

TITLE OF THE STUDY: Synergy testing between sulbactam and meropenem/colistin in MDR *Acinetobacter baumannii-calcoaceticus* complex isolated from ventilator associated pneumonia- a pilot study

DEPARTMENT: Clinical Microbiology

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INTRODUCTION: *A.baumannii-calcoaceticus* (*Abc*) complex has surfaced as a major nosocomial pathogen causing blood stream infection and ventilators associated pneumonia (VAP) and are associated with increased mortality and morbidity.

Carbapenems are the mainstay of treatment for *Abc complex*. However, there has been an increase incidence of infection with carbapenem resistant strains. Combination antibiotic therapy is an approach frequently employed in the treatment of MDR strains, which attempts to achieve synergy, particularly against MDR strains. To support this clinical practice, in vitro combinations of antibiotics have been examined using checkerboard methods, E-tests, and the reference, time-to-kill assay

OBJECTIVES: To determine the minimum inhibitory concentration (MIC) of meropenem, sulbactam and colistin by broth micro-dilution technique on the isolates of MDR *Acinetobacter baumannii-calcoaceticus* complex (*Abc* complex). To determine the presence of synergy between sulbactam plus meropenem and sulbactam plus colistin combinations by time kill assay at a sub-inhibitory concentration and micro-broth checkerboard assay.

METHODOLOGY: A prospective study was conducted for a duration of one year. Twenty five isolates of carbapenem resistant *Abc* complex cultured from endotracheal aspirates of patients admitted in medical and surgical intensive care units diagnosed with ventilator associated pneumonia were collected. Isolates were tested for MIC by micro-broth dilution method for meropenem, sulbactam and colistin. Synergism between sulbactam plus meropenem and sulbactam plus colistin was tested by micro-broth checkerboard assay and the reference, time kill assay.

Data was analyzed using SAS software. MIC₅₀, MIC₉₀, MIC range and mean MIC were estimated for the tested antimicrobial agents. Proportion tests were used to compare the outcomes between antibiotics combination. Concordance rate of checker board assay and time kill assay were presented.

RESULTS: MIC ranges ($\mu\text{g/ml}$) for sulbactam, meropenem, and colistin were 16-512, 16-256, and 0.5-64 respectively. MIC_{50} for sulbactam, meropenem, and colistin were 128, 128 and 1 correspondingly, and MIC_{90} for sulbactam, meropenem, and colistin were 256, 256 and 2 respectively. In the checkerboard assay, synergy of 52% and 16% for the combination of sulbactam plus meropenem and sulbactam plus colistin, respectively, were noted. Time-kill assay showed a synergy of 68% and 32% for the combination of sulbactam plus meropenem and sulbactam plus colistin, respectively. Bactericidal activity of 80% for sulbactam plus meropenem and 96% for sulbactam plus colistin was observed. Antagonism was not detected with any combination or any method.

CONCLUSION: Against MDR isolates of *Abc* complex commendable synergy was seen with time kill assay for sulbactam plus meropenem combination. Significant bactericidal activity was observed for both sulbactam plus meropenem/colistin. Therefore, *in-vitro* combinations of antimicrobial agents are most effective than the single agent against multidrug resistant organism

Keywords: MDR *Abc* complex, VAP, Time Kill assay, Checkerboard assay