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Pharmacodynamic Activity of Ertapenem vs. Multi-Drug Resistant (MDR) Genotypically Characterized Extended Spectrum ß-lactamase (ESBL) Producing *Escherichia coli* at Standard and High Inocula Using an In Vitro Model.

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ABSTRACT

Background: Carbapenems such as ertapenem (Erta) possess in vitro activity against ESBL producing *E. coli.* This study assessed the pharmacodynamic (PD) activity of Erta against molecularly characterized MDR ESBL at standard (std) and high inocula using an in vitro PD model (IVPM).

Methods: 6 ESBL *E.coli* were studied with ertapenem MICs (CLSI 2013) ranging from 0.06 mg/L to 0.25 mg/L. All ESBL strains were CTX-M-15 or CTX-M-14 genotypes and demonstrated a MDR phenotype with concomitant resistance to ciprofloxacin MIC (mg/L) ≥ 4 mg/L, trimethoprim/sulfamethoxazole MIC ≥ 4 mg/L and gentamicin MIC ≥ 16 mg/L. The IVPM was inoculated with a std inocula (1x10^6 CFU/mL) or high inocula (1x10^7 CFU/mL) of *E. coli.* Erta was dosed once daily at 0 and 24 hours to simulate free (*f*) serum C_{max} and $t_{1/2}$ obtained after a standard 1 gram dose in healthy volunteers (*f*C_{max} 14 ug/mL [90% protein binding], $t_{½}$ 4 hrs). Sampling was performed over 48 h to assess viable growth.

Results: Erta MICs at Std inoculum and high inocula were unchanged, while for cefepime and piperacillin/tazobactam MICs increased by ≥ 8 (range 8-512, median 32) fold versus all ESBL strains. Erta PD parameters $T_{>MIC}$ ≥ 75% (ertapenem MIC ≤0.5mg/L) resulted in bacterial killing (log_{10} killing assessed relative to the starting inoculum at 12, 24 and 48 hours) of ≥4.0, ≥4.0 and 3.5, respectively. No difference in rate or extent of Erta killing occurred at std versus high inocula. No regrowth occurred with std or high inocula exposures over the 48 h study period.

Conclusion: Erta MICs at std versus high inocula were unchanged in contrast to cefepime and piperacillin/tazobactam where ESBL *E. coli* MICs increased by ≥ 8 (range 8-512, median 32) fold. Erta 1 gram daily was bactericidal at all timepoints over the 48 hour testing period against MDR ESBL *E. coli* whether assessed at standard (1x10^6 CFU/mL) or high (1x10^7 CFU/mL) inocula.

INTRODUCTION

Extended-spectrum ß-lactamase (ESBL) producing *E. coli* have rapidly spread in the community, extended-care facilities and hospital settings.[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] Little data are available regarding the pharmacodynamic outcomes with ertapenem against MDR ESBL producing *E. coli* with elevated MICs to ertapenem and no data are available regarding the pharmacodynamic activity of ertapenem versus MDR ESBL producing *E. coli* at high inocula.

PURPOSE

The purpose of this study was to assess the pharmacodynamic activity of ertapenem against molecularly characterized MDR ESBL producing *E. coli* at standard and high inocula using an in vitro pharmacodynamic model.

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

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] In the CANWARD study, any *E. coli* with a ceftriaxone MIC ≥ 1 mg/L was identified as a putative ESBL.[3] Putative ESBL phenotypes were confirmed by the disk diffusion method as described by Clinical and Laboratory Standards Institute (CLSI). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were the control strains. Genotypic characterization of ESBLs was performed by PCR and sequencing of bla_{SHV}, bla_{TEM}, bla_{CTX}, bla_{OXA} and bla_{VEB} genes as previously described.[3] A BLAST search of the DNA sequence was conducted to determine the specific ESBL genotype. All putative carbapenemase-producing *E. coli* and *K. pneumoniae* were screened for the presence of bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{IMI}, bla_{NDM}, bla_{GES}, and bla_{OXA-48} by multiplex PCR as described by Denisuik *et al.*[3] 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) and six MDR ESBL producing *E. coli* with ertapenem MICs 0.03-0.25 mg/L.

For 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 (standard inoculum), while a bacterial count of 1x10^7 CFU/mL was the high inoculum. A growth control was included in every experiment. Growth controls peaked at ~ 1-5 x 10^9 CFU/mL and were maintained over the 48 h experiment.

Antibiotic preparations and susceptibility testing: Antibiotic agents were obtained as laboratory-grade powders from their respective manufacturers (Ertapenem, Merck, Montreal, Quebec). Stock solutions were made according to the CLSI M7-A6 method and MICs were determined by the CLSI-approved broth microdilution method. All MICs were performed in triplicate on separate days.[2,3] MIC testing was performed at both the standard inocula and high inocula.

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 1 gram once daily) (Table 1).[4,5] Protein free (unbound) serum concentrations were simulated using known protein binding fractions (ertapenem ~90%).[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 0.87. The intra-day and inter-day coefficients of variation were 2.8-6.0% and 2.3-5.2%, 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.

In vitro pharmacodynamic model/pharmacodynamic experiments: The in vitro pharmacodynamic model used in this study has been previously described.[5] Logarithmic phase cultures were diluted into fresh cation-supplemented Mueller Hinton broth to achieve a final inoculum of approximately 1x10^6 CFU/mL (standard inoculum) and 1x10^7 CFU/mL (high inoculum). Only free (protein unbound) serum concentrations were simulated. Pharmacodynamic experiments were performed in duplicate (on separate days) in ambient air at 37°C. Sampling was performed at 0, 1, 2, 4, 6, 12, 18, 24, 36 and 48 h as previously described.[5] The lowest dilution plated was 0.1 mL of undiluted sample and the lowest level of detection was 200 CFU/mL (20 colonies of 0.1 mL undiluted sample). Antibiotic carryover was minimized by diluting samples withdrawn from the model or by repeated washing and centrifugation. No difference in antibiotic carryover was observed between dilution and washing. Measurement of antibacterial effects was assessed as log_{10} changes in bacterial counts at 6, 12, 24 and 48 h with respect to time 0.

RESULTS

Figure 1. Ertapenem killing of ESBL *E. coli* strain 61567 using standard inocula.

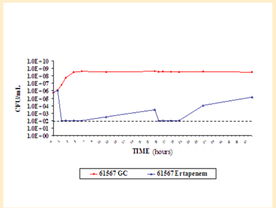

Figure 2. Ertapenem killing of ESBL *E. coli* strain 61567 using high inocula.

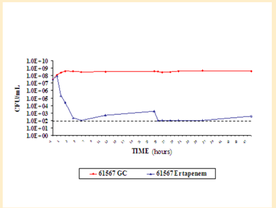

Figure 3. Ertapenem killing of ESBL *E. coli* strain 62188 using standard inocula.

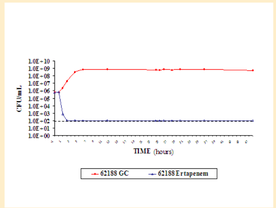

Figure 4. Ertapenem killing of ESBL *E. coli* strain 62188 using high inocula.

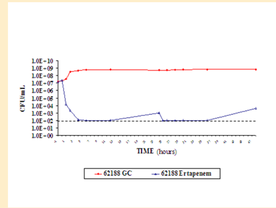

Table 1. MICs (mg/L) of ESBL producing *E. coli* performed at standard inocula.

Strain	Erta	Pip/Tazo	Cefepime	Amox/Clav	Aztreonam
79768	0.03	1	0.25	2	0.12
64197	0.03	32	4	16	16
61567	0.06	128	16	32	64
59096	0.12	2	16	8	64
62188	0.12	2	32	8	>64
63106	0.12	16	>64	16	>64
64771	0.25	32	>64	16	>64

Table 2. MICs (mg/L) of ESBL producing *E. coli* performed at high inocula.

Strain	Erta	Pip/Tazo	Cefepime	Amox/Clav	Aztreonam
79768	0.03	64	2	4	16
64197	0.03	>512	>64	32	>64
61567	0.06	128	>64	>32	>64
59096	0.12	>512	>64	16	>64
62188	0.12	>512	>64	8	>64
63106	0.12	>512	>64	32	>64
64771	0.5	>512	>64	32	>64

Table 3. Ertapenem pharmacodynamic parameters simulated (using MICs obtained at standard inocula).

Strain	Genotype	Erta MIC (mg/L)	$T_{>MIC}$ h [%]	*f*C_{max} /MIC	*f*AUC_{24} /MIC
79768	wild type	0.03	24 [100]	457	2200
64197	CTX-M-15,TEM-1	0.03	24 [100]	457	2200
61567	CTX-M-15,TEM-1	0.06	24 [100]	230	1100
59096	CTX-M-14,TEM-1	0.12	24 [100]	115	550
62188	CTX-M-15,OXA-1	0.12	24 [100]	115	550
63106	CTX-M-15,OXA-1	0.12	24 [100]	115	550
64771	CTX-M-15,TEM-1	0.25	22.1 [92]	57.5	275

Table 4. Ertapenem pharmacodynamic parameters simulated (using MICs obtained at high inocula).

Strain	Genotype	Erta MIC (mg/L)	$T_{>MIC}$ h [%]	*f*C_{max} /MIC	*f*AUC_{24} /MIC
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64197	CTX-M-15,TEM-1	0.03	24 [100]	457	2200
61567	CTX-M-15,TEM-1	0.06	24 [100]	230	1100
59096	CTX-M-14,TEM-1	0.12	24 [100]	115	550
62188	CTX-M-15,OXA-1	0.12	24 [100]	115	550
63106	CTX-M-15,OXA-1	0.12	24 [100]	115	550
64771	CTX-M-15,TEM-1	0.5	18.1 [75.4]	28.8	138

Table 5. Ertapenem killing of ESBL *E. coli* at standard inocula simulating free serum concentrations Log_{10} killing at 6, 12, 24 and 48 h, respectively[a].

Strain (Ertapenem MIC mg/L)	$T_{>MIC}$ (%)	6 h	12 h	24 h	48 h
79768 (0.03)	100	≥4.0	≥4.0	≥4.0	≥4.0
64197 (0.03)	100	≥4.0	≥4.0	≥4.0	≥4.0
61567 (0.06)	100	≥4.0	≥4.0	≥4.0	3.1 ± 0.5 (0.06*)
59096 (0.12)	100	≥4.0	≥4.0	≥4.0	3.0 ± 0.4 (0.12)
62188 (0.12)	100	≥4.0	≥4.0	≥4.0	≥4.0
63106 (0.12)	100	≥4.0	≥4.0	≥4.0	3.0 ± 0.4 (0.12)
64771 (0.25)	92	≥4.0	≥4.0	≥4.0	3.0 ± 0.5 (0.25)

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

Table 6. Ertapenem killing of ESBL *E. coli* at high inocula simulating free serum concentrations Log_{10} killing at 6, 12, 24 and 48 h, respectively[a].

Strain (Ertapenem MIC mg/L)	$T_{>MIC}$ (%)	6 h	12 h	24 h	48 h
79768 (0.03)	100	≥4.0	3.9 ± 0.5	3.9 ± 0.5	3.8 ± 0.5
64197 (0.03)	100	≥4.0	≥4.0	≥4.0	≥4.0
61567 (0.06)	100	≥4.0	≥4.0	≥4.0	3.2 ± 0.5 (0.06*)
59096 (0.12)	100	≥4.0	≥4.0	≥4.0	3.2 ± 0.4 (0.12)
62188 (0.12)	100	≥4.0	≥4.0	≥4.0	3.9 ± 0.6 (0.12)
63106 (0.12)	100	≥4.0	≥4.0	≥4.0	3.1 ± 0.4 (0.12)
64771 (0.5)	75.4	≥4.0	≥4.0	≥4.0	3.4 ± 0.6 (0.5)

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

CONCLUSIONS

1. Ertapenem MICs vs MDR ESBL producing *E. coli* were unchanged at high inocula compared to standard inocula.
2. Ertapenem 1 gram daily was bactericidal at all timepoints over the 48 hour testing period against MDR ESBL *E. coli* at standard (1 x 10^6 CFU/mL) inocula.
3. Ertapenem 1 gram daily was bactericidal at all timepoints over the 48 hour testing period against MDR ESBL *E. coli* when assessed at high (1 x 10^7 CFU/mL) inocula.

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