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

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REVISED ABSTRACT

Background: DOR, a recently approved carbapenem, has been shown to have intrinsic stability to most β-lactamases. The purpose of this study was to determine the DOR susceptibilities of CRS-EC, AmpC-EC and ESBL-EC versus comparator antimicrobial agents.

Methods: From January 2007 through December 2008, 12 sentinel hospitals (10 centers – 2008) 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. In 2007 and 2008, a total of 1560 and 1132 EC, respectively, were collected. Susceptibility testing was performed using CLSI broth microdilution methods. PCR and sequencing of β-lactamase resistant genes was used to determine AmpC and ESBL genotypes. CRS (defined as ETP MIC ≥0.12 µg/ml) isolates were tested for KPC by PCR.

Results:

The activity of DOR and comparators are:

Drug	MIC $_{50}$/MIC $_{90}$ (µg/ml) Total EC (n=2692)	AmpC-EC (n=76)	ESBL-EC (n=108)	CRS-EC (n=30)
A/C[a]	4/8[c]	8/16	8/16	8/32
Cefepime	≤1/≤1	≤0.25/16	16/>32	16/>32
Ceftazidime	≤0.5/1[d]	2/>32[e]	16/>32[f]	>32/>32
Ceftobiprole	≤1/≤1	0.12/32	32/>32	16/>32
Ceftriaxone	≤1/2	≤0.25/>64	>64/>64	>64/>64
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	0.12/0.5
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=1656; [d]: Results reflect n=1132; [e]: Results reflect n=63; [f]: Results reflect n=55

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.

INTRODUCTION

The incidence of β-lactam resistant *E. coli* (EC) has become an increasing problem in the hospital. With EC remaining the leading pathogen causing infections in Canada (http://www.can-r.ca/), close monitoring while it evolves with the possibility of developing antimicrobial resistance is imperative. Resistance to β-lactams can arise through many different mechanisms including the production of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases. Doripenem, a recently approved carbapenem, has been shown to have intrinsic stability to most β-lactamases. Its antimicrobial spectrum most closely resembles meropenem (MER) and imipenem (IMP) then ertapenem (ETP) (1). Like other β-lactams, doripenem interacts with penicillin-binding proteins (PBPs) to form stable acyl-enzymes (PBP2 in EC). Inactivation of PBPs causes the bacterial cell wall to weaken, causing it to rupture due to osmotic pressure forces. DOR, along with other carbapenems (MER, IMP, ETP), represents the first line of defense against Enterobacteriaceae producing AmpC or extended-spectrum β-lactamases (ESBLs).

Of more recent concern is EC with resistance to carbapenems. Carbapenemases have a very broad spectrum of activity (2). These enzymes protect the bacteria from the lethal action of β-lactam antibiotics, such as penicillins, cephalosporins and carbapenems by hydrolyzing the β-lactam ring, rendering the antibiotic inactive. Carbapenemases are divided into 4 classes (A, B, C, and D) based on amino acid sequence. Class A, C, and D enzymes have a serine in the active site whereas class B (metalloenzyme) enzymes require zinc in their active site.

Carbapenem resistance can arise through the production of acquired carbapenemases. Resistance can also arise when bacterial strains express carbapenemases or AmpC/ESBL enzymes in combination with porin mutation (3). AmpC/ESBL enzymes with porin mutation in absence of a carbapenemase can also result in a carbapenem resistant phenotype.

The purpose of this study was to determine the doripenem susceptibilities of CRS-EC, AmpC-EC and ESBL-EC versus comparator antimicrobial agents.

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MATERIALS AND 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. 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). 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 *E. coli* 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-S19. Vol.29 No.3.). Any *E. coli* with a cefoxitin MIC ≥ 32 µg/ml was identified as a putative AmpC-producer. Any *E. coli* with an ertapenem MIC ≥ 0.12 µg/ml was defined as carbapenem reduced susceptible.

Characterization of…:

ESBLs: 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 (4).

AmpCs: 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 (5).

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 (6).

CRS-EC: Phenotypic characterization of CRS-EC was performed using modified hodge test (MHT). Genotypic characterization of CRS-EC 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 XbaI as previously described (7, 8).

CONCLUSIONS

1. The prevalence of CRS, ESBL, and AmpC *E. coli* in 2007 and 2008 were 1.3% and 2.0%, 3.4% and 4.9% and 0.8% and 5.6%, respectively.
2. CRS isolates were more commonly isolated from blood and urine specimens collected from patients greater than 65 years of age on medical wards.
3. Of the CRS isolates, 56.7% and 63.3% were ESBL and AmpC producers, respectively.
4. Doripenem displayed potent in vitro activity against CRS-, AmpC- and ESBL-EC and showed comparable activity to meropenem.
5. The carbapenems (DOR, MER, ETP) sustained their level of activity against ESBL-, AmpC-producing and CRS-EC.

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RESULTS

Table 1: *E. coli* isolates from CANWARD 2007 and 2008

***E. coli* isolates submitted**	**# of *E. coli* isolates 2007/2008**	**% of isolates**
Overall within objectives	1560/1132	19.8/21.4% of all isolates
With ETP results	524/1132	33.6/100% of all EC isolates
With ETP MIC ≥ 0.12 µg/ml (CRS)	7/23	1.3/2.0% of EC w/ ETP results
ESBL producers	53/55	3.4/4.9% of all isolates
AmpC producers	13/63	0.8/5.6% of all isolates

Table 2: Demographics, hospital ward and specimen source types from patients with AmpC-EC, ESBL-EC and CRS-EC infections in Canadian hospitals

Parameter	Value	Number (%) of patients AmpC-EC n=76	ESBL-EC n=108	CRS-EC n=30
Sex	Male	33 (43.4)	52 (48.1)	13 (43.3)
	Female	43 (56.6)	56 (51.9)	17 (56.7)
Age	≤ 17	6 (7.9)	3 (2.8)	2 (6.7)
	18 - 65	30 (39.5)	46 (42.6)	12 (40.0)
	≥ 66	40 (52.6)	59 (54.6)	16 (53.3)
Patient Region*	BC/AB	23 (30.3)	30 (27.8)	10 (33.3)
	SK/MB	13 (17.1)	16 (14.8)	3 (10.0)
	ONT	19 (25.0)	46 (42.6)	9 (30.0)
	QC/Mari	21 (27.6)	16 (14.8)	8 (26.7)
Hospital Ward	Medical Wards	29 (38.2)	48 (44.4)	13 (43.3)
	Surgical Wards	5 (6.6)	11 (10.2)	1 (3.3)
	Intensive Care Units	9 (11.8)	9 (8.3)	8 (26.7)
	Emergency Rooms	23 (30.3)	28 (25.9)	5 (16.7)
	Outpatient Clinics	10 (13.2)	12 (11.1)	3 (10.0)
Specimen Source	Urine	30 (39.5)	39 (36.1)	9 (30.0)
	Blood	36 (47.4)	62 (57.4)	16 (53.3)
	Wound	7 (9.2)	2 (1.9)	2 (6.7)
	Respiratory	3 (3.9)	5 (4.6)	3 (10.0)

*: BC/AB, British Columbia/Alberta; SK/MB, Saskatchewan/Manitoba; ONT, Ontario; QC/Mari, Quebec/Maritimes

Table 3: Activity of doripenem vs comparators (Total *E. coli*)

	Total EC (n=2692)				
	MIC 50/90 µg/ml		% S | I | R		
Drug	**50**	**90**	**S**	**I**	**R**
Cefazolin	2	64	84.4	3.1	12.5
Ceftazidime[a]	≤0.5	1	95.9	0.6	3.5
Ceftriaxone	≤1	2	91.4	1.5	7.1
Cefepime	≤1	≤1	96	2.2	1.8
Ceftobiprole	≤1	≤1	---	---	---
Cefoxitin[b]	4	8	91.3	3.7	5
Doripenem	≤0.12	≤0.12	---	---	---
Ertapenem[b]	≤0.12	≤0.12	100	0	0
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.[b]	4	8	95.4	3.9	0.8
Pipercillin/Tazo.	2	4	97.3	1.4	1.3
Ciprofloxacin	≤0.06	>16	76.2	0.3	23.5
Levofloxacin	≤0.06	16	76.5	0.7	22.8
Moxifloxacin	≤0.06	>16	---	---	---
Trimethoprim/Sulf.	≤0.12	>8	73	---	27
Tigecycline	0.5	1	99.8	0.2	0
Amikacin	≤2	4	99.5	0.3	0.2
Gentamicin	≤0.5	16	89.3	0.4	10.3
Colistin[b]	0.5	1	99.4	---	0.6
Nitrofurantoin[b]	16	32	96.5	2.4	1.1

Tables 3-6: a: n=1132; b: n=1656; c: n=63; d: n=55; ---: no breakpoints available

Table 4: Activity of doripenem vs comparators (AmpC *E. coli*)

	AmpC-EC (n=76)				
	MIC 50/90 µg/ml		% S | I | R		
Drug	**50**	**90**	**S**	**I**	**R**
Cefazolin	64	>128	27.6	9.2	63.2
Ceftazidime[c]	2	>32	65.1	4.8	30.2
Ceftriaxone	≤1	>64	67.1	14.5	18.4
Cefepime	≤1	16	89.5	1.3	9.2
Ceftobiprole	0.12	32	---	---	---
Cefoxitin	>32	>32	0	0	100
Doripenem	≤0.12	≤0.12	---	---	---
Ertapenem	≤0.12	0.25	100	0	0
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.	8	16	77.6	15.8	6.6
Pipercillin/Tazo.	4	32	85.5	10.5	3.9
Ciprofloxacin	16	>16	44.7	0	55.3
Levofloxacin	8	>32	44.7	0	55.3
Moxifloxacin	16	>16	---	---	---
Trimethoprim/Sulf.	0.5	>8	63.2	---	36.8
Tigecycline	0.5	1	100	0	0
Amikacin	≤2	4	98.7	0	1.3
Gentamicin	1	>32	77.6	0	22.4
Colistin	0.5	1	98.7	---	1.3
Nitrofurantoin	16	64	89.5	7.9	2.6

Table 5: Activity of doripenem vs comparators (ESBL *E. coli*)

	ESBL-EC (n=108)				
	MIC 50/90 µg/ml		% S | I | R		
Drug	**50**	**90**	**S**	**I**	**R**
Cefazolin	>128	>128	0	0	100
Ceftazidime[d]	16	>32	47.3	7.3	45.5
Ceftriaxone	>64	>64	10.2	10.2	79.6
Cefepime	16	>32	46.3	26.9	26.9
Ceftobiprole	32	>32	---	---	---
Cefoxitin	8	32	82.4	6.5	11.1
Doripenem	≤0.12	≤0.12	---	---	---
Ertapenem	≤0.12	0.25	100	0	0
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.	8	16	78.7	19.4	1.9
Pipercillin/Tazo.	4	16	90.7	6.5	1.9
Ciprofloxacin	>16	>16	13.9	0.9	85.2
Levofloxacin	16	32	13.9	0.9	85.2
Moxifloxacin	16	>16	---	---	---
Trimethoprim/Sulf.	>8	>8	28.7	---	71.3
Tigecycline	0.5	1	99.1	0.9	0
Amikacin	4	16	95.4	3.7	0.9
Gentamicin	32	>32	44.4	0.9	54.6
Colistin	0.5	1	99.1	---	0.9
Nitrofurantoin	16	32	94.4	4.6	0.9

Table 6: Activity of doripenem vs comparators (CRS *E. coli*)

	CRS-EC (n=30)				
	MIC 50/90 µg/ml		% S | I | R		
Drug	**50**	**90**	**S**	**I**	**R**
Cefazolin	>128	>128	0	3.3	96.7
Ceftazidime	>32	>32	20	0	80
Ceftriaxone	>64	>64	13.3	16.7	70
Cefepime	16	>32	46.7	6.7	46.7
Ceftobiprole	16	>32	---	---	---
Cefoxitin	32	>32	23.3	13.3	63.3
Doripenem	≤0.12	≤0.12	---	---	---
Ertapenem	≤0.12	0.5	100	0	0
Meropenem	≤0.12	≤0.12	100	0	0
Amoxicillin/Clav.	8	32	60	26.7	13.3
Pipercillin/Tazo.	8	64	70	20	10
Ciprofloxacin	>16	>16	26.7	0	73.3
Levofloxacin	16	32	26.7	0	73.3
Moxifloxacin	16	>16	---	---	---
Trimethoprim/Sulf.	2	>8	50	---	50
Tigecycline	0.5	1	100	0	0
Amikacin	≤2	8	96.7	0	3.3
Gentamicin	2	>32	53.3	0	46.7
Colistin	0.5	1	96.7	---	3.3
Nitrofurantoin	16	32	93.3	6.7	0

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