

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



Dr. G. G. Zhanel Health Sciences Centre MS673 - 820 Sherbrook St. Winnipeg, MB, Canada R3A 1R9 (204) 787-4902 ggzhanel@pcs.mb.ca



G. G. ZHANEL¹, A. DENISUIK¹, S. VASHISHT¹, C. YACHISON¹, N. LAING¹, H.J. ADAM^{1,2} and D.J. HOBAN^{1,2} University of Manitoba¹ and Health Sciences Centre², Winnipeg, Manitoba, Canada

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 carbapenemase 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 1/2 obtained after a standard 1 gram intravenous once daily dose in healthy volunteers (fCmax 15 mg/L, t 1/2 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 log10 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 log10 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 mg/L) resulted in bactericidal (≥3 log10 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 β -lactamase (ESBL) producing E. coli in the community, extended-care facilities and hospital settings has been well documented. ESBL producing E. coli are frequently multi-drug resistant-MDR (defined as resistant to 3rd generation cephalosporins and ≥ 2 other unrelated antimicrobial classes). 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. However, ESBL producing E. coli may demonstrate elevated MICs to carbapenems such as ertapenem and resistance to carbapenems is a concern. 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. Extensive protein binding of ertapenem extends the half-life and allows for once daily dosing. Clinical trials have demonstrated that ertapenem has equivalent efficacy and safety compared to ceftriaxone and piperacillin/tazobactam against a variety of community acquired infections.

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. ESBL E. coli were phenotypically and genotypically characterized as previously described. 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). 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. 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.

Pharmacokinetics of ertapenem in the in vitro pharmacodynamic model

Experiments were performed simulating peak serum concentrations (Cmax) and AUC24 of ertapenem, achieved in human serum after standard intravenous doses (ertapenem 1gram once daily) (Table 1). Protein free-f (unbound) serum concentrations were simulated using known protein binding fractions (ertapenem ~90%). Ertapenem clearance was simulated using a reported serum half-life of 4 h. 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. 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 fAUC24 (mg.h/L) for ertapenem was calculated using the trapezoidal rule. The fAUC24/MIC was calculated for ertapenem against the specific E. coli studied. The in vitro pharmacodynamic model used in this study has been previously described.

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. Denisuk AJ, Lagacé-Wiensi 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, Johannsson C, Embil J et al. Ertapenem: Review of a new carbapenem. Expert Rev Anticancer 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: Results 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;7:1108.

Table 1. Ertapenem pharmacodynamic parameters simulated

Table with 6 columns: Strain, Genotype, Erta MIC (mg/L), T_MIC h [%], fCmax /MIC, fAUC24 /MIC. Rows include 79768 wild type, 85332 CTX-M-14,TEM-1, 80083 CTX-M-15,OXA-1, 87164 CTX-M-15,TEM-1, 88273 CTX-M-15,TEM-1, OXA-1, 90087 CTX-M-15,OXA-1, 80960 CTX-M-15,TEM-1, 89439 CTX-M-15,OXA-1, 91191 CTX-M-14,TEM-1, 92756 CTX-M-14,TEM-1, 90789 KPC-3,TEM-1, 92969 CTX-M-15,OXA-1, 98550 CTX-M-15,OXA-1, N10-1631 CTX-M-15,OXA-1, 95882 KPC-3, TEM-1, ECMH01 NDM-1.

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

Table with 6 columns: Strain (Ertapenem MIC mg/L), Log10 killing at 6, 12, 24 and 48 h, respectively. Rows include 79768 (0.03), 85332 (0.12), 80083 (0.25), 87164 (0.5), 88273 (0.5), 90087 (0.5), 80960 (1.0), 89439 (1.0), 91191 (1.0), 92756 (1.0), 90789 (2.0), 92969 (2.0), 98850 (2.0), N10-1631 (4.0), 95882 (8.0), ECMH01 (256).

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

RESULTS

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

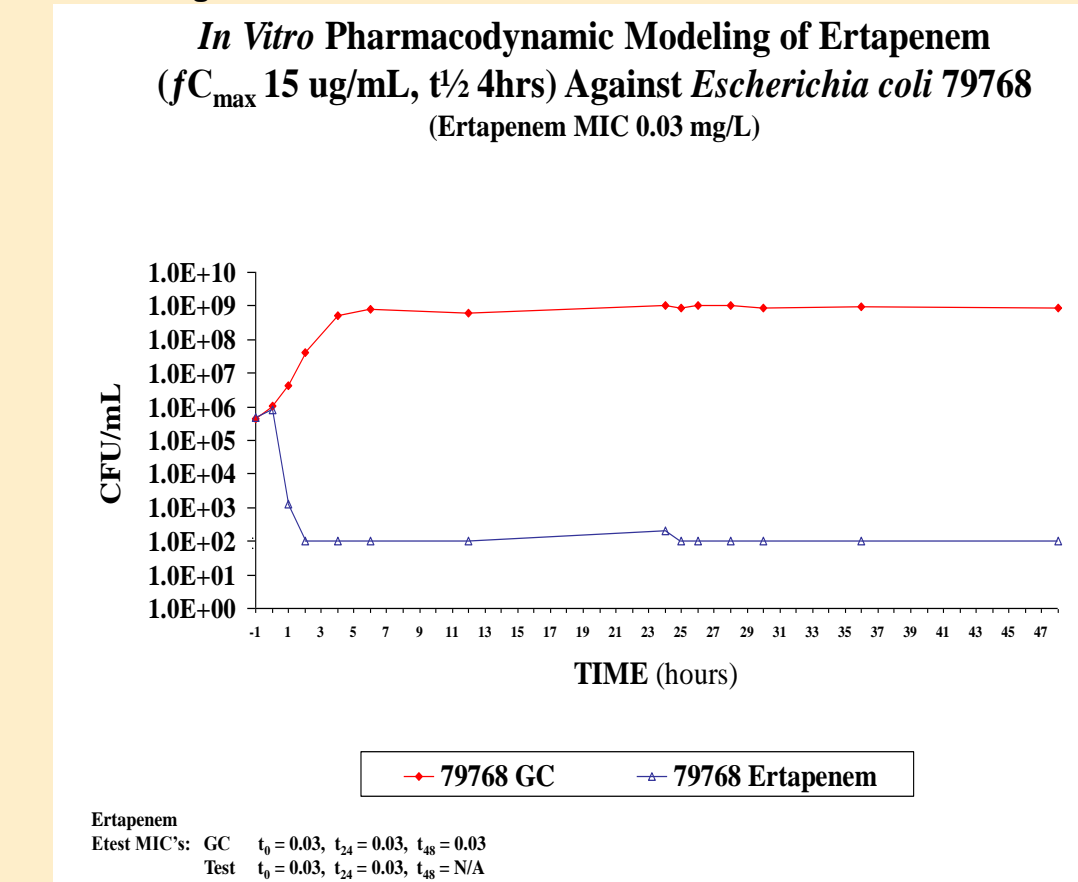


Figure 3. Ertapenem killing of KPC E. coli strain 95882 simulating free T>MIC of 100%

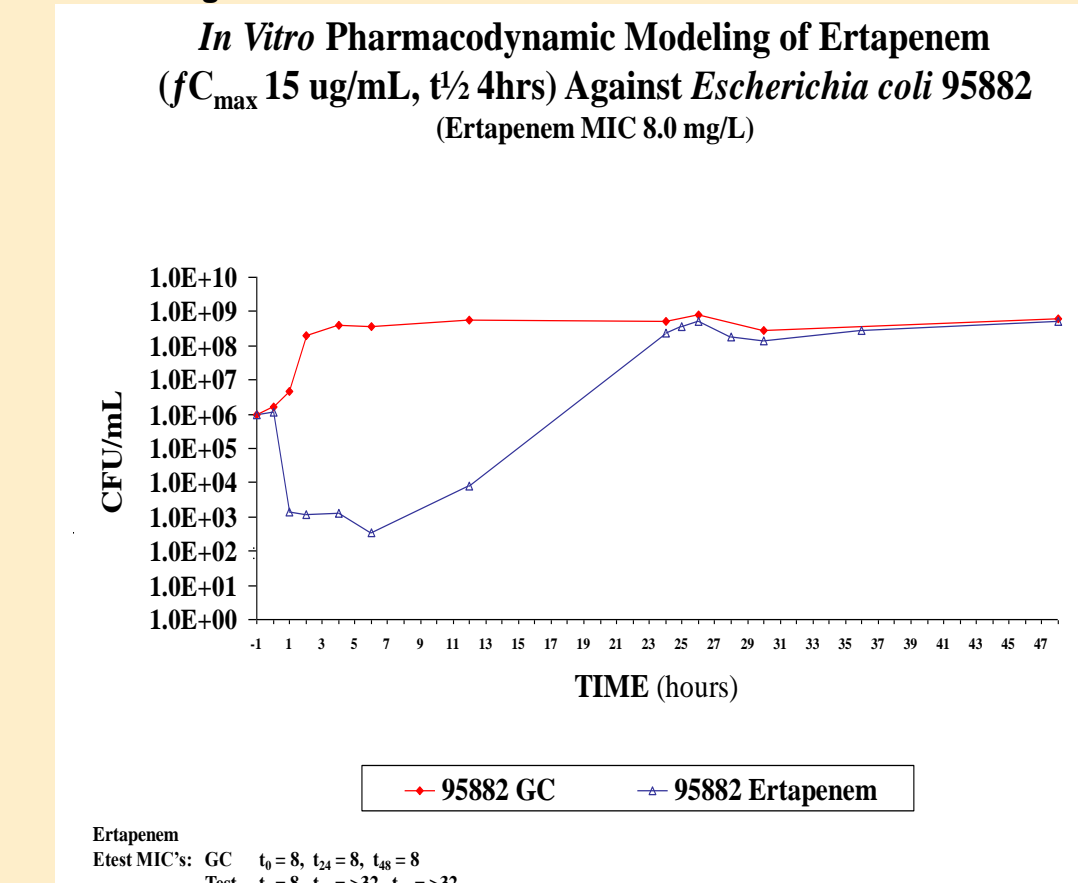


Figure 2. Ertapenem killing of ESBL E. coli strain 92756 simulating free T>MIC of 100%

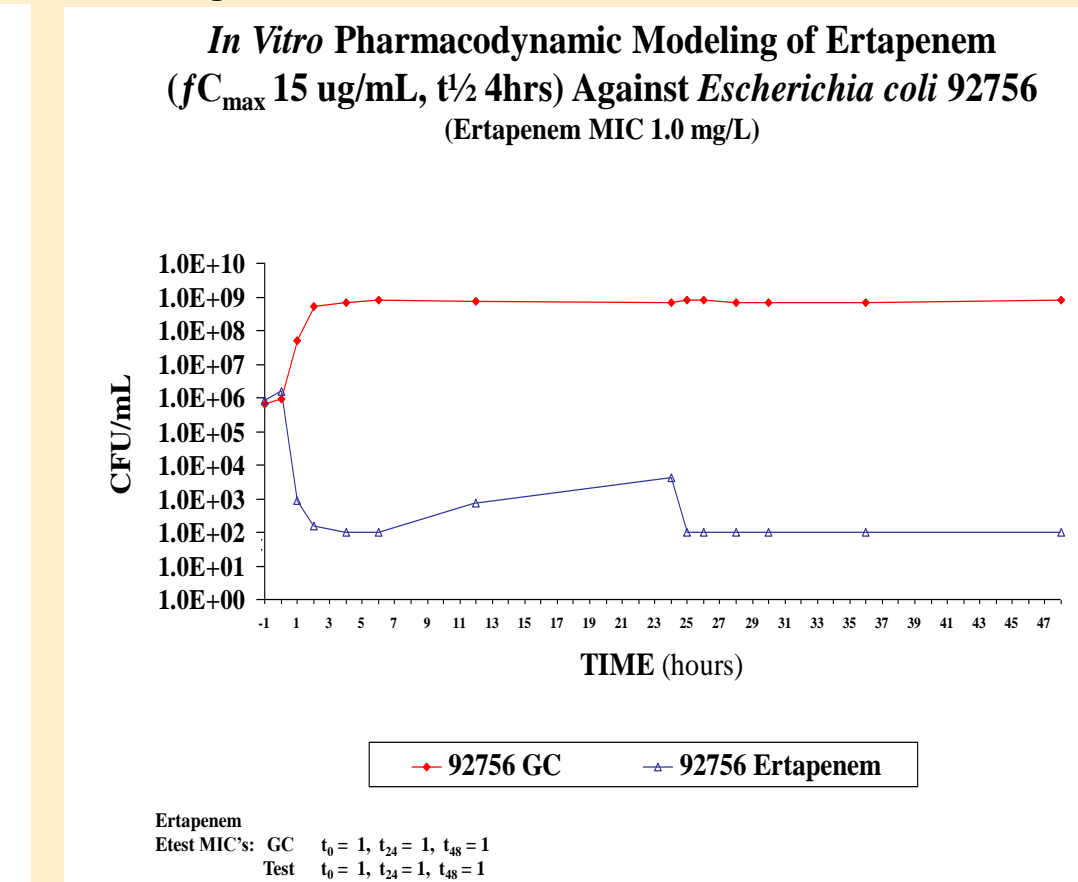
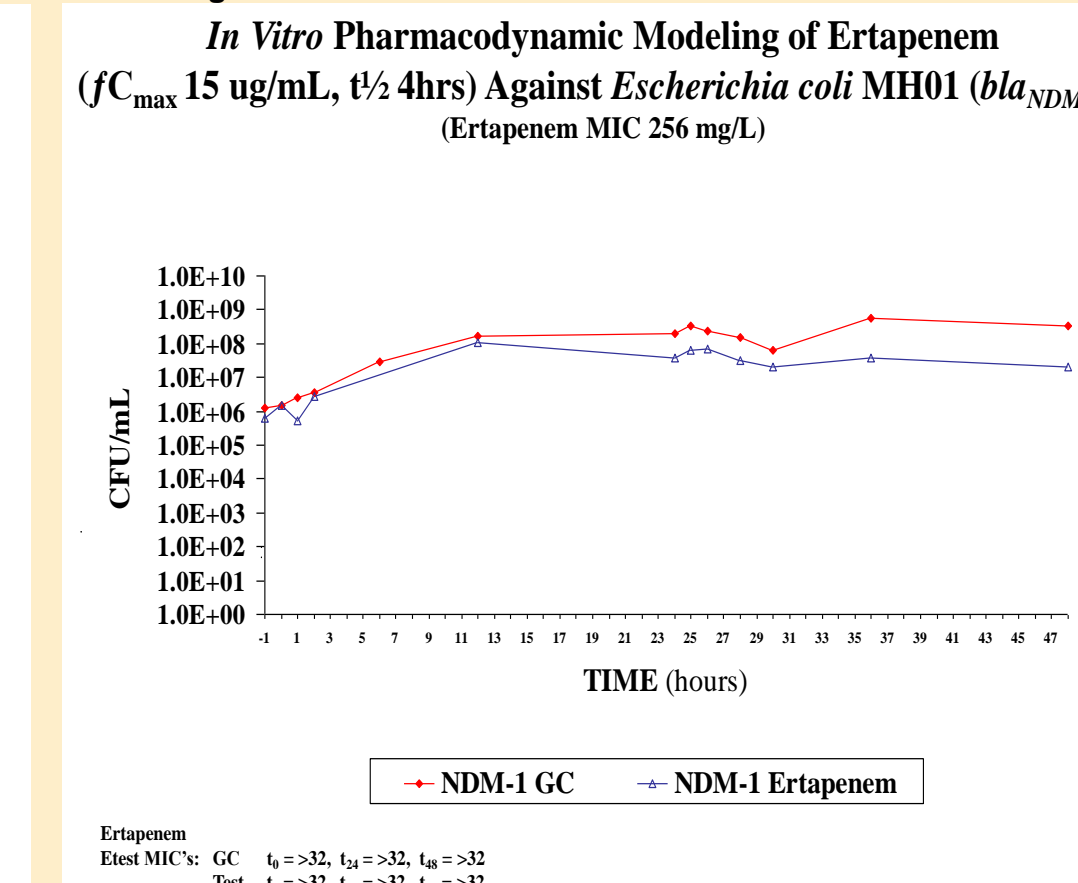


Figure 4. Ertapenem killing of NDM-1 E. coli strain ECMH01 simulating free T>MIC of 100%



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).

ACKNOWLEDGMENTS

The authors would like to thank Barbara Weshnoweski, Ravinder Vashisht and Franil Tailor for technical assistance. CANWARD data are also displayed at www.can-r.ca, the official website of the Canadian Antimicrobial Resistance Alliance (CARA). Funding for this study was provided in part by the University of Manitoba and Merck Canada Inc., Kirkland, QC.