2)	Pseudomonas aeruginosa	514 (4.2)	Staphylococcus epidermidis	681 (4.6)	Streptococcus pneumoniae	494 (4.4)		
	Enterococcus faecalis	89 (3.4)	Pseudomonas aeruginosa	429 (3.8)	Staphylococcus epidermidis	415 (3.4)	Streptococcus pneumoniae	614 (4.1)	Pseudomonas aeruginosa	393 (3.5)		
Staphylococcus hominis	Staphylococcus hominis	82 (3.2)	Enterococcus faecalis	402 (3.5)	Streptococcus pneumoniae	352 (2.9)	Pseudomonas aeruginosa	610 (4.1)	Enterococcus faecalis	360 (3.2)		
	Streptococcus agalactiae	81 (3.1)	Enterobacter cloacae	319 (2.8)	Enterobacter cloacae	294 (2.4)	Enterobacter cloacae	386 (2.6)	Enterobacter cloacae	300 (2.7)		
Streptococcus pyogenes	Streptococcus pyogenes	81 (3.1)	Enterococcus faecium	294 (2.6)	Enterococcus faecium	282 (2.3)	Enterococcus faecium	357 (2.4)	CoNS	253 (2.2)		
	Viridans group streptococci	77 (3.0)	CoNS ^a	280 (2.5)	Proteus mirabilis	256 (2.1)	CoNS	350 (2.4)	Enterococcus faecium	245 (2.2)		
Other	Other	859 (33.3)	Other	2989 (26.3)	Other	2859 (23.6)	Other	4011 (27.0)	Other	2792 (24.9)		

^aCoNS, coagulase-negative staphylococci (not speciated).

Table 4. Top 10 pathogens causing bloodstream infection among patients admitted to or evaluated at Canadian hospitals (CANWARD), stratified by study time period

Study years									
2007–09		2010–12		2013–15		2016–18		2019–23	
Organism	Number (% of total)	Organism	Number (% of total)	Organism	Number (% of total)	Organism	Number (% of total)	Organism	Number (% of total)
<i>Escherichia coli</i>	1850 (22.5)	<i>Escherichia coli</i>	1156 (22.2)	<i>Escherichia coli</i>	1000 (25.0)	<i>Escherichia coli</i>	1030 (26.9)	<i>Escherichia coli</i>	1343 (27.9)
<i>Staphylococcus aureus</i>	1457 (17.7)	<i>Staphylococcus aureus</i>	870 (16.7)	<i>Staphylococcus aureus</i>	761 (19.1)	<i>Staphylococcus aureus</i>	660 (17.2)	<i>Staphylococcus aureus</i>	767 (15.9)
<i>Klebsiella pneumoniae</i>	597 (7.3)	<i>Klebsiella pneumoniae</i>	383 (7.4)	<i>Klebsiella pneumoniae</i>	316 (7.9)	<i>Klebsiella pneumoniae</i>	302 (7.9)	<i>Klebsiella pneumoniae</i>	462 (9.6)
CoNS ^a	474 (5.8)	<i>Staphylococcus epidermidis</i>	310 (6.0)	<i>Staphylococcus epidermidis</i>	205 (5.1)	<i>Staphylococcus epidermidis</i>	205 (5.4)	<i>Pseudomonas aeruginosa</i>	199 (4.1)
<i>Streptococcus pneumoniae</i>	470 (5.7)	<i>Candida albicans</i>	258 (5.0)	<i>Streptococcus pneumoniae</i>	170 (4.3)	<i>Enterococcus faecalis</i>	141 (3.7)	<i>Enterococcus faecalis</i>	187 (3.9)
<i>Enterococcus faecalis</i>	388 (4.7)	<i>Streptococcus pneumoniae</i>	222 (4.3)	<i>Enterococcus faecalis</i>	164 (4.1)	<i>Enterobacter cloacae</i>	129 (3.4)	<i>Staphylococcus epidermidis</i>	185 (3.8)
<i>Pseudomonas aeruginosa</i>	332 (4.0)	<i>Enterococcus faecalis</i>	211 (4.1)	<i>Pseudomonas aeruginosa</i>	160 (4.0)	<i>Streptococcus pneumoniae</i>	127 (3.3)	Viridans group streptococci	153 (3.2)
<i>Staphylococcus epidermidis</i>	310 (3.8)	<i>Pseudomonas aeruginosa</i>	189 (3.6)	<i>Enterobacter cloacae</i>	102 (2.6)	<i>Pseudomonas aeruginosa</i>	123 (3.2)	<i>Enterococcus faecium</i>	145 (3.0)
<i>Enterobacter cloacae</i>	193 (2.3)	<i>Enterobacter cloacae</i>	123 (2.4)	<i>Enterococcus faecium</i>	96 (2.4)	<i>Enterococcus faecium</i>	94 (2.5)	<i>Enterobacter cloacae</i>	140 (2.9)
Viridans group streptococci	162 (2.0)	<i>Enterococcus faecium</i>	114 (2.2)	<i>Streptococcus agalactiae</i>	90 (2.3)	<i>Klebsiella oxytoca</i>	87 (2.3)	<i>Streptococcus pneumoniae</i>	119 (2.5)
Other	1988 (24.2)	Other	1370 (26.3)	Other	930 (23.3)	Other	932 (24.3)	Other	1116 (23.2)

^aCoNS, Coagulase-negative staphylococci (not speciated)

Table 5. Temporal trends in the antimicrobial susceptibility profile of common bacterial pathogens recovered from the bloodstream of patients admitted to or evaluated at Canadian hospitals (CANWARD)

	% Susceptible (n/N)					
Organism	2007–09	2010–12	2013–15	2016–18	2019–23	P value
<i>Escherichia coli</i>						
ESBL ^a	4.5 (84/1850)	4.9 (57/1156)	9.7 (97/1000)	10.2 (105/1030)	12.7 (170/1343)	<0.001
Amoxicillin-clavulanate	94.6 (1448/1531)	82.1 (949/1156)	81.8 (818/1000)	75.9 (782/1030)	62.5 (671/1073)	<0.001
Cefazolin	69.0 (1277/1850)	74.9 (866/1156)	74.3 (743/1000)	69.8 (719/1030)	61.1 (821/1343)	<0.001
Ceftazidime	95.4 (1015/1064)	94.6 (1093/1156)	92.3 (923/1000)	89.5 (922/1030)	86.2 (1157/1343)	<0.001
Ceftriaxone	93.5 (1730/1850)	92.7 (1072/1156)	89.0 (890/1000)	88.2 (908/1030)	84.4 (1133/1343)	<0.001
Ciprofloxacin	75.8 (1402/1850)	76.0 (879/1156)	75.4 (754/1000)	75.8 (781/1030)	72.5 (974/1343)	0.062
Ertapenem	99.8 (1526/1529)	99.9 (1155/1156)	99.8 (998/1000)	99.4 (1024/1030)	99.7 (1339/1343)	0.165
Gentamicin	89.1 (1648/1849)	89.9 (1039/1156)	90.3 (903/1000)	91.3 (940/1030)	90.6 (1217/1343)	0.076
Meropenem	100 (1850/1850)	100 (1156/1156)	100 (1000/1000)	99.9 (1029/1030)	99.9 (1341/1343)	ND ^b
Piperacillin-tazobactam	96.6 (1788/1850)	97.3 (1125/1156)	96.9 (969/1000)	93.8 (966/1030)	95.1 (1277/1343)	<0.001
SXT ^c	70.1 (1296/1850)	72.2 (835/1156)	73.0 (730/1000)	74.5 (767/1030)	71.6 (962/1343)	0.1222
<i>Staphylococcus aureus</i>						
MRSA ^a	24.4 (356/1457)	19.9 (173/870)	17.9 (136/761)	17.4 (115/660)	19.4 (149/767)	<0.001
Ceftobiprole ^d	100 (1456/1456)	100 (382/382)	100 (243/243)	100 (660/660)	100 (766/766)	ND
Clindamycin ^e	80.2 (1168/1456)	86.6 (744/859)	89.2 (677/759)	94.1 (621/660)	94.9 (727/766)	0.038
Daptomycin	100 (1456/1456)	99.9 (858/859)	100 (759/759)	99.8 (659/660)	99.7 (764/766)	ND
Doxycycline	No data	99.2 (482/486)	98.7 (750/760)	98.8 (652/660)	99.0 (758/766)	0.698
Linezolid	100 (1456/1456)	100 (859/859)	100 (759/759)	100 (660/660)	99.9 (765/766)	ND
SXT	97.2 (1415/1456)	99.2 (861/868)	99.2 (754/760)	99.4 (656/660)	98.4 (754/766)	0.003
Vancomycin	99.9 (1455/1456)	100 (859/859)	99.9 (758/759)	99.8 (659/660)	100 (766/766)	ND
<i>Klebsiella pneumoniae</i>						
ESBL ^a	2.0 (12/597)	4.4 (17/383)	6.3 (20/316)	8.3 (25/302)	7.1 (33/462)	<0.001
Amoxicillin-clavulanate	97.6 (483/495)	92.1 (352/382)	91.8 (290/316)	88.4 (267/302)	88.0 (315/358)	<0.001
Cefazolin	86.3 (515/597)	86.7 (332/383)	84.5 (267/316)	81.8 (247/302)	82.2 (379/461)	0.018
Ceftazidime	98.2 (327/333)	95.3 (365/383)	93.7 (296/316)	92.7 (280/302)	91.5 (422/461)	<0.001
Ceftriaxone	97.7 (583/597)	94.8 (363/383)	92.7 (293/316)	91.7 (277/302)	91.5 (422/461)	<0.001
Ciprofloxacin	88.6 (529/597)	88.5 (339/383)	88.6 (280/316)	88.1 (266/302)	88.7 (409/461)	0.957
Ertapenem	99.6 (493/495)	98.7 (378/383)	98.1 (310/316)	98.7 (298/302)	98.9 (456/461)	0.272
Gentamicin	97.7 (583/597)	96.6 (370/383)	95.3 (301/316)	95.7 (289/302)	96.5 (445/461)	0.179
Meropenem	99.8 (596/597)	99.7 (382/383)	99.1 (313/316)	99.3 (300/302)	99.1 (457/461)	ND
Piperacillin-tazobactam	94.3 (563/597)	92.2 (353/383)	93.4 (295/316)	89.4 (270/302)	93.9 (433/461)	0.379
SXT	91.6 (547/597)	90.1 (344/382)	88.9 (281/316)	88.7 (267/301)	88.5 (408/461)	0.064
<i>Streptococcus pneumoniae</i>						
Amoxicillin-clavulanate	99.1 (435/439)	96.8 (209/216)	98.8 (163/165)	97.6 (121/124)	99.1 (116/117)	ND
Ceftriaxone	100 (439/439)	99.1 (214/216)	100 (165/165)	100 (124/124)	99.1 (116/117)	ND
Clarithromycin	87.4 (383/438)	74.9 (161/215)	71.5 (118/165)	81.5 (101/124)	76.9 (90/117)	0.002
Levofloxacin	99.8 (438/439)	98.6 (213/216)	99.4 (162/163)	100 (124/124)	100 (117/117)	ND
Penicillin (IV) ^f	99.3 (436/439)	97.4 (188/193)	100 (165/165)	100 (124/124)	99.1 (116/117)	ND
Penicillin (oral) ^g	86.1 (378/439)	87.0 (168/193)	92.7 (153/165)	89.5 (111/124)	88.0 (103/117)	0.1719
SXT	89.6 (398/444)	84.7 (182/215)	83.4 (136/163)	87.1 (108/124)	82.1 (96/117)	0.032
Vancomycin	100 (445/445)	100 (216/216)	100 (163/163)	100 (124/124)	100 (117/117)	ND
<i>Staphylococcus epidermidis</i>						
Ceftobiprole ^c	100 (310/310)	100 (169/169)	100 (75/75)	100 (204/204)	99.4 (177/178)	ND
Clindamycin	55.8 (173/310)	54.5 (168/308)	60.0 (120/200)	64.7 (132/204)	66.9 (119/178)	0.002
Daptomycin	100 (310/310)	100 (308/308)	100 (200/200)	100 (204/204)	99.4 (177/178)	ND
Doxycycline	No data	96.4 (135/140)	96.0 (192/200)	96.6 (197/204)	91.0 (162/178)	0.035
Linezolid	100 (310/310)	100 (308/308)	100 (200/200)	100 (204/204)	100 (178/178)	ND
SXT	59.0 (183/310)	57.3 (177/309)	61.5 (123/200)	64.7 (132/204)	67.4 (120/178)	0.020
Vancomycin	100 (310/310)	100 (308/308)	100 (200/200)	100 (204/204)	100 (178/178)	ND

Continued

Table 5. Continued

Organism	% Susceptible (n/N)					P value
	2007–09	2010–12	2013–15	2016–18	2019–23	
<i>Enterococcus faecalis</i>						
Amoxicillin-clavulanate ^h	99.0 (309/312)	100 (208/208)	99.4 (162/163)	100 (141/141)	100 (132/132)	ND
Daptomycin	98.7 (378/383)	98.5 (202/205)	98.2 (160/163)	97.2 (137/141)	99.5 (184/185)	ND
Linezolid	99.5 (381/383)	94.1 (193/205)	99.4 (162/163)	100 (82/82) ⁱ	98.4 (182/185)	ND
Vancomycin	100 (383/383)	100 (205/205)	100 (163/163)	100 (141/141)	100 (185/185)	ND
<i>Pseudomonas aeruginosa</i>						
DTR ^{a,j}	5.5 (18/329)	2.6 (5/189)	1.9 (3/160)	3.3 (4/123)	2.0 (4/198)	ND
Ceftazidime	88.1 (290/329)	90.5 (171/189)	91.3 (146/160)	86.2 (106/123)	83.3 (165/198)	0.088
Ceftolozane-tazobactam	99.7 (328/329)	98.9 (187/189)	98.8 (158/160)	98.4 (121/123)	98.5 (195/198)	ND
Ciprofloxacin	71.7 (238/332)	84.1 (159/189)	81.9 (131/160)	94.3 (116/123)	91.9 (182/198)	<0.001
Colistin ^k	92.6 (262/283)	97.4 (184/189)	92.5 (148/160)	100 (123/123)	97.0 (192/198)	0.014
Meropenem	82.5 (274/332)	86.8 (164/189)	83.8 (134/160)	91.1 (112/123)	79.8 (158/198)	0.850
Piperacillin-tazobactam	88.6 (294/332)	91.0 (172/189)	90.0 (144/160)	91.9 (113/123)	87.4 (173/198)	0.875
Tobramycin	93.6 (308/329)	94.2 (178/189)	98.8 (158/160)	97.6 (120/123)	91.4 (181/198)	0.874
<i>Enterobacter cloacae</i>						
Ceftazidime	86.7 (91/105)	81.3 (100/123)	82.2 (83/101)	82.9 (107/129)	71.4 (100/140)	0.009
Ceftriaxone	77.7 (150/193)	76.4 (94/123)	74.3 (75/101)	80.6 (104/129)	70.0 (98/140)	0.286
Ciprofloxacin	92.2 (178/193)	90.2 (111/123)	93.1 (94/101)	96.1 (124/129)	91.4 (128/140)	0.592
Ertapenem	95.2 (157/165)	89.4 (110/123)	96.0 (97/101)	92.2 (119/129)	88.6 (124/140)	0.109
Gentamicin	98.4 (190/193)	96.7 (119/123)	98.0 (99/101)	98.4 (127/129)	95.7 (134/140)	ND
Meropenem	100 (193/193)	99.2 (122/123)	100 (101/101)	100 (129/129)	99.3 (139/140)	ND
Piperacillin-tazobactam	81.9 (158/193)	82.9 (102/123)	89.1 (90/101)	82.9 (107/129)	77.9 (109/140)	0.486
SXT	91.7 (177/193)	91.9 (113/123)	91.1 (92/101)	94.6 (122/129)	87.1 (122/140)	0.384
<i>Enterococcus faecium</i>						
Amoxicillin-clavulanate ^h	16.5 (22/133)	15.9 (18/113)	7.4 (7/95)	11.7 (11/94)	8.4 (10/119)	0.027
Daptomycin ^l	100 (152/152)	100 (112/112)	100 (95/95)	98.9 (93/93)	97.9 (142/145)	ND
Linezolid	97.4 (148/152)	83.0 (93/112)	93.7 (89/95)	84.0 (79/94)	90.3 (131/145)	0.084
Vancomycin	84.9 (129/152)	76.8 (86/112)	78.9 (75/95)	72.3 (68/94)	69.0 (100/145)	0.001

^aValues provided in the table indicate the percent of isolates with a particular resistance phenotype (e.g. ESBL, MRSA or DTR).

^bND, not done. Statistical analysis was not performed due to too few isolates testing not susceptible.

^cSXT, trimethoprim-sulfamethoxazole.

^dHealth Canada breakpoints were applied for ceftobiprole (MIC ≤ 4 mg/L is considered susceptible). While these breakpoints were used for both *S. aureus* and *S. epidermidis*, they are only approved for use by Health Canada versus *S. aureus* (i.e. breakpoints not defined for *S. epidermidis*).

^eTesting for inducible clindamycin resistance was not performed.

^fCLSI parenteral non-meningitis breakpoints were applied to determine IV penicillin susceptibility.

^gCLSI oral penicillin breakpoints were applied to determine oral penicillin susceptibility.

^hCLSI ampicillin breakpoints were used to determine susceptibility to amoxicillin-clavulanate.

ⁱData provided are for 2017 and 2018 only. No data available for isolates obtained from 2016.

^jDTR was defined as isolates testing not susceptible (intermediate or resistant) to all of the following antimicrobials: meropenem, piperacillin-tazobactam, ceftazidime and ciprofloxacin.

^kFor colistin versus *P. aeruginosa*, CLSI does not have a susceptible breakpoint. The percentages provided in the table reflect the proportion of *P. aeruginosa* isolates testing intermediately susceptible to colistin.

^lFor daptomycin versus *E. faecium*, CLSI does not have a susceptible breakpoint. The percentages provided in the table reflect the proportion of *E. faecium* isolates testing susceptible-dose dependent.

Discussion

The most common bloodstream pathogens recovered from patients admitted to or evaluated at Canadian hospitals between 2007 and 2023 in descending order of frequency were *E. coli*, *S. aureus*, *K. pneumoniae*, *S. epidermidis* and *S. pneumoniae*.

The data presented here are generally in keeping with other publications in the literature. Pfaller *et al.*¹⁸ evaluated the frequency of occurrence and antimicrobial susceptibility profile of bacterial pathogens recovered from the bloodstream of patients at 16 sentinel hospitals in the USA (10 sites), Europe (4 sites), Mexico

Table 6. Distribution of antimicrobial-resistant phenotypes among patients admitted to or evaluated at Canadian hospitals (CANWARD), stratified by ward type

Organism/Phenotype	% with phenotype (n/N)				
	Ward type ^a				
	ICU	Surgical ward	Medical ward	ER	Clinic
<i>E. coli</i> (% ESBL)	9.2 (51/557)	9.1 (36/395)	10.2 (214/2103)	6.6 (199/3006)	4.1 (13/317)
<i>S. aureus</i> (% MRSA)	22.8 (135/592)	25.5 (95/372)	23.2 (381/1645)	16.8 (267/1586)	16.0 (51/318)
<i>K. pneumoniae</i> (% ESBL)	8.0 (22/275)	3.3 (6/180)	7.3 (55/755)	2.7 (20/740)	3.6 (4/110)
<i>P. aeruginosa</i> (% DTR) ^b	7.3 (14/191)	6.7 (5/75)	2.3 (10/432)	1.2 (3/248)	3.5 (2/57)
<i>E. faecium</i> (% VRE) ^c	29.0 (47/162)	17.9 (12/67)	24.6 (67/272)	15.9 (11/69)	10.7 (3/28)

^aNo location data were provided for one *E. coli* and two *S. aureus* isolates. Vancomycin susceptibility was not determined for four *E. faecium* isolates.
^bDTR was defined as isolates testing not susceptible (intermediate or resistant) to all of the following antimicrobials: meropenem, piperacillin-tazobactam, ceftazidime and ciprofloxacin.
^cVRE were defined as *E. faecium* isolates having a vancomycin MIC of ≥ 8 mg/L.

(1 site) and South Korea (1 site) between 2012 and 2017. The five most common pathogens across all study years were *S. aureus*, *E. coli*, *Enterococcus* spp., *Klebsiella* spp. and coagulase-negative staphylococci.¹⁸ Ohnuma et al.² assessed the distribution of pathogens causing bloodstream infections among patients at 183 hospitals in the USA using data from the Premier Healthcare database (2016–20). The top five pathogens recovered from bacteraemic patients in this publication were *E. coli*, *S. aureus*, *Streptococcus* spp., *Klebsiella* spp. and *Enterococcus* spp.² Of note, coagulase-negative staphylococci were excluded from this analysis due to challenges in determining whether they represented true pathogens or contaminants. Variation in the rank order of pathogens across studies is potentially explained by differences in the study populations (including differences in the proportion of inpatients versus outpatients) and protocols.

While the rank order of the top five pathogens varied slightly over different time periods of our study, the organisms themselves were fairly consistent. Older data in the literature support the consistency of pathogens recovered from bacteraemic patients going back to at least the 1990s. Weinstein et al.⁵ evaluated 843 episodes of bacteraemia among 707 patients at three healthcare centres in the USA between 1992 and 1993. The five most common pathogens recovered from patients in their study were *S. aureus*, *E. coli*, coagulase-negative staphylococci, *K. pneumoniae* and *Enterococcus* spp.⁵

E. coli, *S. aureus* and *K. pneumoniae* were the pathogens most frequently recovered from the bloodstream of both inpatients and outpatients in our study. However, among outpatients (ER and clinic patients), a greater proportion of bloodstream infections were due to *S. pneumoniae* and beta-haemolytic streptococci (e.g. *S. agalactiae* and *S. pyogenes*) relative to inpatients. Among inpatients (ICU, medical wards and surgical wards), a relatively greater proportion of bloodstream infections were due to *E. faecium*, *P. aeruginosa*, *E. cloacae* and *C. albicans*. We hypothesize that differences in the pathogen distribution by ward location may be related to differences in the presenting clinical syndromes. We presume that bacteraemia due to community-acquired pneumonia and soft tissue infections

would be more common among outpatients and bacteraemia due to complicated post-operative infections, device-related infections (central line infections and urinary catheter infections), hospital-acquired pneumonia and ventilator-associated pneumonia would be more common among inpatients. It is also possible that inpatients would have received prior antimicrobial treatment, predisposing them to more antimicrobial-resistant organisms.

Over 17 years of the CANWARD study, we observed an increase in the proportion of *E. coli* and *K. pneumoniae* bloodstream isolates that harboured an extended-spectrum beta-lactamase enzyme. The increase in infections due to ESBL-producing *E. coli* described here has similarly been reported in the USA and other parts of the world.^{9–12} It is thought to be due, at least in part, to successful spread of the ST131 clone.¹¹ In a prior evaluation of 671 ESBL-producing *E. coli* clinical isolates obtained as a part of CANWARD (all specimen types, 2007–18), ST131 was the most frequent sequence type, accounting for 62.4% (419/671) of isolates.⁹ Further, CTX-M-type beta-lactamases were the dominant enzymes encountered, with 92% (617/671) of ESBL-producing *E. coli* possessing one of these (most commonly CTX-M-15, CTX-M-14 or CTX-M-27).⁹ We also observed an increase in the proportion of *E. faecium* isolates that were vancomycin resistant over time. An increase in bloodstream infections due to vancomycin-resistant Enterococci among Canadian patients has been previously reported by the Canadian Nosocomial Infection Surveillance Program.¹⁹ This may be linked to a change in infection control practices, including discontinuation of screening and isolation for VRE at some acute care facilities in Canada.¹⁹

There are several important limitations to this work that deserve attention. The sites participating in the CANWARD study and the number of bloodstream isolates requested from participating sites on an annual basis varied somewhat depending on the study year. This potentially may have influenced trends in pathogen distribution and antimicrobial susceptibility over time. There were no unified criteria across all study sites for determining clinical significance of isolates (e.g. requirement that bacteria considered common blood culture contaminants such as coagulase-negative staphylococci be recovered from at least

two sites). Rather, this attribution was made by the submitting site based on local criteria. As such, it cannot be excluded that some of the organisms described here were actually contaminants rather than true pathogens. In early study years, CANWARD received a number of bloodstream isolates that were simply identified as coagulase-negative staphylococci, while in later years coagulase-negative staphylococci were typically identified to the species level. We speculate that this is due to the widespread implementation of MALDI-TOF across Canada for organism identification over the last two decades. It is possible that some of the coagulase-negative staphylococci isolates may have in fact been *S. epidermidis*, thereby introducing some error into the number of *S. epidermidis* isolates reported here. Some clinically relevant antimicrobials were not included on the susceptibility panels used here due to space limitations. Susceptibility data were not available for certain antimicrobials versus a small number of isolates. Finally, clinical information on patients from whom the isolates were obtained was not collected. Hence, we were unable to stratify pathogen distribution by source of bacteraemia/presenting clinical syndrome.

In summary, the most common pathogens recovered from the bloodstream of patients admitted to or evaluated at Canadian hospitals between 2007 and 2023 were *E. coli*, *S. aureus*, *K. pneumoniae*, *S. epidermidis* and *S. pneumoniae*. The distribution of pathogens did not vary much over the course of the study, but some variation in the pathogen distribution was observed when comparing inpatients with outpatients. ESBL-producing *E. coli* and *K. pneumoniae* isolates were recovered more frequently in later study years, with a corresponding reduction over time in the susceptibility of third generation cephalosporins versus these species. An increase in the proportion of *E. faecium* bloodstream isolates that were resistant to vancomycin was also observed over time. However, the proportion of *S. aureus* isolates that were MRSA and *P. aeruginosa* isolates that had a DTR phenotype remained fairly stable over the course of the study. Knowledge of the common pathogens causing bloodstream infections and their susceptibility profiles may assist clinicians in selecting empiric antimicrobial therapy for patients presenting with severe sepsis, although local/institution level data remains preferable for guiding treatment decision.

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Disclaimer

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