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Activity of Doripenem (DOR) Against Molecularly Characterized AmpC, ESBL Producing and Carbapenem Reduced-Susceptible (CRS) *E. coli* (EC) Across Canada from 2007-2009

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ABSTRACT

Background: DOR is a recently approved carbapenem. The purpose of this study was to determine the activity of DOR against CRS-EC, AmpC-EC and ESBL-EC.

Methods: From 2007-2009 inclusive, a total of 3,930 EC were collected from medical centres across Canada as part of a national surveillance program assessing antimicrobial resistance in Canadian hospitals. Susceptibility testing was performed using CLSI methods. PCR and sequencing of β-lactamase resistant genes was used to determine AmpC and ESBL genotypes. CRS (defined as ertapenem [ETP] MIC ≥0.12 µg/ml) isolates were tested for KPC by PCR. SDS-PAGE and sequencing assessed OmpF and OmpC among CRS isolates. PFGE was used to determine genetic relatedness.

Results:

The activity of DOR and comparators are:

Drug	MIC$_{50}$/MIC$_{90}$ (µg/ml)			
	Total EC (n=3930)	AmpC-EC (n=71)	ESBL-EC (n=155)	CRS-EC (n=46)
A/C[a]	4/8[c]	16/32	8/16	16/32
Cefepime	≤1/≤1	≤1/≤1	16/>32	16/>32
Ceftazidime	≤0.5/1[d]	4/>32[e]	16/>32[f]	>32/>32
Ceftobiprole	≤1/≤1	≤1/≤1	32/>32	16/>32
Ceftriaxone	≤1/≤1	8/64	>64/>64	>64/>64
Cefoxitin	4/8[c]	>32/>32	8/32	32/>32
Doripenem	≤0.12/≤0.12	≤0.12/≤0.12	≤0.12/≤0.12	≤0.12/≤0.12
Ertapenem	≤0.06/≤0.06[c]	≤0.06/0.25	≤0.06/0.25	0.12/1
Meropenem	≤0.12/≤0.12	≤0.12/≤0.12	≤0.12/≤0.12	≤0.12/≤0.12
P/T[b]	2/4	4/32	4/16	8/64

[a]: A/C, Amoxicillin/Clavulanic Acid; [b]: P/T, Piperacillin Tazobactam; [c]: Results reflect n=2828; [d]: Results reflect n=2229; [e]: Results reflect n=67; [f]: Results reflect n=103

Conclusions: DOR displayed potent *in vitro* activity against CRS-EC, AmpC-EC and ESBL-EC isolates and displayed comparable activity to meropenem. DOR represents a potential option for infections caused by CRS-EC, AmpC-EC and ESBL-EC isolates.

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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). From this family, *Escherichia coli* (EC) remains the leading pathogen causing infections in Canada (http://www.can-r.ca). EC is associated with many infections including urinary tract infections, bacteremia, pneumonia, meningitis, and diarrheal diseases. Traditional treatment includes the use of β-lactam antibiotics (penicillins, cephalosporins) with the main concern being the development of resistance towards these agents with production of extended-spectrum β-lactamases (ESBLs) and/or AmpC β-lactamases by these organisms. Doripenem, a recently approved carbapenem, has been shown to have intrinsic stability to most β-lactamases including ESBLs and AmpCs. Its antimicrobial spectrum most closely resembles meropenem (MER) and imipenem (IPM) than ertapenem (ETP) (2). With the increase in use of carbapenems, the greatest concern is the development of carbapenem resistance or reduced susceptibility (defined as an MIC of ETP ≥ 0.12 µg/ml). Carbapenem resistance can arise through the production of carbapenemases which include the *Klebsiella pneumoniae* carbapenemases (KPCs), as well as ESBL and/or AmpC production coupled with porin modification (3). Genes encoding KPCs are carried on plasmids and have the ability to hydrolyze virtually all β-lactam antibiotics including carbapenems (3, 4). Plasmids may also carry other resistant determinants such as resistance to aminoglycosides, fluoroquinolones, tetracyclines, and trimethoprim sulfamethoxazole conferring a MDR profile. For this reason, treatment can be very problematic and limited to only few agents such as tigecycline and colistin (1, 5). The purpose of this study was to determine the DOR susceptibilities of CRS-EC, ESBL-EC and AmpC-EC verses comparator antimicrobial agents.

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 November 2009, participating Canadian hospitals 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. All isolates were identified at the originating centre using routine procedures performed at each site. A total of 7881, 5282, and 5375 isolates were collected for 2007, 2008, and 2009, respectively. Of these, 3930 were *E. coli* (EC).

Susceptibility Testing: 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. 2010 CLSI breakpoints were used for interpretation excluding the ones mentioned following. 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). Health Canada interpretation breakpoints were used for ceftobiprole (S: ≤1 µg/ml, I: 2 µg/ml, R: ≥4 µg/ml). Any EC 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 with a cefoxitin MIC ≥32 µg/ml was identified as a putative AmpC-producer. Any EC with an ertapenem MIC ≥0.12 µg/ml was defined as carbapenem reduced susceptible.

Characterization of ESBLs and AmpCs: Genotypic characterization of ESBLs was performed by PCR and sequencing of bla_{SHV}, bla_{TEM}, bla_{CTX-M}, and bla_{OXA} genes as previously described (6). All isolates with an MIC ≥ 32 µg/ml were screened for genes encoding EBC (ACT-1/MIR-1-related genes), DHA (DHA-related genes), FOX (FOX-related genes) and CIT (CMY-2-related genes) phylogenetic groups of AmpC acquired enzymes using a previously described multiplex PCR (7). A BLAST search of the DNA sequence was conducted to determine specific genotypes.

PCR for the amplification of the ampC promoter region was preformed as previously described on plasmidic AmpC negative isolates (8).

Characterization of CRS-EC: Phenotypic characterization of CRS-EC 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 were assessed by pulsed-field gel electrophoresis (PFGE) following digestion with *Xba*I as previously described (9).

Outer Membrane Porin (OMP) Analysis of CRS-EC: Cells grown in nutrient broth overnight at 37°C were sonicated and OMPs were analyzed using SDS-PAGE and sequencing of OmpF/C of EC (10, 11).

RESULTS

Table 1. Antimicrobial profiles among total-EC, AmpC-EC, ESBL-EC, and CRS-EC from CANWARD 2007-2009

Drug	Total EC (n=3930) MIC 50/90 µg/ml		% S | I | R		
	50	90	S	I	R
Cefazolin	2	32	32.8	33.8	33.4
Ceftazidime[a]	≤0.5	1	95.6	0.5	4
Ceftriaxone	≤1	≤1	91.1	0.5	8.5
Cefepime	≤1	≤1	96.6	1.9	1.5
Ceftobiprole	≤1	≤1	93	0.4	6.6
Cefoxitin[b]	4	8	91.8	3.6	4.6
Doripenem	≤0.12	≤0.12	100	0	0
Ertapenem[b]	≤0.06	≤0.06	99.6	0.2	0.2
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.[b]	4	8	94.3	4.7	1
Pipercillin/Tazo.	2	4	97.6	1.3	1.1
Ciprofloxacin	≤0.06	>16	76.7	0.3	23
Levofloxacin	≤0.06	16	77	0.7	22.3
Trimethoprim/Sulf.	≤0.12	>8	72.9	N/A	27.2
Tigecycline	0.5	1	99.9	0.1	0
Amikacin	≤2	4	99.5	0.3	0.2
Gentamicin	≤0.5	8	89.7	0.4	10
Colistin[c]	0.5	1	98.4	N/A	0.6

Drug	AmpC-EC (n=70) MIC 50/90 µg/ml		% S | I | R		
	50	90	S	I	R
Cefazolin	>128	>128	1.4	0	98.6
Ceftazidime[c]	2	>32	54.5	1.5	43.9
Ceftriaxone	4	64	45.7	1.4	52.9
Cefepime	0.25	1	97.1	1.4	1.4
Ceftobiprole	0.12	1	92.9	2.9	4.3
Cefoxitin	>32	>32	0	0	100
Doripenem	≤0.12	≤0.12	100	0	0
Ertapenem	≤0.06	0.25	94.3	2.9	2.9
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.	16	32	44.3	30	25.7
Pipercillin/Tazo.	4	32	88.6	8.6	2.9
Ciprofloxacin	2	>16	50	1.4	48.6
Levofloxacin	4	>32	50	2.9	47.1
Trimethoprim/Sulf.	0.5	>8	63.4	N/A	36.6
Tigecycline	0.5	1	100	0	0
Amikacin	2	4	98.6	0	1.4
Gentamicin	1	32	80	0	20
Colistin	0.5	0.5	100	N/A	0

Drug	ESBL-EC (n=155) MIC 50/90 µg/ml		% S | I | R		
	50	90	S	I	R
Cefazolin	>128	>128	0	0	100
Ceftazidime[d]	16	>32	37.9	8.7	53.4
Ceftriaxone	>64	>64	1.9	2.6	95.5
Cefepime	16	>32	49	27.7	23.2
Ceftobiprole	32	>32	3.2	4.5	92.3
Cefoxitin	8	32	81.3	7.7	11
Doripenem	≤0.12	≤0.12	100	0	0
Ertapenem	≤0.06	0.25	94.8	3.2	1.9
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.	8	16	74.2	23.9	1.9
Pipercillin/Tazo.	4	16	91.6	5.8	1.9
Ciprofloxacin	>16	>16	12.9	0.6	86.5
Levofloxacin	16	32	12.9	1.3	85.8
Trimethoprim/Sulf.	>8	>8	26.5	N/A	73.5
Tigecycline	0.5	1	99.4	0.6	0
Amikacin	4	16	95.5	3.9	0.6
Gentamicin	16	>32	49	0.6	50.3
Colistin	0.5	1	98.7	N/A	1.3

Drug	CRS-EC (n=46) MIC 50/90 µg/ml		% S | I | R		
	50	90	S	I	R
Cefazolin	>128	>128	0	0	100
Ceftazidime	>32	>32	19.6	0	80.4
Ceftriaxone	>64	>64	10.9	0	89.1
Cefepime	16	>32	47.8	6.5	45.7
Ceftobiprole	16	>32	39.1	4.3	56.5
Cefoxitin	32	>32	19.6	15.6	65.2
Doripenem	≤0.12	≤0.12	100	0	0
Ertapenem	0.12	1	73.9	15.2	10.9
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.	16	32	43.5	34.8	21.7
Pipercillin/Tazo.	8	64	67.4	26.1	6.5
Ciprofloxacin	>16	>16	23.9	0	76.1
Levofloxacin	16	>32	23.9	0	76.1
Trimethoprim/Sulf.	0.5	>8	56.5	N/A	43.5
Tigecycline	0.5	1	100	0	0
Amikacin	4	8	95.7	2.2	2.2
Gentamicin	2	>32	58.7	0	41.3
Colistin	0.5	1	97.8	N/A	2.2

***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)**

a: n=2229; b: n=2863; c: n=67; d:n=103

Figure 1. Genetic relationship among CRS-EC from CANWARD 2007-2009

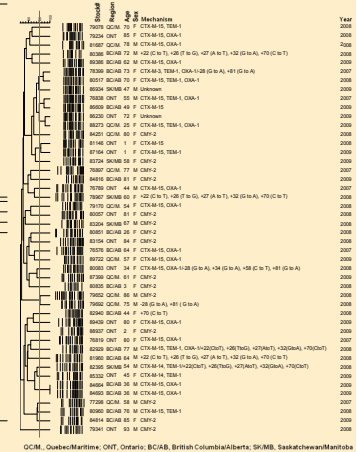

Table 2. EC isolates from CANWARD 2007-2009

E. coli isolates submitted	# of *E. coli* isolates 2007/2008/2009	% of isolates
Overall within objectives	1560/1132/1097	19.8/21.4/20.4% of all isolates
With ETP results	524/1132/1097	33.6/100/100% of all EC isolates
With ETP MIC ≥ 0.12 µg/ml (CRS)	7/23/16	1.3/2.0/1.5% of EC w/ ETP results
ESBL producers	53/55/47	3.4/4.9/4.3% of all isolates
AmpC producers	4/36/30	0.3/3.2/2.7% of all isolates

Table 3. Percentage of ESBL and AmpC among CRS-EC (CANWARD 2007-2009)

	% ESBL	% AmpC	% non-ESBL/non-AmpC
CRS-EC (n=46)	56.5	39.1	4.3

CRS-EC Porin Sequence Data (n=46)

OmpF: 10/46 (21.7%) isolates were shown to have mutations leading to premature termination. 22/46 (47.8%) had a DTAR to GIPE amino acid change within L4. 18/46 (39.1%) had a NKFNT deletion within L6 with these same isolates also showing amino acid changes within L5.

OmpC: 2/46 (4.3%) isolates were shown to have mutations leading to premature termination. 11/46 (23.9%) had a NGYGERY amino acid insertion within L5. 12/46 (26.1%) had a VING amino acid insertion with L7.

CONCLUSIONS

1. CRS-EC is emerging in Canada. However , all isolates were KPC negative by PCR. Many CRS-EC were also ESBL and AmpC-producers. PFGE demonstrated polyclonal banding patterns suggesting that clonal spread is not occurring.
2. Doripenem displayed potent *in vitro* activity against CRS-, ESBL-, and AmpC-EC and showed comparable activity to meropenem.
3. Porin profiles for all CRS isolates showed amino acid changes from wild type. 17.4% of 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 increased MIC to the carbapenems.

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