# Serial Passage of Enterobacteriaceae to Explore Development of Carbapenem Resistance

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## Background

- According to the Center for Disease Control and Prevention's 2019 Antibiotic Resistance Threats in the Unites States, carbapenem-resistant Enterobacteriaceae (CRE) is considered an urgent threat[1]
- Ertapenem-resistant Enterobacteriaceae infections are associated with higher mortality rates and poor clinical response rates when compared to ertapenem-susceptible Enterobacteriaceae infections[2]
- Carbapenems are the drugs of choice for more resistant Enterobacteriaceae such as extended spectrum β-lactamases (ESBL)
- Ertapenem (ERT) resistance in Enterobacteriaceae is known to be primarily caused by mechanisms other than carbapenemases, such as expression of β-lactamases including AmpC or an ESBL combined with porin loss[3]
- Carbapenems are stable to most β-lactamases, but this stability varies between agents, and ertapenem appears to be less stable than other carbapenems
- There is a lack of data comparing the risk of resistance or tolerance selection between ertapenem and meropenem (MER)

## Objectives

- The purpose of this study was to explore the potential development of resistance to carbapenems through a novel serial passage method

## Methods

- Non-duplicate Enterobacteriaceae isolates were selected randomly for from patients within the WVU Medicine health system for inclusion
- Isolate suspensions were prepared in 0.45% sterile saline to a turbidity of 0.5-0.63 McFarland using a DensiCHEK Plus meter (Biomerieux, Durham, NC)
- Isolates were plated to confluence on Mueller Hinton agar (BD, BBL), and ERT (10 mcg) and MER (10 mcg) impregnated discs (BD, BBL) were placed
- Zones of inhibition were recorded following 24-hour incubation at 35C in ambient air
- Growth from the innermost zones of inhibition from each disc was used to prepare subsequent suspensions for serial antimicrobial susceptibility testing
- Innermost zones of growth were chosen for serial propagation to ensure sub-lethal antibiotic exposure allowing for the highest chance of resistance development
- From the initial susceptibility plate, isolates were subdivided to either an ERT origin plate or MER origin plate based on which innermost zone they were taken from initially
- Each subsequent passage had both an ERT and MER disc placed, but the serial passage kept true to the antimicrobial of original exposure
- Serial passage was performed for ten days
- Resistance was defined using standard CLSI guidelines (ERT ≤ 18mm, MER ≤ 19mm)

## Results

Table 1. Zone of inhibition changes dependent on exposure to MER or ERT

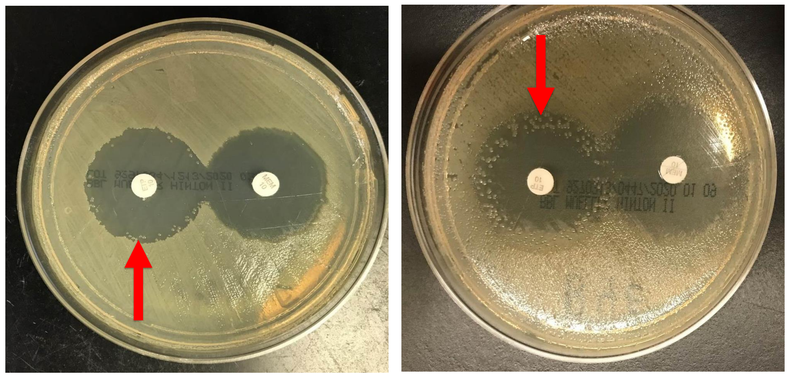
| Isolate | Day 1 | | Day 10 | | Difference (mm) |
|---|---|---|---|---|---|
| | Origin plate | Initial zone size (mm) | Disc measured | Final zone size (mm) | |
| *K. Pneumoniae* | MER | 31 | ERT | 30 | 1 |
| | | | MER | 30 | 1 |
| | ERT | 31 | ERT | 29 | 2 |
| | | | MER | 29 | 2 |
| *K. pneumoniae* | MER | 34 | ERT | 34 | 0 |
| | | | MER | 35 | -1 |
| | ERT | 34 | ERT | 17 | 17 |
| | | | MER | 24 | 10 |
| *K. pneumoniae* | MER | 30 | ERT | 27 | 3 |
| | | | MER | 25 | 5 |
| | ERT | 26 | ERT | 26 | 0 |
| | | | MER | 29 | -3 |
| *K. pneumoniae* | MER | 32 | ERT | 33 | -1 |
| | | | MER | 31 | 1 |
| | ERT | 33 | ERT | 32 | 1 |
| | | | MER | 29 | 4 |
| *K. pneumoniae* | MER | 29 | ERT | 32 | -3 |
| | | | MER | 32 | -3 |
| | ERT | 33 | ERT | 33 | 0 |
| | | | MER | 30 | 3 |
| *K. oxytoca* | MER | 32 | ERT | 35 | -3 |
| | | | MER | 32 | 0 |
| | ERT | 33 | ERT | 34 | -1 |
| | | | MER | 32 | 1 |
| *K. pneumoniae* | MER | 30 | ERT | 34 | -4 |
| | | | MER | 31 | -1 |
| | ERT | 32 | ERT | 35 | -3 |
| | | | MER | 32 | 0 |
| *K. pneumoniae* | MER | 29 | ERT | 32 | -3 |
| | | | MER | 32 | -3 |
| | ERT | 30 | ERT | 30 | 0 |
| | | | MER | 28 | 2 |
| *K. pneumoniae* | MER | 29 | ERT | 32 | -3 |
| | | | MER | 30 | -1 |
| | ERT | 33 | ERT | 33 | 0 |
| | | | MER | 28 | 5 |
| *K. pneumoniae* | MER | 33 | ERT | 38 | -5 |
| | | | MER | 33 | 0 |
| | ERT | 35 | ERT | 37 | -2 |
| | | | MER | 35 | 0 |
| *K. pneumoniae* | MER | 31 | ERT | 32 | -1 |
| | | | MER | 33 | -2 |
| | ERT | 32 | ERT | 34 | -2 |
| | | | MER | 32 | 0 |
| *E. coli* | MER | 32 | ERT | 29 | 3 |
| | | | MER | 29 | 3 |
| | ERT | 33 | ERT | 35 | -2 |
| | | | MER | 33 | 0 |
| *M. morganii* | MER | 31 | ERT | 35 | -4 |
| | | | MER | 34 | -3 |
| | ERT | 32 | ERT | 10 | 22 |
| | | | MER | 11 | 21 |
| *K. oxytoca* | MER | 32 | ERT | 31 | 1 |
| | | | MER | 35 | -3 |
| | ERT | 31 | ERT | 20 | 11 |
| | | | MER | 28 | 3 |
| *E. cloacae* | MER | 27 | ERT | 23 | 4 |
| | | | MER | 28 | -1 |
| | ERT | 23 | ERT | 14 | 9 |
| | | | MER | 22 | 1 |
| *E. cloacae* | MER | 35 | ERT | 14 | 21 |
| | | | MER | 24 | 11 |
| | ERT | 26 | ERT | 14 | 12 |
| | | | MER | 23 | 3 |
| *K. pneumoniae* | MER | 30 | ERT | 32 | -2 |
| | | | MER | 31 | -1 |
| | ERT | 33 | ERT | 34 | -1 |
| | | | MER | 31 | 2 |

## Results

Table 2. Development of resistance

| Organisms (N, number of sub-isolates) | Resistance to ERT | Resistance to MER |
|---|---|---|
| *Klebsiella pneumoniae* (11, 22) | 1 | - |
| *Klebsiella oxytoca* (2, 4) | 1 | - |
| *Enterobacter cloacae* (2, 4) | 3 | - |
| *Escherichia coli* (1, 2) | - | - |
| *Morganella morganii* (1, 2) | 1 | 1 |
| Total (17, 34) | 6 | 1 |
| | P = 0.053 | |

Figure 1. Agar plates depicting isolates that were propagated from the inner most zone of inhibition



## Discussion

- Enterobacteriaceae resistance to ERT can be caused by mechanisms other than carbapenemases with the expression of AmpC and ESBL combined with porin loss being common
- ERT resistant Enterobacteriaceae infections carry a higher rate of mortality compared to ERT sensitive Enterobacteriaceae infections
- Though further research is needed, it seems ERT exposure may put patients at higher risk of carbapenem resistance development compared to MER
- Clinical application of this study may suggest the use of MER over ERT
- ERT use should potentially be reserved for the outpatient setting secondary to its ease of administration
- Limitations include the small number of isolates and in-vitro nature of this study

## Conclusions

- This novel experiment identified the development of nonsignificant reductions in susceptibility with ERT (vs MER) after serial exposure
- Results from this pilot study should encourage larger well-designed studies in this area

## References

1. https://www.cdc.gov/drugresistance/pdf/threats-report/CRE-508.pdf
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