Poster # A-027

Characterization of Carbapenem Reduced-Susceptible (CRS) *E. coli* (EC) and *K. pneumoniae* (KP) from Canadian Hospitals: CANWARD 2007 and 2008

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Revised Abstract

Background: The purpose of this study was to characterize CRS EC and KP isolates from Canadian hospitals.

Methods: A total of 2692 EC and 771 KP were collected from January 2007 - December 2008, inclusive, as part of the national CANWARD Surveillance Study assessing antimicrobial resistance in Canadian hospitals. Antimicrobial susceptibility testing was performed using CLSI broth microdilution methods. Any EC or KP with an ertapenem MIC of ≥0.12 µg/ml were defined as CRS and further analyzed. Carbapenemase-production (CP) was screened by the modified hodge test (MHT) and confirmed by KPC PCR. Isolates were analyzed for outer membrane porin (OMP) protein changes. PFGE was used to assess genetic relatedness.

Results: A total of 30/2692 (1.1%) EC and 6/771 (0.8%) KP were CRS. 22/30 (73.3%) EC and 4/6 (66.7%) KP demonstrated a weak positive result using the MHT. 4/30 (13.3%) EC were uninterpretable by MHT. All isolates were KPC PCR negative. 17/30 (56.7%) EC and 5/6 (83.3%) KP were ESBL producers. Approximately 50% of isolates were Multi-Drug Resistant (MDR- resistance to ≥3 antimicrobial classes) and were determined to be genetically unrelated (< 80% homology). Susceptibility testing for select agents is shown in the table below.

Conclusions: The majority of CRS EC and KP in Canada are MDR with most being ESBL producers. CP was not detected by PCR or MHT. The spread of CRS EC and KP in Canada was found to be polyclonal. The emergence of these isolates demonstrates the need for a more careful use of carbapenems.

Drug*		WT[b]-EC	CRS-EC	WT-KP	CRS-KP
Ceftazidime	$MIC_{50}/_{90}$	≤0.5/≤0.5	>32/>32	≤0.5/≤0.5	32/>32
	%S|I|R[c]	100|0|0	20|0|80	100|0|0	16.7|0|83.3
Ceftriaxone	$MIC_{50}/_{90}$	≤0.25/≤0.25	>64/>64	≤0.25/≤0.25	>64/>64
	%S|I|R	100|0|0	10|0|90	100|0|0	33.3|0|66.7
Ciprofloxacin	$MIC_{50}/_{90}$	≤0.06/≤0.06	>16/>16	≤0.06/≤0.06	0.25/>16
	%S|I|R	100|0|0	26.7|0|73.3	100|0|0	50|0|50
Doripenem	$MIC_{50}/_{90}$	≤0.06/≤0.06	≤0.06/≤0.06	≤0.06/≤0.06	≤0.06/0.12
	%S|I|R	100|0|0	100|0|0	100|0|0	100|0|0
Ertapenem	$MIC_{50}/_{90}$	≤0.06/≤0.06	0.12/0.5	≤0.06/≤0.06	0.25/1
	%S|I|R	100|0|0	80|10|10	100|0|0	83.3|0|16.7
Gentamicin	$MIC_{50}/_{90}$	≤0.5/1	2/>32	≤0.5/≤0.5	≤0.5/>32
	%S|I|R	100|0|0	53.3|0|46.7	100|0|0	66.7|0|33.3
Meropenem	$MIC_{50}/_{90}$	≤0.06/≤0.06	≤0.06/0.12	≤0.06/≤0.06	≤0.06/0.25
	%S|I|R	100|0|0	100|0|0	100|0|0	100|0|0
TMP/SMX[a]	$MIC_{50}/_{90}$	≤0.12/≤0.12	2/>8	≤0.12/0.25	0.5/>8
	%S|I|R	100|-|0	50|-|50	95|-|5	50|-|50

*2010 CLSI breakpoints from M100-S20 Vol. 20 No. 1; carbapenem breakpoints from upcoming supplement

[a]: TMP/SMX, Trimethoprim/Sulfamethoxazole

[b]: WT, Wild-Type (randomly selected isolates [with ertapenem MIC ≤ 0.06 µg/ml] from CANWARD covering various infection sites and geographic locations throughout Canada)

[c]: S, susceptible; I, intermediate; R, resistant

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Background

In the last two decades, carbapenems have been considered the last line of defence against multi-drug resistant (MDR-defined as concomitant resistance to ≥ 3 different antimicrobial classes) infections caused by *Enterobacteriaceae* (1). Examples include two of the most prevalent organisms causing infections in Canada such as *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) (http://www.can-r.ca). Both organisms are associated with urinary tract infections, bacteremia, pneumonia, meningitis, and diarrheal diseases. Traditional treatment includes the use of β-lactam antibiotics (penicillin, cephalosporins) but one of the main concerns is that frequently these organisms produce extended-spectrum β-lactamases (ESBLs) and/or AmpC β-lactamases. Even more worrisome is the production of *Klebsiella pneumoniae* carbapenemases (KPCs) which is becoming more common. KPCs are a group of molecular class A carbapenemases with a broad spectrum of activity. These genes are carried on plasmids and the encoded enzyme has the ability to hydrolyze virtually all β-lactam antibiotics including carbapenems (2, 3). Plasmids may also carry other resistant determinants such as resistance to aminoglycosides, fluoroquinolones, tetracyclines, and trimethoprim sulfamethoxazole conferring a MDR profile. Carbapenem resistance is not limited to KPC production. Other methods of resistance include the modification of outer membrane porins (OMPs) coupled with ESBL and/or AmpC production. This alone can lead to carbapenem resistance or reduced susceptibility even in the absence of a carbapenemase. Carbapenem reduced susceptibility is defined as an MIC of ertapenem ≥ 0.12 µg/ml which represent about an 8x increase in MIC compared to wild type. All of these attributes can contribute to the MDR profiles we see in these particular EC and KP strains. Treatment options for patients infected with these can be very problematic and limited to few agents such as tigecycline and colistin (1, 4). The purpose of this study was to characterize carbapenem reduced susceptible (CRS) isolates of EC and KP from Canadian hospitals. CRS isolates are frequently MDR with porin alteration (non-functional porin or decreased expression of porin) being a contributing factor.

Materials & Methods

Bacterial Isolates:

Isolates were selected from the Canadian Ward (CANWARD) surveillance study which is a national study based at Health Sciences Centre (HSC) in Winnipeg, MB, Canada. From January 2007 through December 2008, 12 sentinel Canadian hospitals (10 centres in 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 the originating centre using routine procedures performed at each site. Isolates were shipped to HSC on Amies charcoal swabs where they were subcultured onto blood agar and stocked in skim milk at -80°C. A total of 7881 and 5282 isolates were collected for 2007 and 2008, respectively. Of these, 2692 were *E. coli* (1560 from 2007 and 1132 from 2008) and 757 were *K. pneumoniae* (441 from 2007 and 316 from 2008). In 2007, if the centers did not collect a minimum of 20 ESBL-producers within the objectives, they were asked to continue collecting until this minimum of 20 was reached.

Susceptibility Testing:

Following two subcultures from frozen stock, the *in vitro* activity of various antimicrobials were determined (CRS isolates tested in duplicate to confirm ETP results) by broth microdilution in accordance with CLSI guidelines (CLSI M07-A8. Vol.29 No.2.). The minimum inhibitory concentration (MIC) was determined using custom designed 96-well microtitre plates. Food and Drug Administration interpretation breakpoints were used for tigecycline (S: ≤2 µg/ml, I: 4 µg/ml, R: ≥8 µg/ml) and colistin (S: ≤2 µg/ml, R: ≥4 µg/ml). Isolates concomitantly resistant to ≥ 3 different antimicrobial classes were defined as Multi-Drug Resistant (MDR). Any EC or KP with a ceftriaxone or ceftazidime MIC ≥ 1 µg/ml were further tested using disk diffusion methods described by CLSI to confirm ESBL phenotype (CLSI M100-S20. Vol.30 No.1.). Any EC or KP with a cefoxitin MIC ≥ 32 µg/ml was identified as a putative AmpC-producer. Any EC or KP with an ertapenem MIC ≥ 0.12 µg/ml was defined as carbapenem reduced susceptible.

Characterization of CRS-EC/-KP:

Phenotypic characterization of CRS-EC/-KP was performed using modified hodge test (MHT). Genotypic characterization of was performed by KPC PCR.

Molecular Subtyping by PFGE:

Genetic relationships of the ESBL-, AmpC-producing and CRS EC and KP were assessed by pulsed-field gel electrophoresis (PFGE) following digestion with *Xba*I as previously described (5).

OMP Analysis: Cells grown in nutrient broth overnight at 37°C were sonicated and OMPs were analyzed using SDS-PAGE and sequencing of OmpK35/36 of KP and OmpF/C of EC (6, 7).

Results

Figure 1. Genetic Relationship among CRS-EC from CANWARD 2007-2008 (n=30)

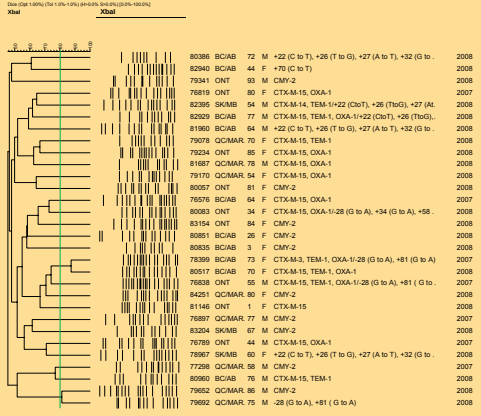


Figure 2. Genetic Relationship among CRS-KP from CANWARD 2007-2008 (n=6)

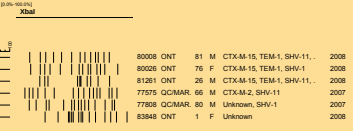
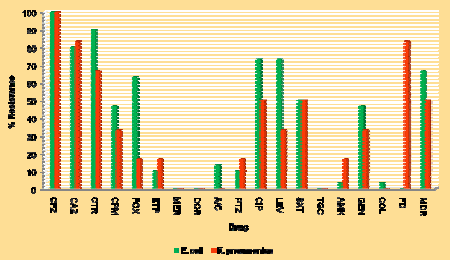


Figure 3. Resistance profiles among CRS-EC and -KP from 2007-2008



*2010 CLSI breakpoints used; ETP (S: ≤ 0.25, I: 0.5, R: ≥ 1 µg/ml); MER + DOR (S: ≤ 1, I: 2, R: ≥ 4 µg/ml)

Table 1. Percentage of ESBLs and AmpC among CRS-EC and -KP from 2007-2008

Organism	% ESBL	% AmpC	% ESBL/AmpC
E. coli (n=30)	36.7	43.3	20.0
K. pneumoniae (n=6)	83.3	N/A	N/A

Sequence Data

K. pneumoniae

OmpK35: One isolate, 77575, was shown to have insertion of G at position 123 leading to a frameshift and premature termination. The remaining isolates were homologous to wild type with a few minor amino acid changes

OmpK36: Isolates were a lot more variable with amino acid changes. Deletions were observed in all isolates in loop (L) 6 of the OmpK36.

E. coli

OmpF: 5/30 isolates were shown to have mutations leading to premature termination. Other major differences found within a high percentage of isolates were within L4, L5, and L6 of OmpF.

OmpC: Isolates were highly variable with about 50% showing major differences in L4, L5 and L7 of OmpC.

Conclusions

1. CRS-EC and -KP are emerging and Canada and are frequently MDR; 66.7% and 50%, respectively.
2. All EC isolates were positive for ESBL and/or AmpC production and negative for KPC by PCR.
3. From the 6 KP isolates, 5 were ESBL positive and all were KPC negative by PCR.
4. The most common ESBL encountered among isolates was CTX-M-15.
5. PFGE shows polyclonal spread of CRS-EC and -KP.
6. Porin profiles for all isolates showed amino acid changes from wild type. Few isolates were shown to have mutations leading to premature termination. A high percentage of isolates were shown to have changes within the external loop structures of the porin which may contribute to carbapenem reduced susceptibility.

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