Population-based surveillance of *Enterobacter cloacae* complex causing blood stream infections in a centralized Canadian region

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Abstract

Active population-based surveillance determined clinical factors, susceptibility patterns, incidence rates (IR), and genomics among *Enterobacter cloacae* complex ($n = 154$) causing blood stream infections in a centralized Canadian region (2015–2017). The annual population IR was 1.2/100,000 (95% CI 0.9–16) in 2015, 1.4/100,000 (95% CI 1.1–1.9) in 2016, and 1.5/100,000 (95% CI 1.2–2.0) in 2017, affecting mainly elderly males with underlying comorbid conditions in the hospital setting. *E. cloacae* complex was dominated by polyclonal subspecies (i.e., *E. hormaechei* subsp. *steigerwaltii*, subsp. *hoffmanni* and subsp. *xiangfangesis*). Antimicrobial resistant determinants were rare. This study provided novel information about *Enterobacter* genomics in a well-defined human population.

Keywords: E. cloacae complex, Blood stream infections, Population-based surveillance

Introduction

Enterobacter cloacae complex is the most common species among the genus *Enterobacter* and is a leading cause of antimicrobial resistant (AMR) nosocomial infections [1]. It ranks among the top 10 bacterial causes of blood stream infections [1, 2]. *E. cloacae* complex contains inducible chromosomal AmpC β-lactamases that causes resistance of certain β-lactam antibiotics, complicating the treatment of serious infections [1].

Population-based studies are optimal to determine the occurrence of clinical conditions, such as bloodstream infections, within well-defined populations at risk [3]. 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 [3].

There is published data regarding the global prevalence of *E. cloacae* complex blood stream infections in selected populations [1]. Population-level characteristics associated with different species within *E. cloacae* complex are currently lacking. Active population-based surveillance determined the clinical factors, susceptibility patterns, incidence rates (IR), and genomics among *E. cloacae* complex causing blood stream infections in a centralized Canadian region during 2015–2017. Combining genomics with population-based clinical epidemiology will aid in designing superior prevention strategies for *E. cloacae* complex blood stream infections.

Materials and methods

Study population and clinical data

The Alberta Precision Laboratories is a regional, centralized laboratory system that performs all clinical microbiology services (hospital and community patients) within the Calgary region, Alberta, Canada. There are 4 adult hospitals (with 2595 beds) and 1 pediatric (with 133 beds) hospital in Calgary.

Clinical information corresponding to source patients at the time of the *E. cloacae* complex blood stream infection was obtained using Sunrise Clinical Manager (All-scripts Healthcare Solutions, Inc., Chicago, IL, USA), National Ambulatory Care Reporting System, and Discharge Abstract Database. Patient comorbidities and invasive procedures were transcribed with Canadian ICD-10 codes. A case of *E. cloacae* complex blood stream infection was defined as a patient with systemic inflammatory response and documented growth of an *E. cloacae* complex isolate in a blood culture. Incident cases were defined as Calgary residents with the first isolation of *E. cloacae* complex from blood. Repeat isolates were excluded. Infections were classified as community acquired, healthcare associated, or hospital acquired [4].

Bacterial isolates, identification, and susceptibility testing

All *E. cloacae* complex isolates ($n = 154$) recovered from blood between January 1st 2015 and December 31st of 2017 were available for this study. Initial identification and susceptibility testing were done 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; bioMerieux Vitek Systems Inc., Hazelwood, MO) respectively. Susceptibilities to the following drugs were determined: piperacillin-tazobactam, ceftriaxone, cefepime, meropenem, ertapenem, amikacin, gentamicin, tobramycin, ciprofloxacin, and trimethoprim-sulfamethoxazole. Throughout this study, results were interpreted using Clinical Laboratory Standards Institute criteria for broth dilution [5].

Molecular characterization

The Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA) was used to prepare libraries for sequencing. Samples were multiplexed and sequenced on an Illumina NextSeq500 for 300 cycles (151 bp paired end).

Draft genomes were obtained using SPAdes version 3.14.0. [6]. Species were identified by calculating the average nucleotide identity using JSpecies [7]. To define the presence of genes and mutations, BLAST [8] in combination with following databases or typing schemes were accessed: NCBI Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/ bioproject/PRJNA313047), ResFinder [9], PlasmidFinder [10], and MLST (http:// pubmlst.org/ecloacae/).

Statistical analysis

Incidence rates (IR) per 100,000 person-years and respective 95% confidence intervals (95% CI) were estimated by year, sex, and age group using the Poisson distribution [3]. IRs were also estimated per number of hospital discharges. Denominator data were retrieved from the 2014, 2016, and 2019 City of Calgary Civic Census (https://www.calgary.ca/ca/city-clerks/election-and-information-services/civic-census/censusresults.html) and from the 2018–2019 Alberta Health Services Annual Report (https://www.albertahealthservices.ca/assets/about/ publications/2018–19-annual-report-web-version.pdf). The 2015 and 2017 Census do not stratify the population according to age and sex; it was assumed that the age-sex distribution of Calgary residents in 2015 and 2017 was weighted averages from ones observed in 2014 and 2016 (weight 1:1), and in 2016 and 2019 (weight 2:1), respectively. Fisher's exact or *t*-tests were used to estimate associations between *Enterobacter* species and demographics, healthcare exposure, and clinical presentation of patients [3]. Fisher's exact tests were used to compare the susceptibility profile of different species. For all analyses, statistical significance was set at 5% level.

Results and discussion

E. cloacae complex isolates and susceptibilities

The taxonomy of *E. cloacae* complex is confusing, and uncertainty remains about what species make up this complex. Davin-Regli and colleagues provided an update in 2019 on the taxonomy of *E. cloacae* complex [1]. This complex belongs to 7 species, namely *Enterobacter asburiae*, *E. cloacae* (with 2 subspecies, namely subsp. *cloacae* and subsp. *dissolvens*), *Enterobacter hormaechei* (with 5 subspecies, namely subsp. *hoffmanni*, subsp. *hormaeche*, subsp. *oharae*, subsp. *steigerwaltii*, and subsp. *xiangfangensis*), *Enterobacter kobei*, *Enterobacter ludwiggi*, *Enterobacter mori*, and *Enterobacter nimipressuralis*.

In this study, *E. cloacae* complex was the 4th most common Gram-negative bacterium obtained from blood in the Calgary region; 45/2155 (2.1%) in 2015, 53/2201 (2.4%) in 2016, and 56/2354 (2.4%) in 2017 of the total blood bacterial isolates were identified as *E. cloacae* complex. The most common *E. cloacae* complex species ($n = 154$) in Calgary was *E. hormaechei* subsp. *steigerwaltii* ($n = 64$ [42%]), followed by *E. hormaechei* subsp. *hoffmanni* ($n = 20$ [13%]), *E. hormaechei* subsp. *xiangfangesis* ($n = 17$ [11%]), *E. ludwiggi* ($n = 14$ [9%]), *E. cloacae* subsp. *cloacae* ($n = 13$ [8%]), *E. hormaechei* subsp. *oharae* ($n = 10$ [6%]), *E. kobei* ($n = 7$ [5%]), *E. asburiae* ($n = 3$ [2%]), *E. cloacae* subsp. *dissolvens* ($n = 3$ [2%]), *E.*

mori (*n* = 2 [1%]), and *E. hormaechei* subsp. *hormaechei* (*n* = 1 [1%]) (Table 1). Recent studies from single hospital settings in China [11] and the USA [12] used short read whole genome sequencing to identify *Enterobacter* spp. obtained from blood. The most common species in the Chinese study were *E. hormaechei* subsp. *xiangfangesis* (*n* = 44%) followed by *E. hormaechei* subsp. *hoffmanni* (17%) [11]. The US study reported on different *E. cloacae* complex lineages (A–I) [12].

Enterobacter spp. are mainly responsible for nosocomial infections and especially in ICUs, among immunocompromised patients with comorbidities. Invasive procedures, such as catheterization and intubation, which are frequently found in an ICU, represent a main source of infection. *Enterobacter* spp. infect patients with a long median duration of hospitalization. This time increases the digestive carriage, which represents a high-risk factor for transmission. MDR isolates are associated with previous exposure to broad-spectrum antibiotics [1].

E. cloacae complex blood stream infections in Calgary were more common among males (93 [60%]) than females (61[40%]) with an average age of 57 years (Table 1). Most patients acquired bloodstream infection as inpatients (*n* = 87 [56%]) or in as healthcare associated (*n* = 35 [23%]) (Table 1). Fifty-seven percent of patients (*n* = 88) presented with bloodstream infections with unknown source, followed by upper urinary tract infections (*n* = 24 [16%]), intra-abdominal infections (*n* = 18 [12%]), cellulitis (*n* = 10 [6%]), pneumonia (*n* = 9 [6%]), and infection of the hepatobiliary tract (*n* = 5 [3%]) (Table 1). Solid organ and hematological malignancies, underlying kidney, and cardiovascular diseases were common among patients (Table 1). ICU admission occurred in 27% of patients and 90-day mortality was 29%. Similar clinical results were reported in the Chinese [11] and US [12] studies.

The annual population IR of *E. cloacae* complex bloodstream infections among Calgary residents was 1.2/100,000 (95% CI 0.9–16) in 2015, 1.4/100,000 (95% CI 1.1–1.9) in 2016, and 1.5/100,000 (95% CI 1.2–2.0) in 2017 (Fig. 1 and Supplemental Table 1). The highest IRs/100,000 were found in the elderly, especially among males older than 75 years (Fig. 1). In comparison, the IR of *E. coli* bloodstream infections during 2016 was 48.8/100,000 p-years [95% CI 45.2–52.6] among Calgary residents, affecting mainly the elderly residing in long-term care centers [3]. *E. cloacae* complex bloodstream infections were rare among Calgary long-term care residents (1 case over the 3-year study period) (Table 1). For Calgary inpatients, the IR/100,000 of *E. cloacae* complex bloodstream infections were 31.7/100,000 (CI 23.1–42.5) during 2015, 37.0 per 100,000 (CI 27.7–48.4) during 2016, and 38.9 per 100,000 (CI 29.3–50.4) during 2017 (Supplemental Table 1). The IRs/100,000 of *E. hormaechei* subsp. *hoffmanni* and *E. hormaechei* subsp. *steigerwaltii* blood stream infections increased slightly in 2017 (as compared to 2015 and 2016) (Supplemental Fig. 1).

Table 1. Demographics, healthcare exposure, and clinical presentation of patients with *E. cloacae* complex blood stream infections ($n = 154$) in Calgary, Alberta, Canada (2015–2017)

	E. cloacae* subsp. *cloacae	***E. hormaechei* subsp. *hoffmanni***	***E. hormaechei* subsp. *xiangfangesis***	***E. hormaechei* subsp. *oharae***	***E. hormaechei* subsp. *steigerwaltii***	***E. kobei***	***E. ludwiggi***
n	13 (8%)	20 (13%)	17 (11%)	10 (6%)	64 (42%)	7 (5%)	14 (9%)
Demographic							
Sex: Female	8 (62%)[1]	4 (20%)[2]	8 (47%)	6 (60%)[1]	20 (31%)	4 (57%)	4 (29%)
Avr age yrs (SD)	58 (26)	49 (25)[1]	51 (22)[1]	64 (16)	58 (18)	67 (11)[2]	56 (27)
Year							
2015	3 (23%)	5 (25%)	6 (35%)	1 (10%)	20 (31%)	1 (14%)	7 (50%)
2016	6 (46%)	5 (25%)	5 (30%)	6 (60%)	19 (30%)	4 (57%)	4 (29%)
2017	4 (31%)	10 (50%)	6 (35%)	3 (30%)	25 (39%)	2 (29%)	3 (21%)
Health care exposure:							
Hospital admission	12 (92%)	20 (100%)	16 (94%)	10 (100%)	53 (83%)	7 (100%)	14 (100%)
Ave length of stay (median)	139 (27)	40 (35)	30 (19)	33 (27)	80 (17)	35 (17)	56 (38)
Hospital 1	9 (69%)	10 (50%)	8 (47%)	6 (6%)	35 (55%)	3 (43%)	6 (43%)
Hospital 2	0	4 (20%)	4 (24%)	1 (10%)	13 (20%)	2 (29%)	2 (14%)
Hospital 3	2 (15%)	2 (10%)	2 (12%)	2 (20%)	4 (6%)	2 (29%)	3 (21%)
Hospital 4	1 (8%)	1 (5%)	0	1 (10%)	1 (2%)	0	1 (7%)
Hospital 5	0	3 (15%)	2 (12%)	0	0	0	2 (14%)
ICU admission	2 (15%)	6 (30%)	2 (12%)	3 (30%)	18 (28%)	2 (29%)	5 (36%)
90-day mortality	1 (8%)	5 (25%)	5 (29%)	2 (20%)	19 (30%)	5 (71%)	3 (21%)
Community acquired	4 (31%)	2 (10%)	3 (18%)	6 (60%)	13 (20%)	0	2 (14%)
Healthcare associated[3]	2 (15%)	4 (20%)	7 (41%)	0	15 (23%)	4 (57%)	2 (14%)
Hospital acquired[4]	7 (54%)	14 (70%)	7 (41%)	4 (40%)	36 (56%)	3 (43%)	10 (71%)
LTC resident	0	0	0	0	1 (2%)	0	0

Comorbidities:							
Kidney disease	7 (54%)	12 (60%)	6 (35%)	3 (30%)	31 (48%)	3 (43%)	6 (43%)
Pulmonary	3 (23%)	3 (15%)	3 (18%)	1 (10%)	13 (20%)	0	4 (29%)
Cardiovascular diseases	4 (31%)	6 (30%)	3 (18%)	4 (40%)	27 (42%)	3 (43%)	6 (43%)
Diabetes mellitus	3 (23%)	7 (35%)	4 (24%)	1 (10%)	15 (23%)	1 (14%)	2 (14%)
Hepatobiliary disease	1 (8%)	1 (5%)	1 (6%)	1 (10%)	1 (2%)	0	0
Intestinal disease	1 (8%)	0	0	0	2 (3%)	0	0
Malignancy	4 (31%)[1]	8 (40%)[1]	9 (53%)	4 (40%)[1]	26 (41%)[1]	7 (100%)[2]	7 (50%)[1]
Solid organ malignancy	3 (23%)	4 (20%)	6 (35%)	3 (30%)	15 (23%)	4 (57%)	2 (14%)
Hematological malignancy	1 (8%)	4 (20%)	3 (18%)	1 (10%)	11 (17%)	3 (43%)	5 (36%)
Central nervous system	1 (8%)	0	1 (6%)	1 (10%)	3 (5%)	0	7 (50%)
Transplant	0	1 (5%)	0	0	3 (5%)	0	2 (14%)
Presentation:							
Primary/unknown source	8 (62%)	16 (80%)	10 (59%)	3 (30%)	36 (56%)	2 (29%)	7 (50%)
Urosepsis	4 (31%)	1 (5%)	4 (24%)	3 (30%)	8 (13%)	2 (29%)	2 (14%)
Pneumonia	0	1 (5%)	0	0	6 (9%)	0	2 (14%)
Cellulitis	1 (8%)	1 (5%)	0	0	6 (9%)	2 (29%)	0
Intra-abdominal infection	0	1 (5%)	3 (18%)	3 (30%)	4 (6%)	1 (14%)	3 (21%)
Cholecystitis	0	0	0	1 (10%)	4 (6%)	0	0

- Species not included in the table include *E. asburiae* ($n = 3$), *E. cloacae subsp dissolvens* ($n = 3$), *E. hormaechei subsp hormaechei* ($n = 1$), and *E. mori* ($n = 2$)
- [1–2]Percentages followed by different superscripts differ at the 5% significance level
- [3]Defined as contact with hospital within 30 days prior to blood culture collection
- [4]Defined as > 48 h in hospital prior to blood culture collection

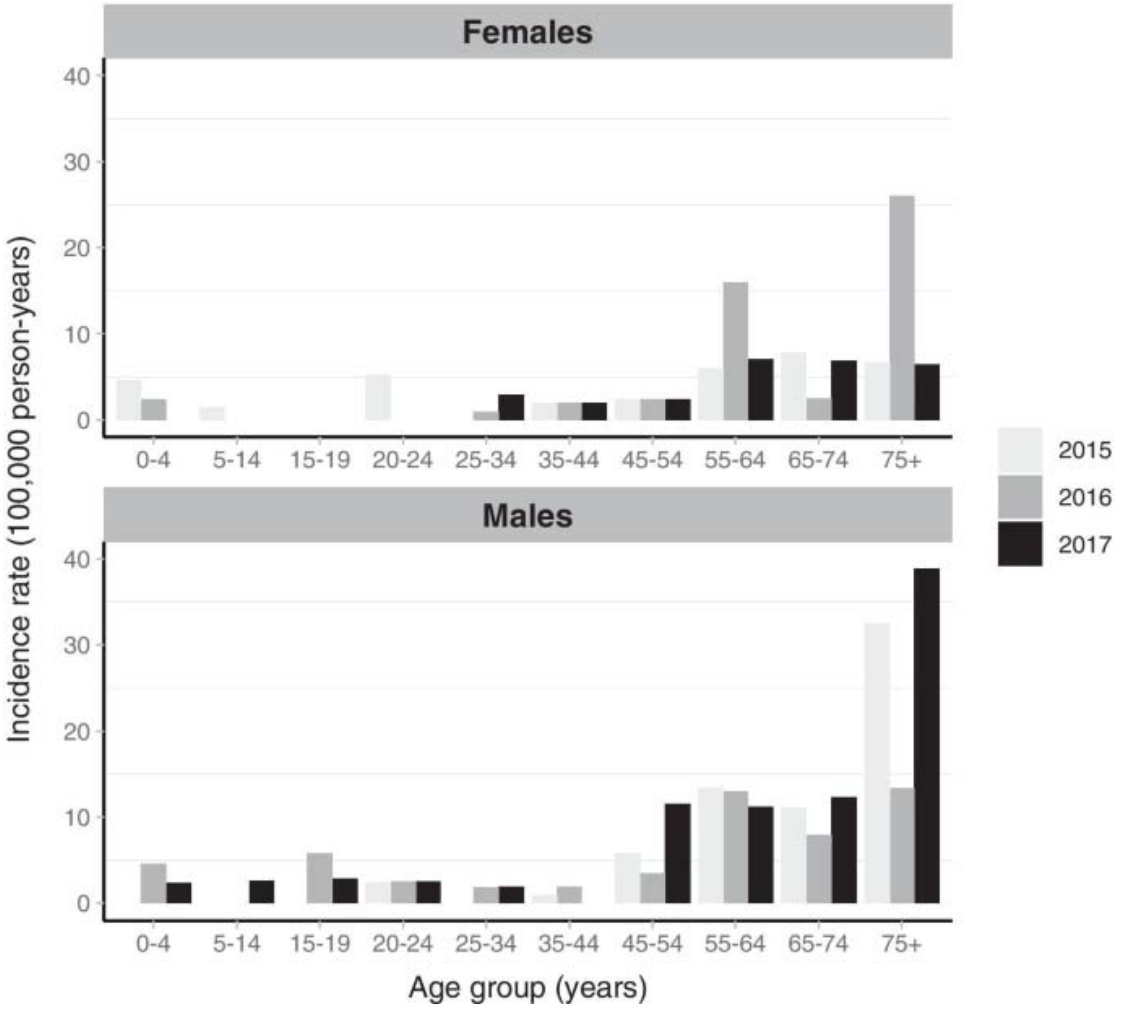

Fig. 1. Age-specific incidence rates of patients with *E. cloacae* complex blood stream infections ($n = 154$) in Calgary, Alberta, Canada (2015–2017)

Overall, high (≥ 20%), intermediate, or resistant (i.e., not susceptible [NS]) rates were found for ceftriaxone (24%), followed by piperacillin/tazobactam (16%), trimethoprim/sulfamethoxazole (10%), and ertapenem (8%) (Table 2). Low NS rates (< 5%) were found for cefepime (3%), ciprofloxacin (3%), gentamicin (1%), and tobramycin (1%). Isolates were susceptible to amikacin and meropenem (Table 2). *E. hormaechei* subsp. *xiangfangesis* was the most resistant subspecie in this cohort especially to trimethoprim/sulfamethoxazole and piperacillin/tazobactam while *E. kobei* showed high NS rates to ceftriaxone and piperacillin/ tazobactam (Table 2). The Chinese study reported high NS rates to piperacillin/tazobactam, cephalosporins, aminoglycosides, fluoroquinolones, and trimethoprim/sulfamethoxazole with *E. hormaechei* subsp. *xiangfangesis* the most resistant subspecie [11].

Non-AmpC β-lactamases (i.e., TEM-1, OXA-1, CARB-2), aminoglycoside-modifying enzymes, and other AMR determinants (except for *fosA*) were rare in this collection (Table 2). *E. hormaechei* subsp. *xiangfangesis* and *E. hormaechei* subsp. *steigerwaltii* contained most of the AMR determinants (Table 2). IncF (e.g., combinations of FIA, FIB, FII replicons) and Col-RNA-1 plasmid types were common among the different subspecies (Table 2).

Table 2. Antimicrobial susceptibilities, resistance determinants, and plasmid types of *E. cloacae* complex (*n* = 154) in Calgary, Alberta, Canada (2015–2017)

	E. cloacae* subsp. *cloacae	***E. hormaechei* subsp. *hoffmanni***	***E. hormaechei* subsp. *xiangfangesis***	***E. hormaechei* subsp. *oharae***	***E. hormaechei* subsp. *steigerwaltii***	***E. kobei***	***E. ludwiggi***
n	13 (8%)	20 (13%)	17 (11%)	10 (6%)	64 (42%)	7 (5%)	14 (9%)
Antimicrobials (NS):							
MEM	0	0	0	0	0	0	0
TZP	2 (15%)	1 (5%)[1]	6 (35%)[2]	0	11(17%)	3 (43%)[2]	2 (14%)
FEP	1 (8%)	1 (5%)	2 (12%)	0	1 (2%)	0	0
CRO	2 (15%)	3 (15%)[1]	7 (41%)	1 (10%)	15 (23%)	4 (57%)[2]	5 (36%)
ERT	2 (15%)	2 (10%)	4 (24%)	0	4 (6%)	1 (14%)	0
CIP	1 (8%)	0	1 (6%)	0	2 (3%)	0	0
SXT	1 (8%)	0[1]	5 (29%)[2]	1 (10%)	6 (9%)[1]	1 (14%)	1 (7%)
GEN	0	0	1 (6%)	0	0	0	0
TOB	0	0	1 (6%)	0	0	0	0
AMK	0	0	0	0	0	0	0
B-lactamases (non-AmpC):							
TEM-1	0	0	3 (18%)	0	6 (9%)	0	1 (7%)
OXA-1	0	0	1 (6%)	0	0	0	0
CARB-2	0	0	1 (6%)	0	0	0	0
Aminoglycoside-modifying enzymes:							
aac(6')Ib-cr	0	0	1 (6%)	0	0	0	0
aac(3)-IVa	0	0	1 (6%)	0	0	0	0
aac(3)-VIa	0	0	0	0	0	1 (14%)	0
aac(3)-IId	0	0	1 (6%)	0	0	0	0
aadA1	0	0	0	0	3 (5%)	1 (14%)	0

aadA2	1 (8%)	1 (5%)	2 (12%)	1 (10%)	1 (2%)	1 (14%)	0
aadA5	0	0	1 (6%)	0	0	0	0
aph(3')-Ia	0	0	1 (6%)	0	0	0	0
aph(3")-Ic	0	0	0	0	1 (2%)	0	0
aph(4')-Ia	0	0	1 (6%)	0	0	0	0
strA	1 (8%)	0	4 (24%)	0	6 (9%)	1 (14%)	1 (7%)
strB	1 (8%)	0	4 (24%)	0	7 (11%)	1 (14%)	1 (7%)
Other determinants							
fosA	13 (100%)	20 (100%)	17 (100%)	10 (100%)	64 (100%)	7 (100%)	14 (100%)
qnrA1	0	0	1 (6%)	0	0	0	0
qnrS1	1 (8%)	0	1 (6%)	0	1 (2%)	0	0
sul1	2 (15%)	1 (5%)	2 (12%)	2 (20%)	4 (6%)	1 (14%)	0
sul2	1 (8%)	0	4 (24%)		5 (8%)	1 (14%)	1 (7%)
dfrA1	1 (8%)	0	0	0	1 (2%)	0	0
dfrA12	0	0	1 (6%)	0	0	0	0
dfrA14	0	0	3 (18%)	0	4 (6%)	0	1 (7%)
dfrA16	0	0	1 (6%)	0	0	0	0
dfrA26	1 (8%)	0	0	0	0	0	0
dfrA27	0	0	0	0	1 (2%)	0	0
tet(A)	1 (8%)	0	0	0	1 (2%)	1 (14%)	0
tet(B)	0	0	1 (6%)	1 (10%)	1 (2%)	0	0
tet(C)	0	0	1 (6%)	0	0	0	0
tet(D)	0	0	1 (6%)	0	3 (5%)	0	1 (7%)
ARR-3	0	0	1 (6%)	0	0	0	0

Plasmid types							
Col-RNA-1	6 (46%)	13 (65%)	10 (59%)	9 (90%)	35 (55%)	0	3 (21%)
FIA	1 (8%)	4 (20%)	1 (6%)	4 (40%)	7 (11%)	0	3 (21%)
FIB	5 (38%)	14 (70%)	9 (53%)	9 (90%)	22 (34%)	3 (43%)	6 (43%)
FII	5 (38%)	10 (50%)	4 (24%)	6 (60%)	25 (39%)	3 (43%)	9 (64%)
IncQ1	0	2 (10%)	2 (12%)	0	4 (6%)	1 (14%)	0
IncH	0	1 (5%)	1 (6%)	1 (10%)	1 (2%)	0	1 (7%)
IncR	1 (1%)	5 (25%)	2 (12%)	3 (30%)	6 (9%)	2 (29%)	0
IncN	0	1 (5%)	0	0	0	1 (14%)	0
IncI	0	0	0	0	1 (2%)	0	0
IncX1	0	0	0	0	1 (2%)	0	0
IncB/O/K/Z	0	0	0	0	1 (2%)	0	0

- *NS*, not susceptible include intermediate or resistant rates; *TZP*, piperacillin-tazobactam; *FEP*, cefepime; *CRO*, ceftriaxone; *ERT*, ertapenem; *MEM*, meropenem; *CIP*, ciprofloxacin; *SXT*, trimethoprim-sulfamethoxazole; *GEN*, gentamicin; *TOB*, tobramycin; *AMK*, amikacin
- Species not included in the table include *E. asburiae* (*n* = 3), *E. cloacae* subsp. *dissolvens* (*n* = 3), *E. hormaechei* subsp. *hormaechei* (*n* = 1), and *E. mori* (*n* = 2)
- [1–2]Percentages followed by different superscripts differ at the 5% significance level

Previous studies identified various *E. cloacae* complex dominant clones among AMR isolates that include ST78, ST93, ST108, ST114, and ST171 [13,14,15,16]. Studies describing the population structure among non-biased *E. cloacae* complex collections are lacking [1, 11]. *E. cloacae* complex ($n = 154$) from this study were polyclonal and consisted of 106 different STs. Global antimicrobial clones were absent (i.e., ST78, ST93) or rare (i.e., ST114, ST171). The most common clones in this study were ST50 ($n = 10$) among *E. hormaechei* subsp. *steigerwaltii*, ST108 ($n = 8$) among *E. hormaechei* subsp. *oharae*, and ST286 ($n = 5$) among *E. hormaechei* subsp. *hoffmanni* (Supplemental Table 2). The average SNP difference within ST50 was 98 (range 46–128), ST108 was 147 (range 53–221), and ST286 was 142 (range 103–177). Patient with blood stream infections due to these common clones were from different time periods and geographic locations and localized nosocomial outbreaks were not considered. ST50 had previously been reported among Chinese extended-spectrum β-lactamase-producing *Enterobacter* spp. [17]. ST108 were present among global collections of *Enterobacter* spp. with carbapenemases [16] and cephalosporin-resistant isolates from Europe [18]. The population structure of *E. cloacae* complex in this cohort different from *E. coli* causing blood stream infections in Calgary during the same period [3]. Over half of *E. coli* population consisted of 5 dominant clones and certain ST131 clades increased significantly over time [19]. The majority of *E. hormaechei* subsp. *oharae* (i.e., 8/10 isolates) from this study belonged to single clone, namely ST108 (Table 1). A previous study showed that *E. hormaechei* subsp. *oharae* isolates ($n = 6$) with carbapenemases, obtained from Australia, China, Spain, and Israel, also belonged to ST108 [16]. The population structure of *E. hormaechei* subsp. *oharae* likely consists of limited number of STs when compared with other *E. cloacae* complex subspecies.

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

In summary, the overall IR/100,000 of *E. cloacae* complex blood stream infections was low (when compared to *E. coli* blood stream infections from the same region). Elderly males with underlying comorbid conditions in the hospital setting were mainly affected and ICU admission rates and 90-day mortality were high. The population structure was dominated by polyclonal *E. hormaechei* subsp. *steigerwaltii* and *E. hormaechei* subsp. *hoffmanni*. AMR determinants and AMR global clones were rare in this unbiased collection. This study provided novel information regarding the population dynamics and genomics of *E. cloacae* complex causing blood stream infections in well-defined human population.

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Ethics

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

Conflict of interest

The authors declare no competing interests.

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