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Pharmacodynamic Activity of Ertapenem vs. Multi-Drug Resistant (MDR) Genotypically Characterized Extended Spectrum ß-lactamase (ESBL) or KPC or NDM producing *Escherichia coli* With Reduced Susceptibility or Resistance to Ertapenem Using an *In vitro* Model

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ABSTRACT

Background: This study assessed the pharmacodynamic activity of ertapenem against multi-drug resistant (MDR) genotypically characterized ESBL/KPC/NDM producing *E. coli* with reduced susceptibility (ertapenem MICs 0.12-0.5 mg/L), intermediate susceptibility (MIC 1.0 mg/L) or resistance to ertapenem (MIC ≥2 mg/L) using an *in vitro* model.

Methods: Fifteen ESBL or carbapenamase producing *E. coli* with CTX-M, KPC or NDM genotypes were studied. All fifteen strains were MDR (defined as resistance to 3rd generation cephalosporins and ≥ 2 other unrelated antimicrobial classes). The *in vitro* pharmacodynamic model was inoculated with ~1x10^6 cfu/mL and ertapenem was dosed once daily at 0 and 24 h to simulate *f* (free) Cmax and t ½ obtained after a standard 1 gram intravenous once daily dose in healthy volunteers (*f*Cmax 15 mg/L, t ½ 4h). Sampling was performed over 48h to assess viable growth and resistance selection.

Results: Ertapenem $T_{>MIC}$ ≥ 75.4% (ertapenem MICs ≤0.5 mg/L) resulted in bactericidal (≥3 log_{10} killing) activity at 6, 12, 24 and 48 h against all strains. Ertapenem $T_{>MIC}$ of 61% (ertapenem MICs 1.0 mg/L) resulted in bactericidal (≥3 log_{10} killing) activity at 6, 12 in all four strains but regrowth at 24 and 48 hours occurred in 2 strains. Ertapenem $T_{>MIC}$ 13-43% (ertapenem MICs 2-8 6mg/L) resulted in bactericidal (≥3 log_{10} killing) activity at 6 hours but regrowth (with MIC increases) occurred at 12, 24 and 48 h against all strains. No inhibition of an NDM strain ertapenem $T_{>MIC}$ 0% (ertapenem MIC 256 mg/L) occurred at any time point.

Conclusions: Ertapenem was rapidly bactericidal against MDR ESBL producing *E. coli* (ertapenem MICs ≤0.5 mg/L) when simulating free drug after 1g intravenous once daily dosing. Ertapenem is bactericidal versus strains with MICs 1.0 mg/L, but regrowth may occur. For strains with ertapenem MICs 2-8 mg/L, early bactericidal activity is followed by regrowth at all timepoints.

INTRODUCTION

The emergence and spread of extended-spectrum ß-lactamase (ESBL) producing *E. coli* in the community, extended-care facilities and hospital settings has been well documented.[1-3] ESBL producing *E. coli* are frequently multi-drug resistant-MDR (defined as resistant to 3rd generation cephalosporins and ≥ 2 other unrelated antimicrobial classes).[1-3] Carbapenems such as ertapenem, doripenem, imipenem/cilastatin and meropenem are recognized as the drugs of choice for seriously ill patients with ESBL *E. coli* infections.[4] However, ESBL producing *E. coli* may demonstrate elevated MICs to carbapenems such as ertapenem and resistance to carbapenems is a concern.[5-9] Little data are available regarding the pharmacodynamic outcomes with ertapenem against MDR ESBL producing *E. coli* with elevated MICs to ertapenem or resistance to ertapenem.

Ertapenem demonstrates broad spectrum antimicrobial activity against many Gram-positive and Gram-negative aerobes and anaerobes and is resistant to nearly all β-lactamases including ESBLs and AmpCs.[4] Extensive protein binding of ertapenem extends the half-life and allows for once daily dosing.[4] Clinical trials have demonstrated that ertapenem has equivalent efficacy and safety compared to ceftriaxone and piperacillin/tazobactam against a variety of community acquired infections.[4]

PURPOSE

The purpose of this study was to assess the pharmacodynamic activity of ertapenem against MDR genotypically characterized ESBL, KPC or NDM producing *E. coli* with elevated MICs to ertapenem or resistance to ertapenem using an *in vitro* pharmacodynamic model.

MATERIALS & METHODS

Bacterial strains and culture conditions

The *E. coli* isolates were obtained from the CANWARD study (www.can-r.ca), a national, ongoing Health Canada endorsed surveillance study assessing antimicrobial resistance in Canadian hospitals.[2,3] ESBL *E. coli* were phenotypically and genotypically characterized as previously described.[3] NDM strain was a generous gift from Dr. Johann Pitout. All fifteen ESBL, KPC or NDM *E. coli* strains were chosen because they had elevated MICs to ertapenem ranging from 0.12-256 mg/L. The current CLSI breakpoints for ertapenem and *E. coli* are ≤ 0.5 mg/L susceptible, 1.0 mg/L intermediate and ≥ 2 mg/L resistant (Table 1).[3] We selected one wild-type strain (ertapenem MIC 0.03 mg/L), five strains with reduced susceptibility to ertapenem (MIC 0.12-0.5 mg/L), four strains with intermediate susceptibility to ertapenem (MIC 1.0 mg/L), five strains with low level ertapenem resistance (MIC 2-8 mg/L) and one high-level ertapenem resistant strain (MIC 256 mg/L).

For the pharmacodynamic studies, logarithmic phase cultures at 0.5 McFarland (1 x 10^8 cfu/mL) in cation-supplemented Mueller Hinton broth were prepared as previously described.[5] Viable bacterial counts consistently yielded a starting inoculum of approximately 1x10^6 cfu/mL. A growth control was included in every experiment. Growth controls peaked at ~ 1 x 10^9 cfu/mL and were maintained over the 48 h experiment.

Susceptibility testing

MICs were determined by the CLSI-approved broth microdilution method. All MICs were performed in triplicate on separate days.[3,5]

Pharmacokinetics of ertapenem in the in vitro pharmacodynamic model

Experiments were performed simulating peak serum concentrations (C_{max}) and AUC_{24} of ertapenem, achieved in human serum after standard intravenous doses (ertapenem 1gram once daily) (Table 1).[5] Protein free-*f* (unbound) serum concentrations were simulated using known protein binding fractions (ertapenem ~90%).[4,5] Ertapenem clearance was simulated using a reported serum half-life of 4 h.[5] The pharmacokinetics of ertapenem were evaluated by dosing using standard doses in the central compartment and sampling from this compartment at 0, 1, 2, 4, 6, 12, 18, 24, 36 and 48 h. Ertapenem concentrations were determined in quadruplicate using *Bacillus subtilis* ATCC 6633 as the test organism with a lower limit of quantification of 0.25 mg/L as previously described.[5] The correlation coefficient of this assay was 0.85. The intra-day and inter-day coefficients of variation were 3.0-5.8% and 2.6-5.0%, respectively. The *f*AUC_{24} (mg.h/L) for ertapenem was calculated using the trapezoidal rule.[5] The *f*AUC_{24}/MIC was calculated for ertapenem against the specific *E. coli* studied.

The *in vitro* pharmacodynamic model used in this study has been previously described.[5]

REFERENCES

1. Lynch JP, Clark NM and Zhanel GG. Evolution of antimicrobial resistance among Enterobacteriaceae (focus on ESBLs and carbapenemases). *Expert Opin Pharmacother* 2013;14(2):199-201.
2. Zhanel GG, Adam H, Baxter M *et al.* Antimicrobial susceptibility of 22,746 pathogens from Canadian hospitals: Results of the CANWARD 2007-2011 study. *J Antimicrob Chemother* 2013;68(Suppl 1):7-22.
3. Denisuik AJ, Lagacé-Wiens P, Pitout JD *et al.* Molecular epidemiology of Extended-spectrum β-lactamase-, AmpC β-lactamase-, and Carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Canadian hospitals over a 5 Year Period: CANWARD 2007-2011. *J Antimicrob Chemother* 2013;68(Suppl 1):57-65.
4. Zhanel GG, Johansson C, Embil J *et al.* Ertapenem: Review of a new carbapenem. *Expert Rev Antiinfect Ther* 2005;3:23-39.
5. Zhanel GG, Baudry P, Vashisht V *et al.* Pharmacodynamic activity of ertapenem versus multi-drug resistant (MDR) genotypically characterized extended spectrum β-lactamase (ESBL) producing *Escherichia coli* using an in-vitro model. *J Antimicrob Chemother* 2008;61:643-6.
6. Marimuthu K, Ng T, Teng C *et al.* Risk factors and treatment outcome of ertapenem non-susceptible enterobacteriaceae bacteremia. *J Infection* 2013;66:294-6.
7. Hoban DJ, Lascols C, Nicolle LE *et al.* Antimicrobial susceptibility of enterobacteriaceae including molecular characterization of extended-spectrum beta-lactamases producing species, in urinary tract isolates from hospitalized patients in North America and Europe: Reults from the SMART study 2009-2010. *Diagn Microbiol Infect Dis* 2012;74:62-7.
8. Lee NY, Lee CC, Huang WH A *et al.* Carbapenem therapy for bacteremia due to extended spectrum β-lactamase producing *Escherichia coli* or *Klebsiella pneumoniae*: Implications of ertapenem susceptibility. *Antimicrob Agents Chemother* 2012;56:2888-93.
9. Teo J, Cai Y, Tang S *et al.* Risk factors molecular epidemiology and outcomes of ertapenem-resistant carbapenem susceptible enterobacteriaceae: A case control study. *PLos ONE* 2012;7:108.

RESULTS

Table 1. Ertapenem pharmacodynamic parameters simulated

Strain	Genotype	Erta MIC (mg/L)	$T_{>MIC}$ h [%]	*f*Cmax /MIC	*f*AUC_{24} /MIC
79768	wild type	0.03	24 [100]	457	2200
85332	CTX-M-14,TEM-1	0.12	24 [100]	115	550
80083	CTX-M-15,OXA-1	0.25	22.1 [92]	57.5	275
87164	CTX-M-15,TEM-1	0.5	18.1 [75.4]	28.8	138
88273	CTX-M-15,TEM-1, OXA-1	0.5	18.1 [75.4]	28.8	138
90087	CTX-M-15,OXA-1	0.5	18.1 [75.4]	28.8	138
80960	CTX-M-15,TEM-1	1.0	14.7 [61]	14.4	69
89439	CTX-M-15,OXA-1	1.0	14.7 [61]	14.4	69
91191	CTX-M-14,TEM-1	1.0	14.7 [61]	14.4	69
92756	CTX-M-14,TEM-1	1.0	14.7 [61]	14.4	69
90789	KPC-3,TEM-1	2.0	10.3 [43]	7.2	34
92969	CTX-M-15,OXA-1	2.0	10.3 [43]	7.2	34
98550	CTX-M-15,OXA-1	2.0	10.3 [43]	7.2	34
N10-1631	CTX-M-15,OXA-1	4.0	6.4 [27]	3.6	17
95882	KPC-3, TEM-1	8.0	3.0 [13]	1.8	9
ECMH01	NDM-1	256	0 [0]	0.06	0.3

Table 2. Ertapenem killing of ESBL *E. coli* simulating free serum concentrations

Strain (Ertapenem MIC mg/L)	$T_{>MIC}$ (%)	Log_{10} killing at 6, 12, 24 and 48 h, respectively[a] 6 h	12 h	24 h	48 h
79768 (0.03)	100	≥4.0	≥4.0	≥4.0	≥4.0 (0.03)*
85332 (0.12)	100	≥4.0	3.5 ± 0.3	3.0 ± 0.5 (0.25)	3.0 ± 0.4 (0.25)
80083 (0.25)	92	≥4.0	≥4.0	≥4.0	≥4.0
87164 (0.5)	75.4	≥4.0	3.5 ± 0.4	3.4 ± 0.4 (0.25)	3.0 ± 0.4 (1.0)
88273 (0.5)	75.4	≥4.0	3.0 ± 0.5	3.0 ± 0.4 (0.5)	3.0 ± 0.5 (0.5)
90087 (0.5)	75.4	≥4.0	≥4.0	≥4.0	≥4.0
80960 (1.0)	61	≥4.0	≥4.0	≥4.0	≥4.0
89439 (1.0)	61	≥4.0	3.2 ± 0.3	2.0 ± 0.7 (2.0)	2.0 ± 0.7 (2.0)
91191 (1.0)	61	≥4.0	3.5 ± 0.6	3.0 ± 0.4 (1.0)	3.0 ± 0.5 (1.0)
92756 (1.0)	61	≥4.0	3.1 ± 0.4	3.0 ± 0.4 (1.0)	3.9 ± 0.5 (1.0)
90789 (2.0)	43	3.5 ± 0.6	1.2 ± 0.6	0 (8)	0 (32)
92969 (2.0)	43	≥4.0	0.7 ± 0.8	0.5 ± 1.0 (4)	0 (4)
98850 (2.0)	43	≥4.0	0.5 ± 0.8	0 (4)	0 (32)
N10-1631 (4.0)	27	≥4.0	1.0 ± 0.7	0 (4)	0 (32)
95882 (8.0)	13	3.0 ± 0.6	0.5 ± 0.4	0 (>32)	0 (>32)
ECMH01 (256)	0	0	0	0 (>32)	0 (>32)

[a] = growth reduction relative to initial inoculum, * MIC performed by Etest (on freshly isolated colonies)

Figure 1. Ertapenem killing of wild-type *E. coli* strain 79768 simulating free T>MIC of 100%

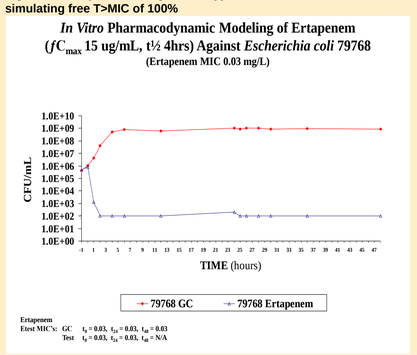


Figure 2. Ertapenem killing of ESBL *E. coli* strain 92756 simulating free T>MIC of 100%

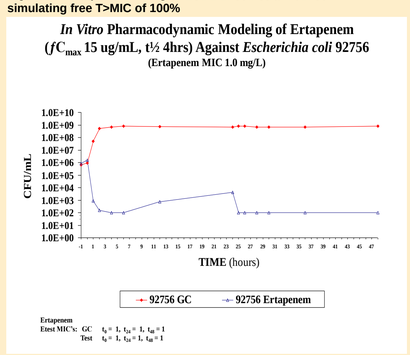


Figure 3. Ertapenem killing of KPC *E. coli* strain 95882 simulating free T>MIC of 100%

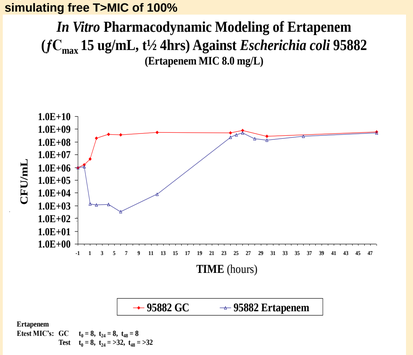
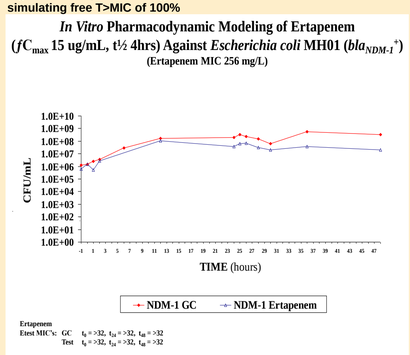


Figure 4. Ertapenem killing of NDM-1 *E. coli* strain ECMH01 simulating free T>MIC of 100%



CONCLUSIONS

- Ertapenem was rapidly bactericidal against MDR ESBL producing *E. coli* (ertapenem MICs ≤0.5 mg/L) when simulating free drug after 1g intravenous once daily dosing.
- Ertapenem was bactericidal versus strains with MICs 1.0 mg/L, but regrowth may occur.
- For strains with ertapenem MICs 2-8 mg/L, early bactericidal activity is followed by regrowth at all timepoints.
- Ertapenem had no effect on an NDM strain (ertapenem MIC 256 mg/L).

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CANWARD data are also displayed at www.can-r.ca, the official website of the Canadian Antimicrobial Resistance Alliance (CARA).

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