Population-based genomic surveillance of *Pseudomonas aeruginosa* causing bloodstream infections in a large Canadian health region

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Abstract

Purpose: Population-based surveillance was undertaken to determine clinical factors, susceptibility patterns, and incidence rates (IR) of *Pseudomonas aeruginosa* causing bloodstream infections (BSIs) in a Canadian region (2010–2018).

Methods: We combined clinical data with genomics to characterize *P. aeruginosa* (BSIs) ($n = 167$) in a well-defined Canadian (Calgary) human population over a 9-year period (2010–2018).

Results: The annual population IR per 100,000 patient years increased from 3.4/100,000 in 2010 to 5.9/100,000 in 2018, with the highest IRs in elderly males from the hospital setting. Over a quarter of patients presented with febrile neutropenia, followed by urinary tract infections and pneumonia. Antimicrobial resistance (AMR) rates and determinants were rare. The *P. aeruginosa* population was polyclonal consisting of three dominant sequence types (STs), namely ST244, ST111, and ST17. Antimicrobial-susceptible ST244 was the most common clone and belonged to three clades (A, B, C). The ST244 IR/100,000 increased over time due to the expansion of clade C. Multidrug-resistant ST111 was the second most common clone and IR/100,000 decreased over time. ST111 belonged to three clades (A, B, C) with clade C containing *bla*$_{\text{VIM-2}}$. Different serotypes were linked to various STs. The IR/100,000 of *P. aeruginosa* that belonged to serotypes O6 increased significantly over time.

Conclusion: An effective multivalent vaccine consisting of five serotypes (O1, O3, O5, O6, O11) would confer protection to > 70% of Calgary residents with *P. aeruginosa* BSIs. This study has provided a unique perspective of the population dynamics over time of *P. aeruginosa* STs, clades, and serotypes responsible for BSIs.

Keywords: *P. aeruginosa* bloodstream infections; Population-based surveillance; Genomics; Dominant clones; Serotypes

Introduction

Pseudomonas aeruginosa ranks among the top ten bacterial causes of bloodstream infections (BSIs) globally [1, 2]. Overall, it is responsible for between 4 and 9% of culture-positive bacterial BSIs [1,2,3]. *P. aeruginosa* BSIs are linked with high mortality rates, ranging from 21 to 62%, and prolonged length of hospital stay [2, 4, 5].

Population-based studies are optimal to determine the occurrence of clinical conditions, such as BSIs, within well-defined populations at risk [6]. The selection bias is minimized by the inclusion of all cases that fulfill a case definition, and incidence rates can be calculated across sex and age groups in a well-defined human community [7, 8]. Population-based studies establish the burden of a disease and facilitate comparisons between different regions and time periods [9]. Combining molecular epidemiology with population-based data is ideal for studying trends and changes in population dynamics over time [10,11,12].

The prevalence, risk factors, mortality rates, and antimicrobial resistance rates of *P. aeruginosa* BSIs in selected populations (i.e., ICU admissions, transplant recipients, cancer patients) are well known [13]. However, *P. aeruginosa* population-based studies in unselected human populations are rare [14]. Furthermore, population-level characteristics and trends over time of BSIs, due to different *P. aeruginosa* sequence types (STs) and serotypes, are currently unknown. Such information will aid in designing better treatment and prevention strategies.

An active population-based surveillance design was utilized to determine clinical factors, susceptibility patterns, and incidence rates (IR) of *P. aeruginosa* STs (i.e., clones), clades, and serotypes causing BSIs in a centralized Canadian region (i.e., Calgary) during 2010, 2014, and 2018. The clinical data was combined with genomics to provide a unique perspective of the molecular epidemiology and population dynamics of *P. aeruginosa* BSIs in a well-defined human population stretching over a 9-year period.

Materials and methods

Study population and clinical data

Alberta Precision Laboratories (previously Calgary Laboratory Services) is a regional, centralized laboratory system that performs all clinical microbiology services (hospital and community patients) within the Calgary region, Alberta, Canada. There are four adult hospitals (with 2595 beds) and one pediatric (with 133 beds) hospital in Calgary. All Calgary *P. aeruginosa* isolates (adult and pediatric) from blood cultures processed by Alberta Precision Laboratories in 2010, 2014, and 2018 were eligible for inclusion.

Clinical information corresponding to source patients at the time of the *P. aeruginosa* BSI was obtained using Sunrise Clinical Manager (All-scripts Healthcare Solutions, Inc., Chicago, IL, USA). A case of *P. aeruginosa* BSI was defined as a patient with a systemic inflammatory response and documented growth of a *P. aeruginosa* isolate in a blood culture. Incident cases were defined as Calgary residents with the first isolation of *P. aeruginosa* from blood per year. Repeat isolates per year were excluded. BSIs were classified as community-acquired, healthcare-associated, or hospital-acquired [15].

Bacterial isolates, identification, and susceptibility testing

All *P. aeruginosa* isolates recovered from blood between January 1 and December 31 of 2010 ($n = 37$), January 1 and December 31 of 2014 ($n = 56$), and January 1 and December 31, 2018 ($n = 74$), were obtained from the frozen depository and included in the study.

Identification and susceptibility testing were conducted using the matrix-assisted laser desorption ionization–time of flight mass spectrometry (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO) and VITEK 2 instrument (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO) respectively. Susceptibilities to the following antimicrobials were determined: piperacillin-tazobactam, ceftazidime, cefepime, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin. Throughout this study, results were interpreted using Clinical Laboratory Standards Institute criteria for broth dilution [16]. Dominant STs were defined as representing ≥ 5% of the total *P. aeruginosa* population.

Molecular characterization

All the *P. aeruginosa* isolates ($n = 167$) underwent short-read (Nextera XT DNA sample preparation kit Illumina, San Diego, CA, USA) whole genome sequencing (WGS), using procedures described previously [17, 18]. Samples were multiplexed and sequenced on an Illumina NovaSeq for 300 cycles (151 bp paired end). Draft genomes were obtained using SPAdes version 3.15.0 [19]. To define the presence of genes and mutations, BLAST [20] in combination with the following databases or typing schemes were accessed: NCBI Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047). The *P. aeruginosa* serotyper (PAst 1.0) was used to identify serotypes [21], and multilocus sequence typing was performed in silico with https://pubmlst.org/organisms/pseudomonas-aeruginosa [22].

We created a core single-nucleotide polymorphism (SNP)–based phylogenetic tree of the dominant STs and identified SNPs by mapping the reads or aligning the genomes against the reference strain PAO1 (GenBank Accession no. NC_002516) using the RedDog pipeline (https://github.com/katholt/RedDog). We included core SNPs that were present in all genomes to create a maximum-likelihood tree using RAxML with the general time-reversible plus gamma substitution model [23]. We visualized the tree by using iTOL version 6 (https://itol.embl.de/). To identify clades, we used a phylogeny-free population genetics approach of core SNPs. The hierarchal Bayesian Analysis of Population Structure clustering analysis [24] was conducted with two nested levels with a priori upper bound of the number of clusters between one-fourth and one-half of the total number of isolates. We defined clades within STs by using the second level of clustering.

Statistical analysis

We estimated incidence rates (IR) per 100,000 person-years and respective 95% confidence intervals (95%CI) by year, sex, age group, hospital discharges, and long-term care status using the Poisson distribution as described before [7, 25]. Denominator data was extracted from the Alberta Health Services Annual Report 2018–2019 (https://www.albertahealthservices.ca/assets/about/publications/2018-19-annual-report-web-version.pdf), the Alberta Health Services Annual Report 2015–2016 (https://www.albertahealthservices.ca/assets/about/publications/ahs-pub-2015-2016-annual-report.pdf), the Alberta Health Services Annual Report 2010–2011

(https://www.albertahealthservices.ca/publications/ahs-pub-annual-rpt-2010-2011.pdf), and Calgary's Civic Census of 2019 (https://www.calgary.ca/info-requests/censusresults.html), which contained age and sex stratified data from previous years. We utilized weighted averages to estimate the age- and sex-stratified population for the years of 2010 (using data from 2009 and 2011, weighted 1:1) and 2018 (using data from 2016 and 2019, weighted 1:2). Comparisons between clinical characteristics of dominant STs were conducted using pairwise Fisher's exact tests, adjusted for multiple comparisons for each outcome. Poisson tests were utilized to compare IRs. Statistical significance was set at 5% level.

Results

Patient characteristics and incidence rates

Incidence rates

The annual population IR per 100,000 patient years of *P. aeruginosa* BSIs among Calgary residents was 3.4/100,000 (95% confidence interval (CI) 2.4–4.7) in 2010, 4.7/100,000 (95% CI 3.5–6.1) in 2014, and 5.9/100,000 (95% CI 4.6–7.4) in 2018 (Table 1, Fig. 1). Overall, the IR/100,000 of *P. aeruginosa* BSIs increased in Calgary residents from 2010 to 2018 ($P = 0.01$). Males over 75 years had the highest overall IR/100,000 over the 9-year period (Fig. 1).

Table 1. Characteristics of patients with *Pseudomonas aeruginosa* bloodstream infections ($n = 167$) in Calgary, Alberta, Canada (2010, 2014, 2018)

	Number (percentage)
Year	
2010	37 (22.1%)
2014	56 (33.5%)
2018	74 (44.3%)
Sex	
Male	120 (71.9%)
Female	47 (28.1%)
Healthcare exposure	
Community-acquired	13 (7.8%)
Hospital-acquired	106 (63.5%)
Healthcare-associated	48 (28.7%)
Long-term care resident	28 (16.8%)
Clinical presentation	
Cellulitis	23 (13.8%)
Febrile neutropenia	43 (25.7%)
Intra-abdominal infection	18 (10.8%)
Pneumonia	28 (16.8%)
Primary sepsis	14 (8.4%)
Urinary tract infection	32 (19.2%)
Others	9 (5.3%)
Average age years (SD)	63.7 (21.1)
Incidence rate/100,000 person-years (95% confidence interval)	
Males	
2010	5.2 (3.4–7.5)
2014	6.9 (5.0–9.3)
2018	8.1 (6.0–10.6)
Females	
2010	1.7 (0.8–3.2)
2014	2.5 (1.4–4.1)
2018	3.7 (2.3–5.5)
Overall	
2010	3.4 (2.4–4.7)
2014	4.7 (3.5–6.1)
2018	5.9 (4.6–7.4)

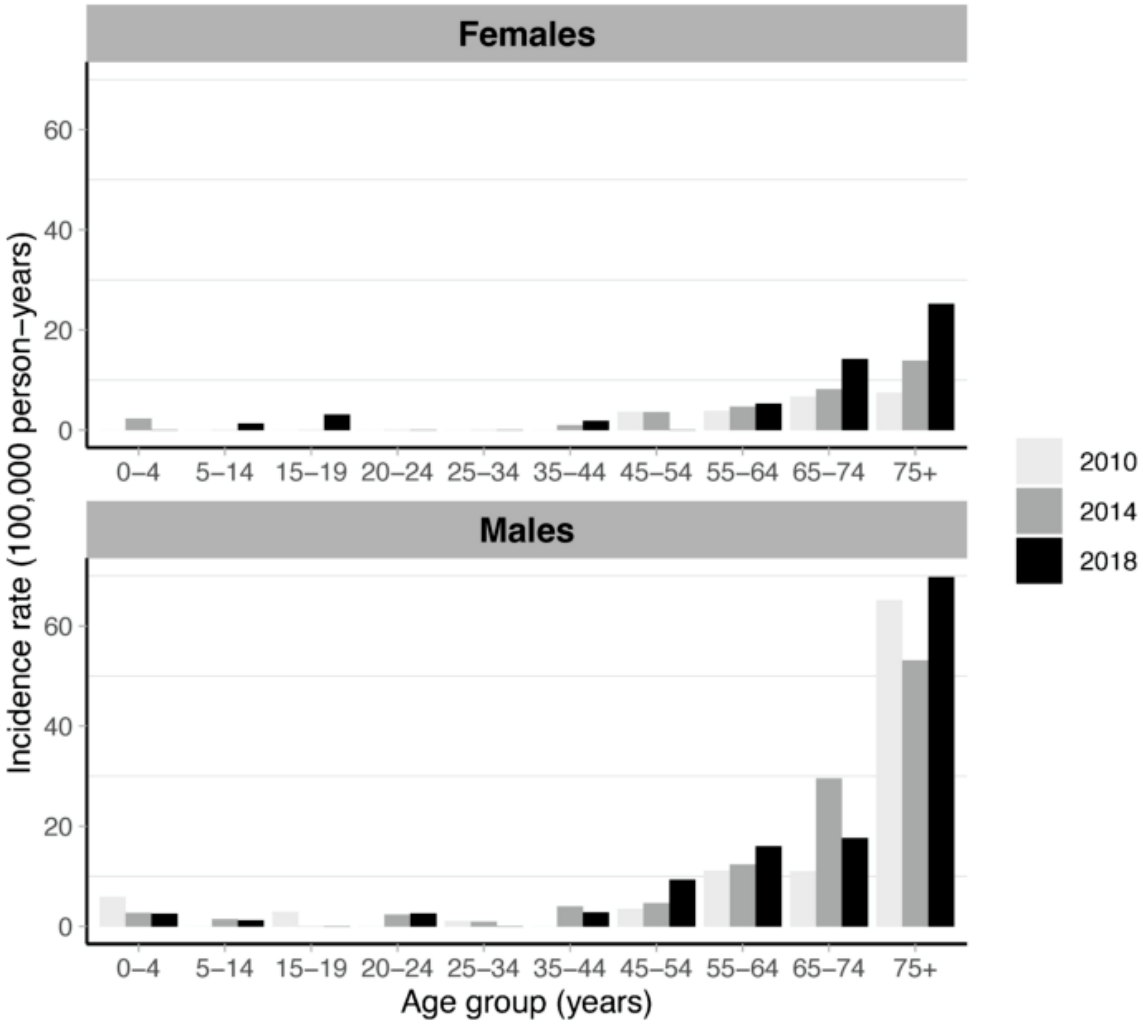

Fig. 1. The annual population incidence rates per 100,000 patient years of *P. aeruginosa* bloodstream infections among Calgary residents (2010–2018)

Patient characteristics

P. aeruginosa BSIs were more common in males (72%) with an average age of 63.7 years (Table 1). Most patients acquired BSIs within the hospital setting (63%), followed by healthcare-associated (29%) and community-acquired infections (8%). Twenty-six percent of patients presented with febrile neutropenia, followed by urinary tract infections (19%), pneumonia (17%), cellulitis (14%), and intra-abdominal infections (11%) (Table 1). Other types of infections included cholangitis (2%), heart valve abscess (0.5%), line infection (2%), and shunt infection (0.5%).

Microbiology and antimicrobial susceptibilities

A total of 167 unique *P. aeruginosa* isolates were obtained from blood cultures during 2010 ($n = 37$), 2014 ($n = 56$), and 2018 ($n = 74$) (Table 2). *P. aeruginosa* was the third most common Gram-negative bacteria in Calgary obtained from blood cultures during 2010, 2014, and 2018 (behind *Escherichia coli* and *Klebsiella pneumoniae*).

Table 2. Characteristics of *Pseudomonas aeruginosa* sequence type bloodstream infections ($n = 167$) in Calgary, Alberta, Canada (2010, 2014, 2018)

	Sequence type						
	ST244	ST111	ST17	ST179	ST253	Others	All
Number (%)	17 (10%)	10 (6%)	8 (5%)	7 (4%)	6 (4%)	119 (71%)	167
Year							
2010	2 (11.8%)	4 (40%)	2 (25%)	2 (28.6%)	3 (50%)	24 (20.2%)	37 (22.2%)
2014	7 (41.2%)	4 (40%)	1 (12.5%)	3 (42.9%)	1 (16.7%)	40 (33.6%)	56 (33.5%)
2018	8 (47.1%)	2 (20%)	5 (62.5%)	2 (28.6%)	2 (33.3%)	55 (46.2%)	74 (44.3%)
Hospital location							
Hospital 1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	5 (4.2%)	6 (3.6%)
Hospital 2	8 (47.1%)[a]	9 (90%)[b]	5 (62.5%)	4 (57.1%)	2 (33.3%)[a]	58 (48.7%)	86 (51.5%)
Hospital 3	3 (17.6%)	1 (10%)	1 (12.5%)	1 (14.3%)	2 (33.3%)	23 (19.3%)	31 (18.6%)
Hospital 4	3 (17.6%)	0 (0%)	0 (0%)	2 (28.6%)	1 (16.7%)	24 (20.2%)	30 (18%)
Hospital 5	1 (5.9%)	0 (0%)	2 (25%)	0 (0%)	0 (0%)	4 (3.4%)	7 (4.2%)
Others	2 (11.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (4.2%)	7 (4.2%)
Serotype							
O1	0 (0%)[a]	0 (0%)[a]	7 (87.5%)[b]	0 (0%)[a]	0 (0%)[a]	21 (17.6%)	28 (16.8%)
O2	8 (47.1%)[a]	0 (0%)[b]	0 (0%)[b]	0 (0%)[b]	0 (0%)[b]	3 (2.5%)	11 (6.6%)
O3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	19 (16%)	19 (11.4%)
O4	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	3 (2.5%)	5 (3%)
O5	9 (52.9%)[a]	0 (0%)[b]	0 (0%)[b]	0 (0%)[b]	0 (0%)[b]	23 (19.3%)	32 (19.2%)
O6	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	7 (100%)[b]	0 (0%)[a]	24 (20.2%)	31 (18.6%)
O9	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (2.5%)	3 (1.8%)
O10	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	6 (100%)[b]	0 (0%)	6 (3.6%)
O11	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	17 (14.3%)	17 (10.2%)
O12	0 (0%)[a]	8 (80%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	0 (0%)	8 (4.8%)
No serotype detected	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)	6 (5%)	7 (4.2%)
Antimicrobials (NS)							
Cefepime	0 (0%)[a]	5 (50%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)	3 (2.5%)	8 (4.8%)
Ceftazidime	0 (0%)[a]	6 (60%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	6 (5%)	12 (7.2%)
Piperacillin-tazobactam	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	6 (5%)	8 (4.8%)
Meropenem	0 (0%)[a]	7 (70%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	6 (5%)	13 (7.8%)
Ciprofloxacin	1 (5.9%)[a]	8 (80%)[b]	1 (12.5%)[a]	0 (0%)[a]	1 (16.7%)[a]	5 (4.2%)	16 (9.6%)
Gentamicin	0 (0%)[a]	6 (60%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	3 (2.5%)	9 (5.4%)
Tobramycin	0 (0%)[a]	7 (70%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	1 (0.8%)	8 (4.8%)
Amikacin	0 (0%)[a]	6 (60%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	1 (0.8%)	7 (4.2%)
OXA β-lactamases							
OXA-2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)	1 (0.6%)
OXA-395	0 (0%)[a]	10 (100%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	10 (8.4%)	20 (12%)
OXA-396	17 (100%)[a]	0 (0%)[b]	0 (0%)[b]	6 (85.7%)[a]	0 (0%)[b]	44 (37%)	67 (40.1%)
OXA-486	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	20 (16.8%)	20 (12%)
OXA-488	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	6 (100%)[b]	14 (11.8%)	20 (12%)
OXA-50	0 (0%)[a]	0 (0%)[a]	8 (100%)[b]	0 (0%)[a]	0 (0%)[a]	29 (24.4%)	37 (22.2%)
Aminoglycoside-modifying enzymes							
aac(6')-29b	0 (0%)[a]	6 (60%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)[a]	0 (0%)	6 (3.6%)
aac(6')-Il	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)	1 (0.6%)
aadA1b	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)	1 (0.6%)
aadA6	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	1 (0.6%)
aadA7	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	0 (0%)	1 (0.6%)
aph(3')-IIb	16 (94.1%)[a]	10 (100%)[a]	8 (100%)	4 (57.1%)[b]	5 (83.3%)	114 (95.8%)	157 (94%)

	Sequence type						
	ST244	ST111	ST17	ST179	ST253	Others	All
aph(3")-Ib	1 (5.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)	2 (1.2%)
aph(6)-Id	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)	1 (0.6%)
Other determinants							
bla_{VIM-2}	0 (0%)[a]	5 (50%)[b]	0 (0%)[a]	0 (0%)[a]	0 (0%)	0 (0%)	5 (3%)
crpP	8 (47.1%)[a]	8 (80%)	8 (100%)[b]	7 (100%)[b]	5 (83.3%)	57 (47.9%)	93 (55.7%)
fosA	16 (94.1%)	10 (100%)	8 (100%)	7 (100%)	5 (83.3%)	112 (94.1%)	158 (94.6%)
qacE	0 (0%)[a]	7 (70%)[b]	1 (12.5%)[a]	0 (0%)[a]	1 (16.7%)	1 (0.8%)	10 (6%)
sul1	0 (0%)[a]	7 (70%)[b]	1 (12.5%)[a]	0 (0%)[a]	2 (33.3%)	1 (0.8%)	11 (6.6%)
Type III secretion system genes							
exoS	17 (100%)[a]	10 (100%)[a]	8 (100%)[a]	7 (100%)[a]	0[b]	106 (89%)	148 (89%)
exoT	17 (100%)	10 (100%)	8 (100%)	7 (100%)	6 (100%)	114 (96%)	162 (97%)
exoU	0[a]	0[a]	0[a]	0[a]	6 (100%)[b]	13 (11%)	19 (11%)
exoY	16 (94.1%)	9 (90%)	8 (100%)	6 (86%)	4 (67%)	109 (92%)	152 (91%)

[a,b]Rates followed by different letters indicate significant differences between dominant STs at the 5% level (adjusted for multiple comparison)
NS non-susceptible

Overall, *P. aeruginosa* was responsible for 3% (167/5621) of bloodstream infections. Antibiotic susceptibilities are shown in Table 2. Low (< 10%) non-susceptible (NS) (i.e., intermediate, or resistant) rates were shown for piperacillin-tazobactam, ceftazidime, cefepime, meropenem, amikacin, gentamicin, tobramycin, and ciprofloxacin.

Genomics

Antimicrobial resistance (AMR) determinants

Overall, acquired AMR determinants were rare in this collection (Table 2). The exceptions were OXA-β-lactamases, *aph(3')-llb* (i.e., aminoglycoside-modifying enzyme), *crpP* (i.e., quinolone resistance determinant), and *fosA* (i.e., fosfomycin-resistant determinant).

Sequence types (STs)

One hundred and two (102) different STs were detected among the 167 isolates including 15 novel STs, namely ST3770, ST3771, ST3772, ST3773, ST3774, ST3775, ST3776, ST3777, ST3778, ST3779, ST3844, ST3867, ST3868, ST3869, and ST3870. Three dominant STs were identified: ST244 ($n = 17$ (10%)), ST111 ($n = 10$ (6%)), and ST17 ($n = 8$ (5%)) (Table 2). ST179 and ST253 did not fulfil our "dominant" ST definition (≥ 5%), but since each ST consisted of 4% of the total population, they were also included in Table 2 and Fig. 2. The IR of ST244 per 100,000 hospital discharges increased over the 9-year period (2.19 in 2010 (95% CI 0.27–7.92) to 5.52 in 2018 (95% CI 2.38–10.88)), but that increase was not statistically significant ($P = 0.33$). A decrease in the IR of ST111 per 100,000 hospital discharges was seen over time (4.39 in 2010 (95% CI 0.17–4.99) to 1.38 in 2018 (95% CI 2.38–10.88)), but the decrease was not statistically significant ($P = 0.21$). IRs of the remaining STs (i.e., ST17, ST179, ST253) remained relatively stable over the 9-year period. ST111 was the most antimicrobial-resistant clone in this cohort, while other STs (i.e., ST244, ST17, ST179, ST253) were susceptible to most drugs tested (Table 2).

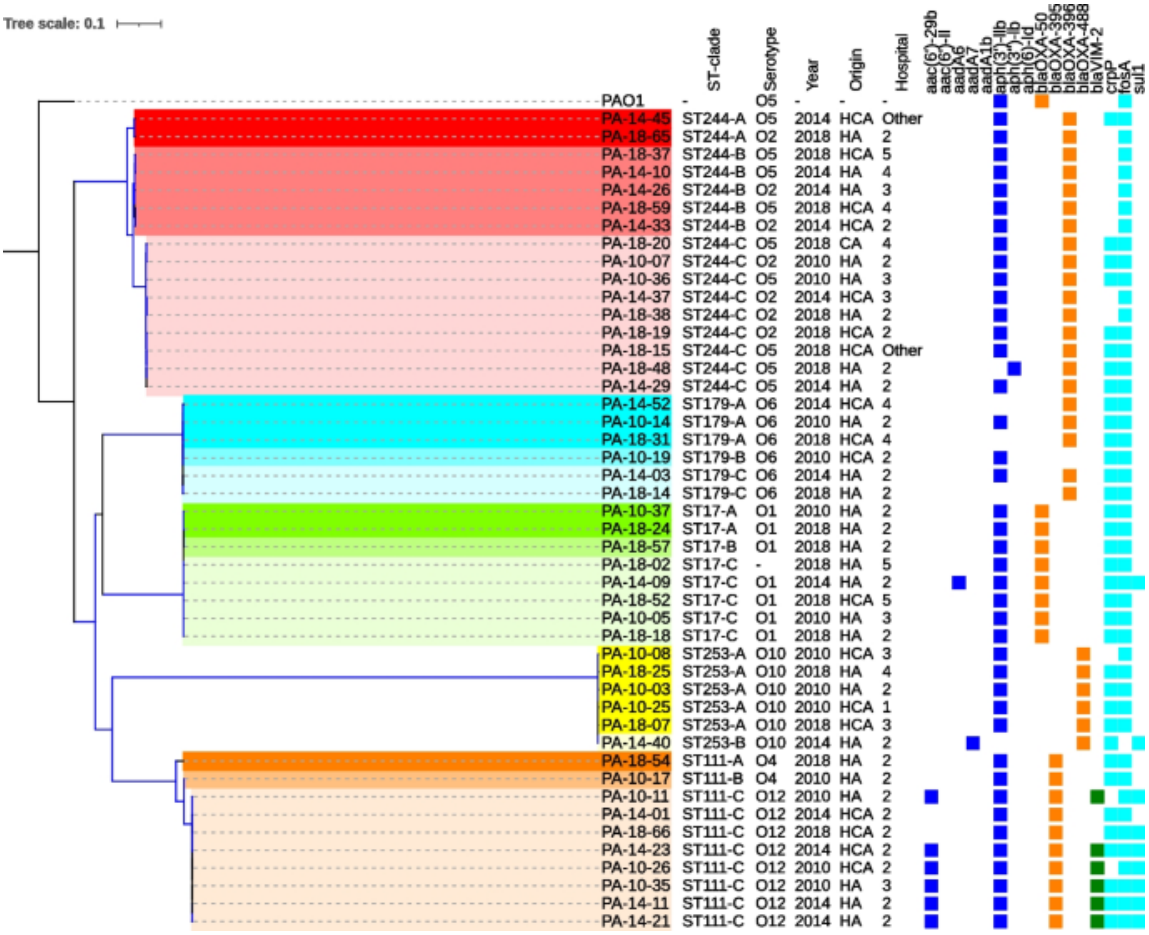

Fig. 2. Single-nucleotide polymorphism–based phylogenetic tree of *P. aeruginosa* isolates causing bloodstream infections in Calgary (2010–2018)

Phylogenetics

Figure 2 shows a phylogenic tree of the dominant STs (i.e., ST244, ST111, ST17) and ST179 and ST253 from this collection. ST224 ($n = 17$) was divided into three clades: ST224-A ($n = 2$), ST224-B ($n = 5$), and ST244-C ($n = 10$). ST224 was obtained from four different Calgary hospitals and contained serotypes O2 and O5 and the following AMR determinants: OXA-396, *aph(3')-llb*, *fosA*, and *crpP* (Table 2, Fig. 2). ST244 serotypes were not linked to specific clades. ST111 ($n = 10$) was divided into three clades namely ST111-A ($n = 1$), ST111-B ($n = 1$), and ST111-C ($n = 8$): clades A and B contained serotype O4, while C contained serotype O12 and *bla*$_{\text{VIM-2}}$ (Fig. 2). ST111 clades clustered in hospital 2 and contained OXA-395, *aac(6')-29b*, *aph(3')-llb*, *fosA*, and *crpP* (Table 2). ST17 ($n = 8$) belonged to three clades namely ST17-A ($n = 2$), ST17-B ($n = 1$), and ST17-C ($n = 5$). ST17 clustered in hospital 2 and contained serotype O1, OXA-50, *aph(3')-llb*, *fosA*, and *crpP* (Fig. 2, Table 2). ST179 also belonged to three clades namely ST179-A ($n = 3$), ST179-B ($n = 1$), and ST179-C ($n = 2$). ST179 clades were obtained from three different Calgary hospitals and contained serotype O6, OXA-396, *aph(3')-llb*, *fosA*, and *crpP*. ST253 belonged to two clades, was obtained from four different Calgary hospitals, and contained serotype O10, OXA-488, *aph(3')-llb*, *fosA*, and *crpP* (Fig. 2, Table 2).

Serotypes

Overall, ten different O serotypes were identified among the 167 isolates (Table 2): serotype O5 was the most common ($n = 32$ (19.1%)), followed by O6 ($n = 31$ (18.5%)), O1 ($n = 28$ (17%)), O3 ($n = 19$ (11%)), and O11 ($n = 17$ (10%)). The IR per 100,000 hospital discharges of O6 isolates increased significantly over the 9-year period (i.e., 4.39 in 2010 (95% CI 1.20–11.23) to 12.42 in 2018 (95% CI 7.36–19.63); $P = 0.05$). The IR per 100,000 hospital discharges of O1 isolates also increased (i.e., 5.48 in 2010 (95% CI 1.78–12.80) to 11.04 in 2018 (95% CI 6.31 – 17.93)) but the increase was not statistically significant ($P = 0.18$). The IR per 100,000 hospital discharges of serotype O5 isolates decreased in 2014 (from 9.87 in 2010 to 5.77 in 2014) followed by an increase in 2018 (from 5.77 in 2014 to 10.37 in 2018). Those fluctuations were not statistically significant. The IR/100,000 of other serotypes remained relatively stable over the years. The O5 serotype isolates belonged to 20 different STs with ST244 (9/32 (28%)) dominating; O6 serotype belonged to 22 different STs with ST179 (7/31 (23%)) dominating; and O1 serotype isolates belonged to 17 different STs with ST17 (7/28 (25%)) and ST27 (5/28 (18%)) dominating. O3 serotype isolates belonged to 16 different STs and O11 belonged to 13 different STs. There was no clustering of STs within O3 and O11 serotypes.

Type III secretion system genes

ExoS, *exoT*, and *exoY* were common among this population while *exoU* was rare (Table 2). The following STs tested positive for *exoU*: ST207 ($n = 1$), ST238 ($n = 1$), ST253 ($n = 6$), ST298 ($n = 1$), ST308 ($n = 2$), ST309 ($n = 2$), ST365 ($n = 1$), ST446 ($n = 2$), ST1248 ($n = 1$), ST3772 ($n = 1$), and ST3773 ($n = 1$).

Discussion

A previous Calgary study described the population-based epidemiology and risk factors of *P. aeruginosa* BSIs from 2000 to 2006 [26]. The annual IR was 3.6/100,000 patient years. Relative to the general population, risk factors for *P. aeruginosa* BSIs ($n = 284$) included male sex, increasing age, hemodialysis, solid organ transplant, diagnosis of cancer, heart disease, HIV infection, diabetes mellitus, and chronic obstructive airway disease [26]. Molecular characterization was not performed in that study.

We report a follow-up population-based epidemiology of *P. aeruginosa* BSIs ($n = 167$) in Calgary, Canada, over a 9-year period (2010–2018) that included genomic characterization of the isolates. The annual population IR per 100,000 patient years increased over the 9-year period. The highest IRs/100,000 in our study were found in the elderly, especially among males older than 75 years. Overall, results from this study were similar to the previously reported Calgary study from 2000 to 2006 [26]. Some differences were noted in this 2010–2018 study: (i) The annual population IR per 100,000 patient years increased over the 9-year period. The IR per 100,000 patient years in the previous Calgary study peaked in 2003 and remained stable from 2004 to 2006 [26]. (ii) The AMR rates of *P. aeruginosa* BSIs were lower in the current study, compared to higher non-susceptibility rates to ceftazidime (17%), ciprofloxacin (18%), gentamicin (16%), and the carbapenems (12%) reported in the previous study [26]. During 2003–2005, Calgary experienced a nosocomial outbreak at hospital 2 due to clonally related VIM-2-producing *P. aeruginosa* [27, 28]. The higher non-susceptibility rates in 2003–2006 were due to the presence of the VIM-2-producing *P. aeruginosa* multidrug-resistant (MDR) clone [26]. This clone was later identified as ST111 (Pitout, unpublished data) and the

IR/100,000 of ST111 in this study decreased over time, likely due to replacing contaminated faucets and sinks in the intensive care and bone marrow units in hospital 2. This explained the differences in AMR rates between the current and previous Calgary studies.

P. aeruginosa has a panmictic population structure [29]. A Spanish study characterized *P. aeruginosa* causing BSIs ($n = 190$) obtained in 2008–2009 [30]. A subset of isolates ($n = 65$) underwent multilocus sequence typing (MLST). The most common MDR *P. aeruginosa* clones were ST175 (23/35 (66%)) and ST111 (3/35 (9%)), while ST244 was the most common clone among antimicrobial-susceptible isolates (4/30 (13%)). A more recent Spanish study from 2016 reported on 64 patients with *P. aeruginosa* BSIs [31]. They identified two dominant clones namely ST235 (11/64 (17%)) and ST175 (10/64 (16%)). Both clones were positive for carbapenemases (i.e., ST235 for GES-5, ST175 for VIM-2). The Calgary *P. aeruginosa* population causing BSIs was polyclonal (i.e., 102 STs among 167 isolates) consisting of three dominant STs (i.e., ST244 (10%), ST111 (6%), and ST17 (5%)). ST175 and ST235 were absent from our collection.

P. aeruginosa ST244 is a global MDR high-risk clone and contains the serotype O2 [32]. ST244 is linked with various types of carbapenemases (i.e., VIMs, IMPs), and more recently, cefiderocol resistance was reported in this clone [33]. ST244 is also frequent among non-MDR *P. aeruginosa* isolates [30]. ST244 was the most common clone in our collection, the IR/100,000 increased during 2014/2018, and this clone was universally susceptible to antimicrobial agents (except for one isolate that was non-susceptible to ciprofloxacin). ST244 was widely distributed among Calgary hospitals. This clone was positive for OXA-395, belonged to three clades (i.e., A, B, C), and contained the O2/O5 serotypes. The O2/O5 serotypes were distributed across the different ST244 clades. ST244 clade C clustered in hospital 2 and was mainly responsible for the increase of ST244 isolates during 2018. We were unable to find a clear source for ST244-C.

P. aeruginosa ST111 is a global MDR high-risk clone and contains serotype O12 [32]. ST111 is linked with VIM-2 and is rare among antimicrobial-susceptible *P. aeruginosa* isolates [30]. ST111 was the second most common and the most MDR clone in this study with high non-susceptibility rates to most antibiotics. ST111 was positive for OXA-395 and half of the isolates contained *bla*$_{\text{VIM-2}}$. Most of the ST111 isolates were obtained from hospital 2. A *P. aeruginosa* pulsotype (named MBLV) with *bla*$_{\text{VIM-2}}$ was previously responsible for Calgary nosocomial outbreaks during 2003–2004 at hospital 2 [34]. The nosocomial outbreak was curtailed by replacing the contaminated faucets and sinks in the intensive care and bone marrow units [34]. ST111 from the current study belonged to three clades: clades A and B contained the O4 serotype, while clade C contained the O12 serotype and *bla*$_{\text{VIM-2}}$. *P. aeruginosa* pulsotype MBLV from the 2003–2004 outbreak belonged to ST111-C (Pitout, unpublished data). ST111 with the O4 serotype is likely the ancestral clade that acquired O12 serotype over time through a large homologous recombination event [35].

The O-polysaccharide is an important *P. aeruginosa* virulence factor responsible for serotype specificity and is a potential target for vaccines [36]. Data describing the distribution of O serotypes among invasive *P. aeruginosa* isolates is currently rare. A recent global study showed that a multivalent vaccine consisting of ten serotypes (i.e., O1, O2, O3, O4, O5, O6, O8, O9, O10, O11) would confer protection against more than 80% of invasive *P. aeruginosa* infections [37]. Such a vaccine would potentially protect 90% of Calgary residents with *P. aeruginosa* BSIs. Certain *P. aeruginosa* serotypes (especially O6 and O1) in Calgary increased over the 9-year period. An effective multivalent vaccine consisting of five serotypes (i.e., O1, O3, O5,

O6, and O11) would confer protection to 76% of Calgary residents with *P. aeruginosa* BSIs. Such serotypes will be important candidates for future *P. aeruginosa* vaccine development.

The type III secretion system (TTSS) is a major *P. aeruginosa* virulence factor [38]. TTSS produces certain cytotoxins (i.e., *exoS*, *exoT*, *exoU*, or *exoY*) with *exoU* having the greatest impact on virulence [38]. A Spanish study showed that the *exoU* + genotype was associated with early mortality among patients with *P. aeruginosa* BSIs [39]. Overall, the *exoU* + genotype was rare, while *exoS* + , *exoT* + , and *exoY* + genotypes were common in our collection. Calgary ST253 tested positive for *exoU* and *exoT* but negative for *exoS*. ST253 is a global MDR high-risk clone that was previously linked with the *exoU* + genotype [40]. Among our collection, ST253 was the fifth most common clone, widely distributed across Calgary hospitals, and was universally susceptible to antimicrobial agents.

This study has several strengths. We included recent unselected *P. aeruginosa* blood isolates obtained from a well-defined human population. We combined clinical with genomic data to determine the population-level characteristics and trends over time of BSIs due to different *P. aeruginosa* sequence types (STs) and serotypes. This provided opportunities to compare the population structure of *P. aeruginosa* BSIs with other Gram-negative pathogens (i.e., *Enterobacter* spp. *E. coli*) within the same human population over similar time periods.

This study has some limitations. Only Calgary patients with positive blood cultures for *P. aeruginosa* complex were included which excluded those patients with BSIs from whom no samples were submitted for culture. Therefore, IRs should be considered conservative estimates of *P. aeruginosa* BSIs in Calgary.

Conclusions

We performed active population-based surveillance that combined clinical data with genomics to characterize *P. aeruginosa* bloodstream infections (BSIs) ($n = 167$) in a well-defined Canadian (Calgary) human population over a 9-year period (2010–2018). This study provided a unique perspective of population dynamics of STs, clades, and serotypes over time that will aid in designing better treatment and prevention strategies for *P. aeruginosa* BSIs. The annual population IR per 100,000 patient years increased over time with the highest IRs in the elderly males from the hospital setting. Over a quarter of patients presented with febrile neutropenia while AMR rates and determinants were rare. The *P. aeruginosa* population was polyclonal consisting of three dominant sequence types (STs) (ST244, ST111, ST17). Antimicrobial-susceptible ST244 was the most common clone and belonged to three clades (A, B, C). The ST244 IR/100,000 increased over time due to the expansion of clade C. ST111 was the second most common clone, belonging to three clades (A, B, C) with clade C containing *bla*$_{\text{VIM-2}}$. ST111 was the most MDR clone and IR/100,000 decreased over time. The *exoU*-positive, *exoS*-negative virulent genotype was linked with antimicrobial-susceptible ST253. Serotypes O6 and O1 were common and their IR/100,000 increased over time. An effective multivalent vaccine consisting of five serotypes (i.e., O1, O3, O5, O6, O11) would confer protection to > 70% of Calgary residents with *P. aeruginosa* BSIs.

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Contributions

All authors designed the study and approved the manuscript. GP, YM, and DN performed WGS, bioinformatics, and statistical analysis. JP, DN, and DC combined the clinical and genomic data. JP wrote the first draft of the manuscript.

Ethics declarations

Ethics approval

Ethics approval for this study was obtained through the University of Calgary Conjoint Health Research Ethics Board (REB18-1123).

Consent to participate

Not applicable.

Consent for publication

This manuscript has not been published and is not being considered for publication elsewhere.

Conflict of interest

The authors declare no competing interests.

Data availability

Sequence data was uploaded to NCBI (BioProject PRJNA988909).

Code availability

Sequence data is available at NCBI (BioProject PRJNA988909).

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