The increase in antimicrobial-resistant organisms has reached alarming proportions. The Public Health Agency of Canada has stated that antimicrobial resistance is an increasingly serious threat to global public health. The World Health Organization identified 12 bacterial groups that are of great concern. The Centers for Disease Control and Prevention identified 18 microorganisms that pose urgent, serious and concerning threats. Enterobacteriaceae that produce extended-spectrum β-lactamase (ESBL) are included on their lists as a “Priority 1: Critical” organism or a “serious threat." Within the Enterobacteriaceae, ESBL-producing *Escherichia coli* is a frequent cause of infection, with bloodstream infections being associated with substantial mortality rates (14%–30%). Extended-spectrum β-lactamases are enzymes that are capable of hydrolyzing most β-lactam antimicrobials, and the plasmids on which they are transported frequently contain other resistance genes. This leaves few treatments available to physicians and increases the likelihood of *E. coli*’s developing resistance to all available drugs.

The prevalence of ESBL production among *E. coli* isolates has increased worldwide and varies between regions. Rates as high as 70% have been reported in regions in Southeast Asia. There is a paucity of recent published Canadian data. Historically, the prevalence of ESBL production in *E. coli* in Toronto temporally correlated with an increase in the ST131 clonal type. Recognition of this dramatic rise is important to inform empiric antibiotic treatment.

**Background:** Enterobacteriaceae that produce extended-spectrum β-lactamase (ESBL) have emerged as a serious threat, with variable rates depending on geographic region. We determined the prevalence of ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca* and *Proteus mirabilis* in bloodstream infections in Toronto from 2006 through 2016.

**Methods:** All patients with *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* isolated from blood in a tertiary care microbiology laboratory in Toronto between 2006 and 2016 (1 isolate per species per patient per year) were included in this retrospective cohort study. Organisms were identified by conventional methods, and susceptibility testing was performed according to Clinical and Laboratory Standards Institute standards. Screening for ESBL and phenotypic confirmatory testing were done with a modified Clinical and Laboratory Standards Institute method. ST131 clonal type was determined by means of an established protocol.

**Results:** The proportion of ESBL-producing *E. coli* isolates increased significantly between 2006 and 2016, from 6.4% (19/296) to 17.3% (89/513) (*p* < 0.001). This trend was seen in both intensive care units and emergency departments. Concurrently, the proportion of ST131 among ESBL-producing *E. coli* also increased significantly, from 31.6% (6/19) in 2006 to 73.0% (65/89) in 2016 (*p* = 0.03). Among ESBL-producing *E. coli*, significant resistance was noted to multiple antimicrobial classes. Comparable increases in the proportion of ESBL-producing *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* were not noted.

**Interpretation:** We observed a significant increase in the proportion of ESBL-producing *E. coli* in bloodstream infections in Toronto temporally correlated with an increase in the ST131 clonal type. Recognition of this dramatic rise is important to inform empiric antibiotic treatment.

**Competing interests:** Susan Poutanen has received honoraria for involvement in advisory boards and speaking events as well as travel reimbursement for speaking events from Merck Pharmaceuticals, advisory board honoraria from Paladin Labs and Ventry Pharmaceuticals, grants and an advisory board honorarium from Accelerate Diagnostics, research support from Bio-Rad Laboratories, nonfinancial research support from bioMérieux and travel reimbursement from Copan Diagnostics, all outside the submitted work. No other competing interests were declared.

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Canada was less than 5% until the last decade.11 Peirano and colleagues12 reported a rate of 14% among bloodstream 
E. coli isolates in 2010, and a national rate of 7.1% among 
E. coli from blood, urine, respiratory and wound specimens 
collected combined from 2007 to 2011 was reported as part 
of the CANWARD national surveillance study.11 In both 
studies, these increases correlated with an increased preva-
lence of the ST131 clone of E. coli, which is thought to be a 
driving force behind the rise in ESBLs worldwide.13–16 Ident-
yfying increases in ESBL prevalence is important to inform 
empiric treatment, as inappropriate therapy has been linked 
to worse outcomes.14–16 The purpose of our study was to 
determine the local prevalence of ESBL-producing E. coli, 
Klebsiella pneumoniae, K. oxytoca and Proteus mirabilis among 
blood culture isolates over the last 11 years in the Toronto 
region.

Methods

Design and setting
This retrospective cohort study was performed through the 
University Health Network/Sinai Health System Department 
of Microbiology’s large tertiary care academic microbiology 
laboratory, which serves 4 acute care hospital sites, 4 rehabili-
tation centres and 3 long-term care/palliative/mental health 
sites in Toronto. With the exception of a neonatal intensive 
care unit, the population served is an adult population.

Study participants and bacterial isolates
All patients with bacteremia due to E. coli, K. pneumoniae, 
K. oxytoca or P. mirabilis between Jan. 1, 2006, and Dec. 31, 
2016 at the University Health Network and Sinai Health Sys-
tem were included in this study. The condition of all patients 
contributing blood culture isolates remained stable over the 
11-year period. We reviewed the records of all patients with 
E. coli, K. pneumoniae, K. oxytoca or P. mirabilis isolates recov-
ered from blood cultures in the laboratory between 2006 and 
2016 as identified in the laboratory information system. Fol-
lowing standard Clinical and Laboratory Standards Institute 
recommendations for annual antibiograms so that data are 
representative of typical antibiograms,17 we included 1 isolate 
per species per patient per year. Organisms isolated between 
January 2006 and March 2013 were identified with the 
Vitek 2 system (bioMérieux), and organisms isolated from 
April 2013 to December 2016 were identified with the Vitek 
MS MALDI-TOF (matrix-assisted laser desorption ionization — 
time of flight) mass spectrometry system (bioMérieux).

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was performed at the time 
of isolation with the Vitek 2 system or disk diffusion (Oxoid) 
according to Clinical and Laboratory Standards Institute stan-
dards current at the time of testing.18 All isolates were tested for 
susceptibility to ciprofloxacin, trimethoprim–sulfamethoxazole, 
tobramycin, gentamicin, and either meropenem or imipenem. 
Ertapenem susceptibility results were available from 2009 to 
2016 only.

Extended-spectrum β-lactamase screening and 
phenotypic confirmatory testing
Before October 2015, E. coli, K. pneumoniae, K. oxytoca and 
P. mirabilis isolates were screened for ESBL production as 
part of routine laboratory procedures. All isolates with a cep-
podoxime minimum inhibitory concentration of 1 mg/L or 
greater with the Vitek 2 system were considered screen-
positive. A double-disk test was used for phenotypic confir-
mation of all screen-positive isolates by means of a modified 
protocol based on the work of M’Zali and colleagues.19 
Mueller–Hinton agar was inoculated with 0.5 McFarland 
bacterial suspension, and an amoxicillin–clavulanic acid disk 
was placed in the centre; cefpodoxime, ceftriaxone, ceftazi-
dime and aztreonam disks were placed around it, with 
15 mm between disk edges. After 18–24 hours of incubation, 
class A ESBL production was identified by potentiation of 
the inhibition zone around the amoxicillin–clavulanic acid 
disk near any of the surrounding disks. After October 2015, 
susceptibility results were reported based on Clinical and 
Laboratory Standards Institute interpretations with the 
Vitek 2 system. All E. coli, K. pneumoniae, K. oxytoca and 
P. mirabilis isolates from this period that were or were not 
susceptible to ceftriaxone were subcultured twice from stock 
stored in glycerol broth at −80°C, and ESBL production was 
identified retrospectively by means of the double-disk test. 
K. oxytoca isolates with a phenotype suggestive of K1 
β-lactamase hyperproduction (positive with the double-disk 
test but susceptible to ceftazidime) were considered non-
class-A ESBLs. Isolates that could not be recovered were 
excluded from the study.

ST131 detection among E. coli producing extended-
spectrum β-lactamase
All E. coli isolates from 2006 through 2016 were subcultured 
twice from stock stored in glycerol broth at −80°C, and DNA 
 extraction was completed with a boiled lysate protocol.20 
ST131 clones were identified by means of a polymerase chain 
reaction assay that targeted the pabB gene based on the 
method previously described by Clermont and colleagues.21 
Isolates that could not be recovered were excluded from the 
ST131 analysis.

Statistical analysis
We used the χ² test for trends to analyze trends in susceptibil-
ity, proportion of ESBL-producing isolates and proportion of 
ST131 E. coli isolates over the study period. We used the 
Fisher exact test to analyze differences in susceptibility 
between ESBL-producing and non–ESBL-producing E. coli. 
All analyses were conducted with GraphPad InStat (Graph-
Pad Software). We performed analysis for all blood culture 
isolates and carried out subset analyses for intensive care unit 
and emergency department isolates. A 2-sided p value of 
< 0.05 was considered statistically significant.

Ethics approval
This study did not involve patients or accessing patients’ med-
ical records; thus, ethics review was not needed.
Results

During the study period, 6754 patients (3427 males [50.7%], 3327 females [49.3%]) met the study inclusion criteria. They had a total of 4615 E. coli, 1541 K. pneumoniae, 261 K. oxytoca and 342 P. mirabilis blood culture isolates. One E. coli isolate and 1 K. pneumoniae isolate from 2016 were excluded because of a presumed loss of the resistance plasmid detected on ESBL confirmatory testing, and 2 K. pneumoniae isolates from 2016 were excluded as they could not be recovered from the glycerol stock; this left a total of 6755 isolates that could be analyzed with regard to ESBL production. Ten ESBL-producing E. coli isolates (1 from each of 2008, 2010 and 2013, 2 from 2009 and 5 from 2011) could not be recovered from the glycerol stock and were excluded from ST131 analysis.

Extended-spectrum β-lactamase production

E. coli (542 [11.7%]) and K. pneumoniae (81 [5.3%]) isolates that produced ESBL predominated. There were 10 (3.8%) ESBL-producing K. oxytoca isolates and 3 (0.9%) ESBL-producing P. mirabilis isolates.

Trends in extended-spectrum β-lactamase production

E. coli

The proportion of ESBL-producing E. coli isolates increased significantly over the study period, from 6.4% (19/296) in 2006 to 17.3% (89/513) in 2016 (p < 0.001, χ² test for trend) (Table 1, Figure 1). This trend was observed in both the intensive care unit and the emergency department, where the proportion of ESBL-producing E. coli isolates increased from 11.1% (1/9) to 25.8% (8/31) (p = 0.02, χ² test for trend) and from 3.3% (6/180) to 14.8% (47/318) (p < 0.001, χ² test for trend), respectively (Table 2, Figure 1). In addition, the frequency of the ST131 clonal type within ESBL-producing E. coli isolates increased from 31.6% (6/19) to 73.0% (65/89) over the study period (p = 0.03, χ² test for trend) (Table 1, Figure 1).

K. pneumoniae, K. oxytoca and P. mirabilis

During the study period, ESBL production among K. pneumoniae isolates showed an upward trend, from 5.0% (6/120) of isolates in 2006 to 9.2% (18/196) in 2016 (p = 0.04, χ² test for trend). The proportion of ESBL-producing K. oxytoca isolates decreased (p = 0.002, χ² test for trend), although the absolute number of ESBL-producing isolates remained low throughout the study period. The proportion of ESBL-producing P. mirabilis isolates remained relatively stable (Table 1). Owing to the small number of these isolates, we did not perform subset analysis of trends in the intensive care unit and the emergency department.

Antimicrobial susceptibility

E. coli

There was a significant decrease over the study period in E. coli susceptibility to ceftriaxone (p < 0.001, χ² test for trend) and ciprofloxacin (p = 0.008, χ² test for trend). Rates of susceptibility to other antimicrobial classes remained relatively stable (Figure 2, A). Susceptibility to ciprofloxacin, trimethoprim-sulfamethoxazole, tobramycin and gentamicin were significantly lower in ESBL-producing E. coli than in non-ESBL-producing E. coli in 2016 (Table 3). Susceptibility to ertapenem and meropenem/imipenem did not differ significantly between ESBL-producing and non-ESBL-producing E. coli. Among the former, a significant increase in aminoglycoside susceptibility was noted between 2006 and 2016 (p < 0.001 for both tobramycin and gentamicin, χ² test for trend). Susceptibility to ciprofloxacin, trimethoprim-sulfamethoxazole, ertapenem and

| Organism                        | 2006     | 2007     | 2008     | 2009     | 2010     | 2011     | 2012     | 2013     | 2014     | 2015     | 2016     | p value* |
|--------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Escherichia coli               | 19/296   | 13/309   | 29/355   | 28/376   | 33/394   | 51/428   | 58/457   | 81/485   | 65/485   | 76/516   | 89/513   | < 0.001  |
| Klebsiella pneumoniae          | 6/120    | 5/109    | 5/87     | 0/115    | 6/139    | 9/136    | 4/129    | 8/162    | 11/154   | 9/191    | 18/196   | 0.04     |
| K. oxytoca                     | 0/4      | 0/3      | 0/4      | 0/17     | 0/23     | 1/30     | 1/30     | 1/30     | 1/30     | 1/30     | 0/33     | 0.002    |
| Proteus mirabilis              | 0/23     | 0/30     | 0/26     | 0/27     | 0/34     | 0/31     | 0/30     | 0/30     | 0/36     | 1/35     | 0.3      |
| ST131 clonal type among ESBL-producing E. coli | 6/19     | 8/13     | 20/28    | 16/26    | 19/32    | 27/46    | 36/58    | 54/80    | 40/65    | 48/76    | 65/89    | 0.03     |

Note: ESBL = extended-spectrum β-lactamase.

*For the trend observed between 2006 and 2016 (χ² test for trend).
meropenem/imipenem remained relatively stable among ESBL-producing *E. coli* (Figure 2, B).

*K. pneumoniae, K. oxytoca and P. mirabilis*
Rates of *K. pneumoniae, K. oxytoca* and *P. mirabilis* susceptibility to ciprofloxacin, trimethoprim–sulfamethoxazole, tobramycin, gentamicin, ertapenem and meropenem/imipenem remained relatively stable over the study period. In 2016, *K. pneumoniae* the rates of global susceptibility to these antibiotics were 90.8%, 85.2%, 93.4%, 95.9%, 100.0% and 99.5%, respectively. For *K. oxytoca*, in 2016, the susceptibility rate was 100.0% for all 6 antimicrobial agents, and for *P. mirabilis*, the susceptibility rates were 71.4%, 77.1%, 97.1%, 97.1%, 100.0% and 100.0%, respectively. Given the small numbers of each of these species, we did not perform subset analysis of ESBL producers compared to non–ESBL-producers.

**Interpretation**
We found a significant increase in the proportion of ESBL-producing *E. coli* isolates in bloodstream infections from 2006 to 2016 in Toronto. The most striking increase was seen in the intensive care units served by our laboratory, where 25.8% of *E. coli* isolates were ESBL producers in 2016, compared to...
Figure 2: Antimicrobial susceptibilities of *Escherichia coli* blood culture isolates, 2006–2016. (A): Extended-spectrum β-lactamase (ESBL) producers and non–ESBL-producers combined. (B): Non–ESBL-producers and ESBL producers separately.
11.1% in 2006. The proportion of ESBL-producing *E. coli* isolates also increased significantly in the emergency departments served by our laboratory, with a prevalence of 14.8% in 2016 compared to 3.3% in 2006. The fact that an increase was observed in both settings suggests that this phenomenon is not being driven by 1 ward alone and is likely being driven by outpatients as well as inpatients. In parallel with this rise in prevalence of ESBL producers, the proportion of the ST131 clone among ESBL-producing *E. coli* also increased, from 31.6% in 2006 to 73.0% in 2016. Interestingly, we noted a significant increase in aminoglycoside susceptibility in ESBL-producing *E. coli* between 2006 and 2016. This may reflect a change in circulating strains or a change in resistance-selective pressure owing to changing prescribing practices.

Clinicians should be aware of their local ESBL prevalence rates and susceptibility patterns as this informs their empiric choice of treatment, preventing delays in appropriate therapy. In 2016, 17.3% of blood culture *E. coli* isolates from centres served by our laboratory were ESBL-positive; this suggests that ceftriaxone may not be an appropriate empiric choice for treatment of suspected *E. coli* bacteremia in our patient population, especially immunocompromised patients and those presenting with sepsis, in whom agents with lower resistance rates should generally be used. Clinicians should work with their local antimicrobial stewardship teams to integrate local data, guidelines and the clinical status of patients to inform empiric treatment decisions.

Our finding of increasing ESBL prevalence is in keeping with the global trend of rising antimicrobial resistance among gram-negative bacteria. The importance of regional data is illustrated by the study by Peirano and colleagues, which showed a prevalence of ESBL production of 14% among *E. coli* blood culture isolates in the Calgary region in 2010, in contrast to the rate of 2.9% among *E. coli* isolated from all specimen types in the same year reported as part of the CANWARD study. There is a paucity of published data on ESBLs from the Toronto region. Lowe and colleagues noted an increase in the incidence rate of ESBL-positive clinical cultures from 2005 to 2009. The CANWARD study includes organisms from sentinel sites across Canada, including our laboratory, but only the first 10 blood culture isolates, (not limited to *E. coli*) per month in the first 10 months of the year are included, and the isolates sent from our laboratory reflect less than 10% of all blood culture isolates each year and represent less than 10% of the CANWARD data. Thus, the current study provides important data on trends in ESBL production by *E. coli* and other organisms in the Toronto region.

The increasing prevalence of ESBL-producing *E. coli* that we observed in our centre is likely related to the globally increasing prevalence of ST131 *E. coli*. This is corroborated by our finding of an increase in the frequency of the ST131 clonal type within ESBL-producing *E. coli*, from 31.6% in 2006 to 73.0% in 2016. ST131 *E. coli* is spreading worldwide, likely related to its multidrug resistance and possibly to virulence factors that provide a survival advantage. There is a temporal correlation of a rise in ESBL prevalence after the introduction of ST131 *E. coli* into a region, which likely contributes to regional differences in ESBL prevalence rates.

**Limitations**

Our data represent local ESBL prevalence rates, reflecting the patient population serviced by the centres supported by our laboratory, which include a large tertiary care transplantation centre, immunocompromised hematology cancer centre and oncology program in a large cosmopolitan city. It is likely that smaller community hospitals or centres that provide care to...
unique patient cohorts would show different rates. Our data are also from a predominantly adult cohort and cannot provide insight to rates among pediatric patients. Furthermore, aside from a polymerase chain reaction assay for ST131 characterization, sequencing or other molecular typing was not performed on isolates, which limits our ability to fully describe the epidemiologic features of the increase in ESBL-producing *E. coli* in our region. In addition, 4 isolates from 2016 were not assessed for ESBL production owing to lack of viability of the glycerol stock or presumed loss of the ESBL-resistance plasmid, and 10 ESBL-producing *E. coli* isolates were excluded from ST131 analysis owing to lack of viability of the glycerol stock; however, given the small numbers of isolates excluded per year, we do not believe this introduced bias into the observed trends. Finally, we cannot comment on where patients may have acquired ESBL-producing *E. coli*, as information on travel history and history of past hospital admissions was not assessed.

**Conclusion**

Our study describes recent ESBL trends in blood cultures from a Canadian region. The significant increase in the proportion of ESBL-producing *E. coli* in bloodstream infections in our region temporally linked with the rise in the ST131 clonal type of *E. coli* highlights the ongoing threat posed by antimicrobial-resistant organisms. Recognition of this significant increase is important to inform empiric antibiotic treatment of bloodstream infections. Further investigations are needed to elucidate the epidemiologic features of ESBL-producing *E. coli* in order to inform modification of practices aimed at reducing the prevalence and limiting the spread of ESBL-producing organisms.

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