

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)	NS
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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Supplementary data

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

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