

Extended-Spectrum β -Lactamase- and AmpC- β -Lactamase- producing *E. coli*
in Canadian Intensive Care Units from 2005-2010P. J. SIMNER, F. TAILOR, P. LAGACÉ-WIENS, G. G. ZHANEL and D. J. HOBAN
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ABSTRACT

Background: The purpose of this study was to compare the prevalence and resistance profiles of Extended-Spectrum β -Lactamase- (ESBL-EC) and AmpC β -Lactamase- producing *E. coli* (AmpC-EC) in Canadian Intensive Care Units (ICUs) from 2005 to 2010.

Materials and Methods: 920 *E. coli* isolates were collected from Canadian ICUs from September 2005 to November 2010, inclusive, as part of two national surveillance studies: CAN-ICU & CANWARD. Susceptibility testing was performed by broth microdilution (CLSI). Putative ESBL-EC and AmpC-EC were further characterized by PCR and DNA sequencing to detect resistance genes. Strains were typed using PFGE and a PCR assay for the *pabB* gene specifically belonging to the ST131 clone.

Results: Overall 4.8% (2005/06: 3.7% to 2010: 6.7%) and 3.9% (2005/06: 5.5% to 2010: 2.9%) of *E. coli* isolated from ICUs were ESBL and AmpC producers, respectively. Resistance occurred to fluoroquinolones in 86.4% and 13.9% ($P<0.0001$), trimethoprim-sulfamethoxazole in 63.6% and 28.6% ($P=0.0017$) and gentamicin in 26.8% and 5.6% ($P=0.00163$) of ESBL-EC and AmpC-EC, respectively. 93.2% of ESBL-EC and 27.8% of AmpC-EC were identified as multidrug resistant ($P<0.0001$). The majority (>97%) of isolates remained susceptible to colistin, tigecycline and the carbapenems. 88.9 % of AmpC-EC carried *bla*_{CMY-2}, 8.3% had mutations in the chromosomal *ampC* gene and 2.8% carried *bla*_{ACT-1}. 97.7% of ESBL-EC carried a CTX-M enzyme with 70.5% of those producing *bla*_{CTX-M-15}. Generally isolates were genetically unrelated (< 80% similarity) by PFGE however 59.1% of ESBL-EC and 16.7% of AmpC-EC belonged to the ST131 clone ($\geq$ 60% similarity; $P=0.0002$). Patient demographics for ESBL-EC were: mean age 56 years with a female/male ratio of 43.2:56.8%. For AmpC-EC patient demographics were: mean age 65 years, with female/male ratio of 75:25%. 29.5% of the ESBL-EC and 69.4% of AmpC-EC were isolated from urine ($P=0.0006$).

Conclusion: The prevalence of ESBL-EC has increased while the prevalence of AmpC-EC has decreased among Canadian ICUs from 2005-2010. ESBL-EC were significantly more likely to be MDR and belong to the ST131 clone whereas Amp-EC were more likely to be isolated from urine and females.

BACKGROUND

Escherichia coli is among the top-ranked pathogens causing significant infections worldwide. Resistance in *E. coli* to the β -lactam antibiotics such as the clinically important cephalosporins is a continuing cause of public health concern, with resistance increasingly seen in community- and nosocomial-acquired infections (1). Resistance to cephalosporins in *E. coli* is often mediated by the production of extended-spectrum β -lactamases (ESBL) or AmpC β -lactamases (AmpC). In *E. coli*, constitutive overexpression of AmpC can occur due to either the deregulation of the chromosomally encoded *ampC* gene (derepressed ampC mutants), or by acquisition of a transferable *ampC* gene on a plasmid or other transferable elements (plasmid-mediated AmpC) (2). Usually, AmpC producers differ from ESBL producers phenotypically, yielding a negative ESBL disk test, are resistant to cefoxitin, and are usually susceptible to cefepime (3). However, the ESBL phenotypic disk test does not exclude the possibility of the presence of multiple β -lactamase enzymes, and may result in false-negative tests, especially when organisms produce both ESBL and AmpC enzymes (1). The increasing association of resistance to other classes of antimicrobials in cephalosporin-resistant *E. coli* giving rise to multidrug-resistant (MDR) strains is of concern, as these strains severely limit therapeutic options. Even more alarming is the emergence of reduced susceptibility or resistance to carbapenems among ESBL and AmpC producers, further limiting options for treatment of such infections (4).

The purpose of this study was to compare the prevalence and resistance profiles of ESBL-EC and AmpC-EC in Canadian ICUs from 2005 to 2010.

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MATERIALS & METHODS

Bacterial Isolates:

E. coli isolates were collected from Canadian ICUs from September 2005 to November 2010 as part of two national surveillance studies: CAN-ICU & CANWARD.

CAN-ICU: From September 2005 through August 2006, 19 medical centres, with active ICUs, from all regions of Canada submitted pathogens. Each centre was to collect a maximum of 300 consecutive pathogens isolated from blood, urine, tissue/wound, and respiratory specimens (one pathogen per cultured site per patient) of ICU patients. A total of 4,092 clinical isolates were obtained. Of the 4,092 isolates, 493 (12%) were *E. coli* isolates.

CANWARD: From January 2007 through November 2010, tertiary-care centres from across Canada submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units. Annually, each centre was asked to submit pathogens (consecutive, one per patient/infection site) from blood, respiratory, urine, and wound/IV infections. 7718, 5282, 5375 and 4868 isolates were collected for 2007, 2008, 2009 and 2010, respectively. A total of 4,807 *E. coli* were collected as part of CANWARD with 427 (8.9%) being isolated from ICUs.

Antimicrobial Susceptibility Testing:

The *in vitro* activity of selected antimicrobial agents was determined by reference broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI, 2006) guidelines. Antimicrobial MIC interpretative standards were defined according to CLSI (2010) breakpoints. Strains concomitantly resistant to ≥ 3 antimicrobial classes were defined as MDR. All *E. coli* displaying ceftriaxone and/or ceftazidime MICs $\geq 1\mu\text{g/mL}$ were identified as putative ESBL producers and were further confirmed by the CLSI (2010) confirmatory disk test. Any putative ESBL-producing *E. coli* that was negative for the ESBL confirmatory disk test and resistant to cefoxitin ($\geq 32\mu\text{g/mL}$) was identified as a putative AmpC producer. The term 'AmpC-producing *E. coli*' defines both derepressed ampC mutants and plasmid-mediated AmpC producers in this study.

Molecular Characterization of ESBL- and AmpC-producing *E. coli*:

Genotypic characterization of ESBLs was performed by PCR and sequencing of *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}, and *bla*_{OXA-like} genes as previously described (5). Putative AmpC producers were screened for plasmid mediated AmpC genes and for mutations within the chromosomal *ampC* promoter and/or attenuator region by PCR and sequencing as previously described (3, 6). All ESBL- and AmpC-producing *E. coli* isolates were typed by pulsed-field gel electrophoresis (PFGE) using a standardized protocol, as previously described (5). Screening for the ST131 clone of *E. coli* among ESBL and AmpC producers was performed using a O25b-ST131 clone allele-specific PCR to amplify a 347-bp fragment of *pabB* as previously described (7).

Statistical analysis

Statistical significance was calculated by χ^2 analysis or Fisher exact test using VassarStats (<http://faculty.vassar.edu/lowry/VassarStats.html>). No statistically significant difference observed will be denoted NS (NS is defined as a *P* value >.05).

REFERENCES

- Paterson DL, Bonomo RA. 2005. Clin Microbiol Rev 18(4):657–686.
- Perez-Perez FJ, Hanson ND. 2002. J Clin Microbiol 40:2153–2162.
- Baudry PJ, Nichol K, DeCorby M, Mataseje L, Mulvey MR, Hoban DJ, Zhanel GG. 2008. Antimicrob Agents Chemother 52:1846–1849.
- Pitout JDD. 2008. Expert Rev 6:657–669.
- Mulvey MR, Bryce E, Boyd D, Ofner-Agostini M, Christianson S, Simor AE, Paton S. 2004. Antimicrob Agents Chemother 48:1204–1214.
- Caroff N, Espaze E, Bérard I, Richet H, Reynaud A. 1999. FEMS Microbiol Lett 173:459–465.
- Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, Mulvey MR, Nordmann P, Ruppé E, Sarthou JL, Frank T, Vimont S, Arlet G, Branger C, Woodford N, Denamur E. 2009. J Antimicrob Chemother 64:274–277.

Figure 1: Prevalence of ESBL- and AmpC β -Lactamase-producing *E. coli* in Canadian ICUs from 2005-2010

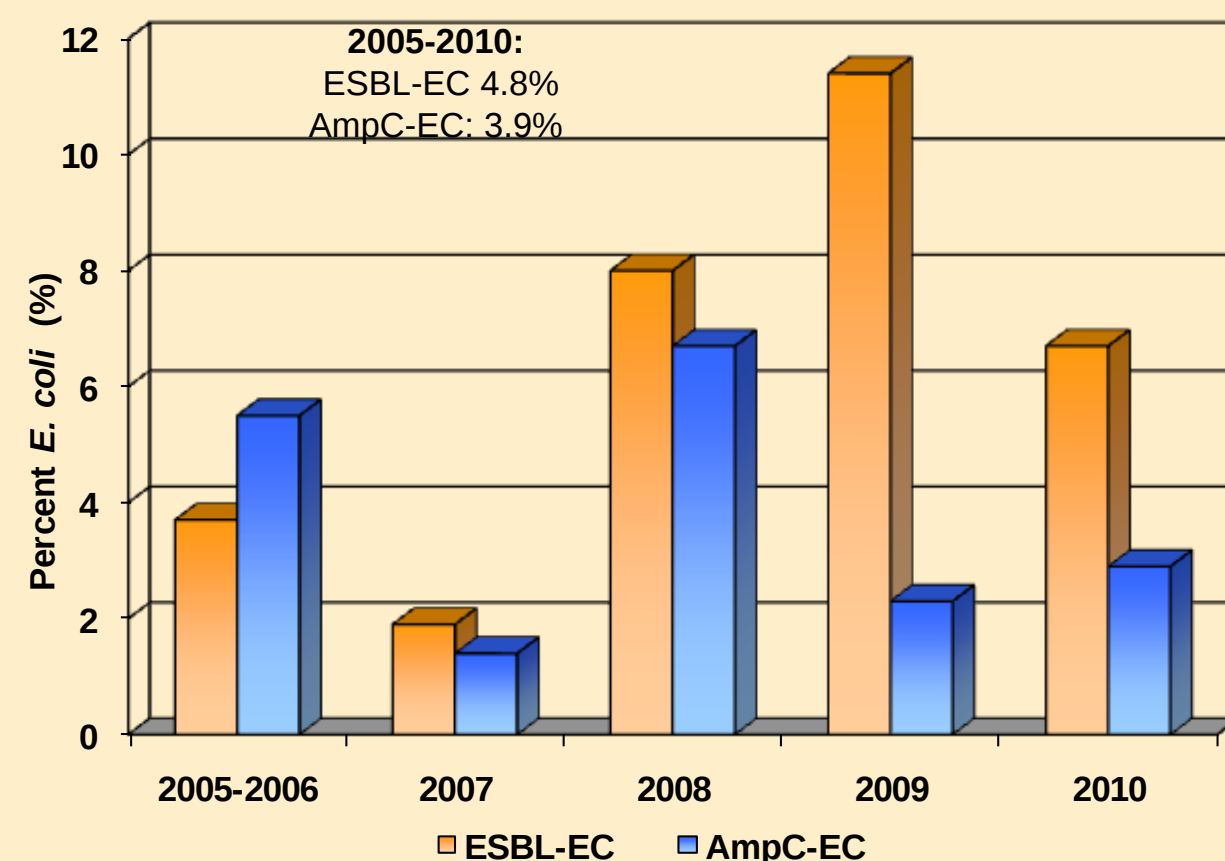


Figure 2: Resistance Profiles Among ESBL- and AmpC β -Lactamase- producing *E. coli* in Canadian ICUs from 2005-2010

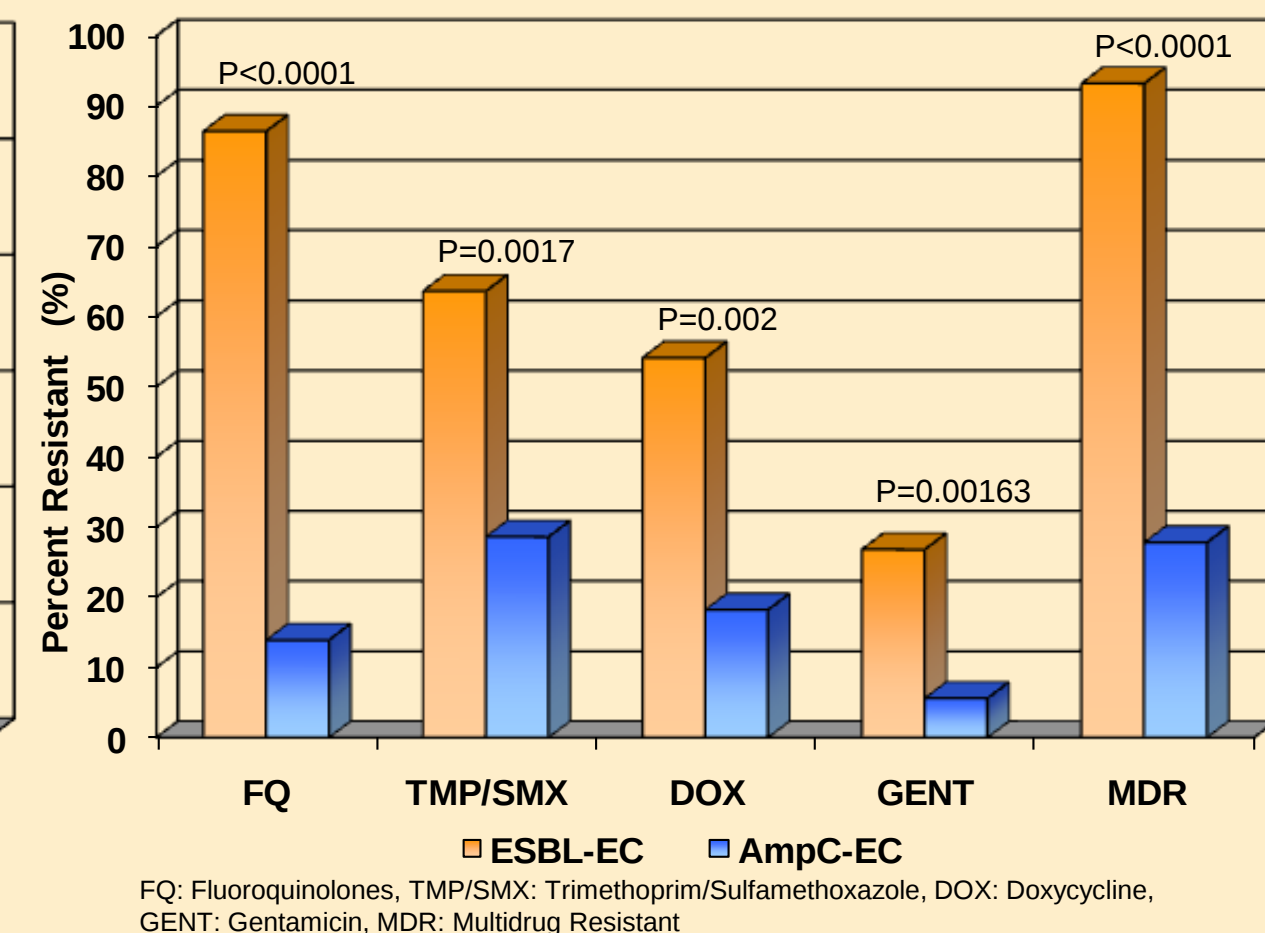


Table 1: Comparison of Patient Demographics, Region of Isolation, and Specimen Source of ESBL and AmpC β Lactamase- producing *E. coli* in Canadian ICUs from 2005-2010

	Patient Demographics												
	Age		Gender - % Isolates		Region-% Isolates					Specimen Source- % Isolates			
	Mean Age	Range	Female	Male	BC/AB	SK/MB	ONT	QB	Maritimes	Urine	Blood	Wound	R.T.
ESBL- <i>E. coli</i> (n=44)	56	1-85	43.2	56.8	18.2	6.8	36.4	15.9	22.7	29.5	36.4	9.1	25
AmpC- <i>E. coli</i> (n=36)	65	1-96	75	25	5.6	11.1	13.9	5.6	63.9	69.4	22.2	2.8	5.6
P value	NS		<0.008		NS	NS	<0.04	NS	<0.0004	<0.0006	NS	NS	NS

BC/AB: British Columbia/Alberta, SK/MB: Saskatchewan/Manitoba, ONT: Ontario, QB: Quebec, Maritimes: Nova Scotia and New Brunswick

R.T.: Respiratory Tract

NS: Not Statistically Significant

CONCLUSIONS

- The prevalence of ESBL-EC has increased while the prevalence of AmpC-EC has decreased among Canadian ICUs from 2005-2010.
- CTX-M and CIT-type were the predominant enzymes being produced, with CTX-M-15 and CMY-2 being the predominant genotypes among ESBL-EC and AmpC-EC, respectively.
- AmpC-EC were more likely to be isolated from females, urine and the Maritimes, whereas ESBL-EC were more likely to be isolated from Ontario.
- AmpC-EC infections are more susceptible to fluoroquinolones, doxycycline, gentamicin and TMP/SMX whereas ESBL-EC infections were more likely to be MDR.
- The majority (>97%) of ESBL-EC and AmpC-EC remained susceptible to colistin, tigecycline and the carbapenems.
- ESBL-EC were more likely to be genetically related and belong to the ST131 clone ($P=0.0002$).

Figure 3: PFGE Dendrogram of ESBL-producing *E. coli*

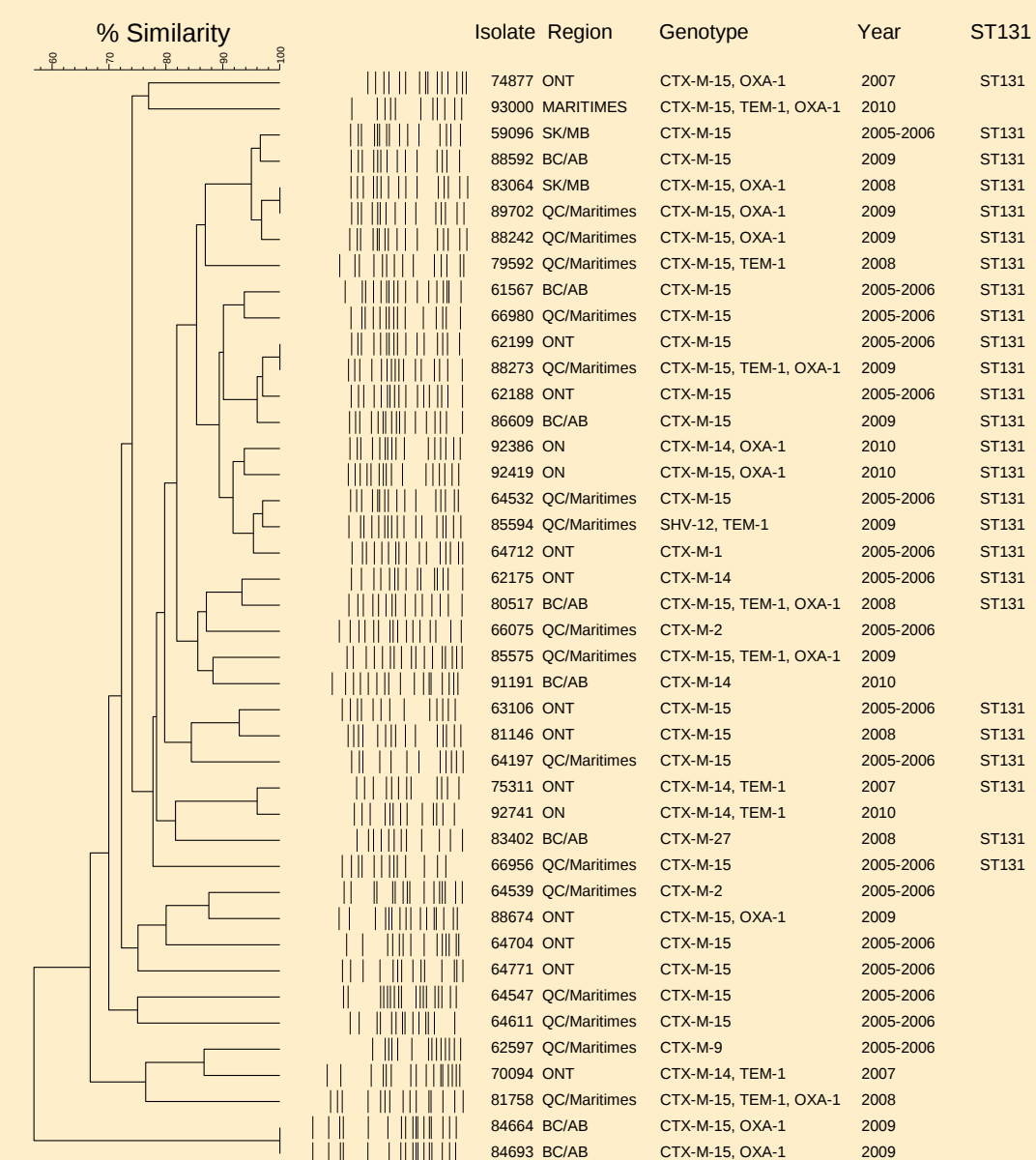


Figure 4: PFGE Dendrogram of AmpC β -lactamase-producing *E. coli*

