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Comparison of Virulence Factors & Antimicrobial Susceptibilities Among ST131 and non-ST131 ESBL-producing *E. coli*

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Abstract

Introduction: The pandemic dissemination of the O25b-ST131 ESBL-producing *E. coli* (mainly CTX-M-15 producers) clone has become a major health concern. The purpose of this study was to compare virulence factors (VF) & antimicrobial susceptibilities among ST131 ESBL-producing *E. coli* (ESBL-EC) and other non-ST131 ESBL-EC.
Materials and Methods: 126 ESBL-EC isolates collected from the national CAN-ICU 2005/2006 and CANWARD 2007 and 2008 surveillance studies. Susceptibility testing was performed by broth microdilution. Isolates were phylogrouped by multiplex PCR and screened by PCR for the five key VF of extraintestinal pathogenic *E. coli* (ExPEC). ExPEC were defined as the presence of ≥ 2 of the 5 VF. All isolates were typed by PFGE and a PCR assay for the *pabB* gene specifically belonging to the O25b-ST131 clone was performed.
Results: Of the 126 ESBL-EC, 65 (51.2%) were identified as the ST131 clone: 12 of 18 (66.7%) from CAN-ICU, 26 of 53 (49.1%) from CANWARD 2007 and 27 of 55 (49.1%) from CANWARD 2008. Of the 65 ST131 ESBL-EC, 52 (80.0%) were CTX-M-15 producers, 6 (9.2%) were CTX-M-14 producers, 2 (3.1%) were CTX-M-27 producers and one of each were CTX-M-1, CTX-M-3, CTX-M-65, SHV2a producers and one unknown (7.7%). All 65 ST131 clones cluster together by PFGE at ≥ 60% similarity. The ST131 ESBL-EC clones were significantly more likely to harbour the *iutA* aerobactin receptor gene ($P < 0.0001$), *kpsMTII* group 2 capsule gene ($P < 0.0001$), *afa/draBC* Dr-binding adhesion genes ($P = 0.0127$) and were significantly more likely to be defined as ExPEC ($P < 0.0001$) than non-ST131 ESBL-EC. They are also more commonly fluoroquinolone (FQ) resistant ($P < 0.0001$) than non-STI31 ESBL-EC.
Conclusions: The association of FQ resistance and VF genes *iutA, kpsMII* and *afa/draBC* participate in the high virulence potential of ST131 strains and may play an important role in the pandemic dissemination of these ESBL-producers in comparison to other non ST131 ESBL-EC.

Background

Extraintestinal pathogenic *E. coli* (ExPEC) and commensal *E. coli* usually differ with respect to phylogenetic groups and virulence factors (1). Phylogenetic analyses have shown that *E. coli* fall into four phylogenetic groups: A, B1, B2 and D. Extraintestinal *E. coli* are commonly associated with the more virulent phylogentic groups B2 and to a lesser extent D. Whereas, commensal strains largely belong to groups A and B1 (2). Antimicrobial resistant strains are often associated with the less virulent phylogenetic groups A and B1, suggesting a trade-off between resistance and virulence (3).
Numerous virulence factors have been identified in extraintestinal *E. coli* including adhesins and fimbriae, toxins, siderophores, capsules and invasins. The presence of 2 of the 5 key virulence genes, including *papA/papC* (P fimbriae structural subunit and assembly; analyzed collectively), *sfa/foc* (S and F1C fimbriae), *afa/draBC* (Dr-binding adhesins), *iutA* (aerobactin receptor), and *kpsMTII* (group 2 capsule), defines an isolate as an extraintestinal pathogenic *E. coli* (ExPEC) (2).
Recent studies have demonstrated that the highly virulent *E. coli* O25:H4-ST131 belonging to phylogenetic group B2 is responsible for the pandemic dissemination of $bla_{CTX\text{-}M\text{-}15}$ (1). The O25b-ST131 $bla_{CTX\text{-}M\text{-}15}$ producing *E. coli* clone has been increasing in prevalence and identified worldwide. The fact that this O25b-ST131 clone includes diverse PFGE patterns (all related at ≥ 60% similarity) complicates recognition of its members (3).
The purpose of this study was to compare virulence factors (VF) & antimicrobial susceptibilities among ST131 ESBL-producing *E. coli* and other non-ST131 ESBL-producing *E. coli*.

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Materials and Methods

Bacterial Isolates:
Bacterial isolates were collected as part of two national surveillance studies: the Canadian Intensive Care Unit (CAN-ICU) and the Canadian ward (CANWARD) surveillance studies.
The CAN-ICU study included 19 medical centres from all regions of Canada with active ICUs. 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. CANWARD isolates were collected from January 2007 through December 2008, in which 12 medical centres (10 centres - 2008) 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 (360), respiratory (200), urine (100), and wound/IV (50) infections. All isolates were identified at participating sites by routine procedures performed at each laboratory. Isolates were shipped to the reference laboratory at the Winnipeg Health Sciences Centre on Amies charcoal swabs, subcultured onto blood agar, and stocked in skim milk at -80°C until MIC testing was carried out.
Susceptibility Testing:
Following two subcultures from frozen stock, the *in vitro* activities of various antimicrobials were determined in duplicate by microbroth dilution in accordance with CLSI guidelines (MO7-A8, M100-S19, 2009). Strains concomitantly resistant to ≥ 3 different antimicrobial classes were defined as multi-drug resistant (MDR). Any *E. coli* with a ceftriaxone and/or a ceftazidime MIC ≥ 1 µg/mL was identified as a putative ESBL and underwent further analysis. The putative ESBL phenotype was confirmed by the disk confirmation method as described by CLSI. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were the control strains used in this study.
Characterization of ESBLs:
Genotypic characterization of ESBLs was performed by PCR and sequencing of bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{OXA} genes as previously described (4). A BLAST search of the DNA sequence was conducted to determine specific genotypes.
Phylogenetic Groups and Virulence Factors:
To determine the phylogenetic group and virulence potential of *E. coli* they were phylogrouped by multiplex PCR into one of the four phylogenetic groups: A, B1, B2 or D (4). All isolates were also screened by PCR for the five key virulence factors of extraintestinal pathogenic *E. coli* (ExPEC) as previously described (2). ExPEC were defined as the presence of ≥ 2 of the 5 virulence factors.
Molecular Typing by PFGE:
Genetic relationships of the ESBL-producing *E. coli* were assessed by pulsed-field gel electrophoresis (PFGE) following digestion with *Xba*I as previously described (4).
PCR detection of the O25b-ST131 clone:
Screening of ESBL-producing *E. coli* for the O25b-ST131 clone was done using a O25b-ST131 allele-specific PCR for the *pabB* gene. Primers O25pabBspe.F (5'-TCCAGCAGGTCGTGGAT-CGT-3') and O25pabBspe.R (5'-GCGAAATTTTCGCCGTACTGT-3') were used to amplify a 347bp fragment of *pabB* as previously described (3).

References

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3. Clermont, O et al. 2009. J Antimicrob Chemother. 64:274-277.
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5. Clermont, O et al. 2000. Appl Environ Microbiol. 66:4555-4558.

Results

Figure 1: Phylogenetic Groups Among ESBL-producing *E. coli*.

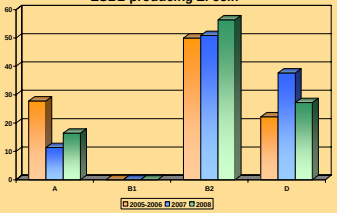


Figure 2: Virulence Factors Among ST131 and non-ST131 ESBL-producing *E. coli*.

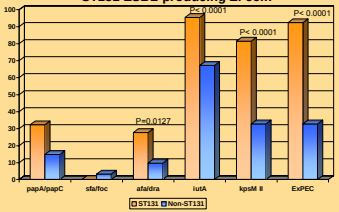
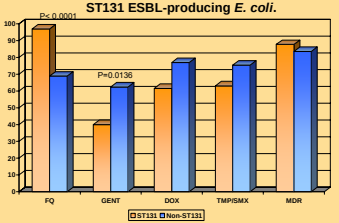
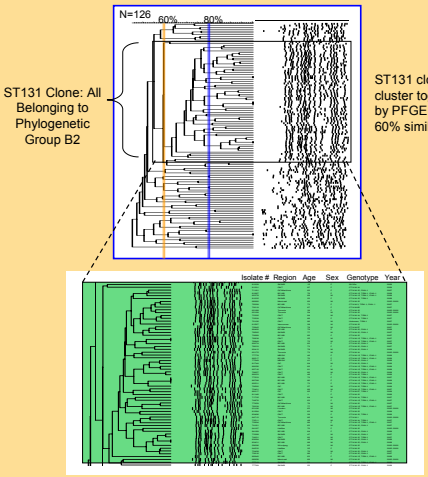


Figure 3: Resistance Profiles Among ST131 and non-ST131 ESBL-producing *E. coli*.



FQ: Fluoroquinolones, GENT: Gentamicin, DOX: Doxycycline, TMP/SMX: Trimethoprim/Sulfamethoxazole, MDR: Multi-drug Resistant

Figure 4: Pulsed Field Gel Electrophoresis Dendrogram of ESBL-producing *E. coli*.



Conclusions

1. Sixty-five (51.2%) of 126 ESBL-producing *E. coli* were identified as the ST131 clone.
2. Of the 65 ST131 ESBL-EC, 52 were CTX-M-15 (80.0%) producers, 6 were CTX-M-14 (9.2%) producers, 2 were CTX-M-27 (3.1%) producers and one of each were CTX-M-1, CTX-M-3, CTX-M-65, SHV2a producers & an unknown (7.7%).
3. ST131 strains were significantly more likely to harbour the aerobactin receptor, the group 2 capsule and the Dr-binding adhesin virulence factor than non-ST131 strains.
4. ST131 strains were significantly more likely to be fluoroquinolone resistant whereas non-ST131 strains were significantly more likely to be gentamicin resistant.
5. The association of fluoroquinolone resistance and virulence factor genes participate in the high virulence potential of ST131 strains and may play an important role in the pandemic dissemination of these ESBL-producers in comparison to other non-ST131 ESBL-producing *E. coli*.

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