EV0394

Rapid killing of extended spectrum beta-lactamase positive (ESBL+), *Escherichia coli* (EC) and *Klebsiella pneumoniae* (Kp) clinical isolates of Amikacin inhaled pulmonary drug concentration.

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ABSTRACT

Background: Inhibition of bacterial growth by either minimum inhibitory (MI) or mutant prevention (MP) concentration (C) are not measurements of kill but rather inhibition of growth. Bacterial eradication is linked to clinical cure and speed of kill likely impacts time to symptom resolution. We measured the killing of ESBL+, Ec and Kp strains by MIC, MPC and amikacin inhaled pulmonary drug concentrations (DC) achieved after inhalation using amikacin inhale (BAY41-6551), a unique drug-device combination of a specially formulated amikacin inhalation solution and a Pulmonary Drug Delivery System device.

Materials/Methods: Clinical isolates of ESBL+, Ec and Kp were tested to determine the MIC using the recommended method by the Clinical and Laboratory Standards Institute; basically 10^5 cfu/ml in Mueller-Hinton broth were exposed to doubling drug concentrations. For MPC testing, 10^{10} CFUs were applied to agar plates containing doubling drug concentrations. Following incubation under ambient conditions, the lowest concentration blocking growth was either the MIC or MPC, depending on method. For kill experiments 10^5 cfu/ml of bacteria were exposed to drug and the $\log_{10}$ (% kill) reduction in viable cells recorded following 5, 10, 15, 20, 25, 30, 60, 120 and 180 minutes. All measurements were performed in triplicate and results averaged.

Results: 3 clinical strains each of ESBL+, Ec and Kp were used and all measurements were in triplicate yielding 9 independent measurements at each time point: results were subsequently averaged. Killing by the MIC drug concentration was slow and incomplete. For ESBL+, Ec at MPC DC, 50% (0.34 $\log_{10}$) of cells were killed by 10 minutes of drug exposure as compared to >99% killing (3.8 $\log_{10}$) at a pulmonary DC of 976 µg/ml. For Kp strains, 22% of cells were killed by 10 minutes at MPC DC versus >99% (3.5 $\log_{10}$) at the pulmonary DC of 976 µg/ml. Regrowth was not seen at the MPC or pulmonary DC.

Conclusion: Both MPC and pulmonary DC for amikacin inhale were bactericidal against ESBL+, Ec and Kp strains, >99% of cells were killed following 10 minutes of drug exposure at the pulmonary DC. Rapid bacterial killing likely reduces the risk for resistance selection. Such observations may have important implications for treatment of ventilator associated pneumonia with amikacin inhaled including duration of therapy.

INTRODUCTION

- **Amikacin, an aminoglycoside, is a bactericidal, concentration dependent antimicrobial agent with broad spectrum activity against Gram-negative pathogens.**
- **We have previously reported MIC and MPC values for amikacin tested against ESBL positive strains of *K. pneumoniae*; MPC values ranged from 8-64 µg/ml with MIC values being 0.25-4 µg/ml.**
- **The CLSI susceptibility breakpoint for amikacin for *Enterobacteriaceae* is ≤16 µg/ml and as such, organisms with MIC or MPC values in excess of the susceptibility breakpoint would be considered non-susceptible.**
- **We tested ESBL+ *K. pneumoniae* and *E. coli* clinical isolates with MPC values below and above the susceptibility breakpoint against amikacin to determine the speed and extent of killing using reported pulmonary drug concentration for the amikacin inhale formulation.**

MATERIALS AND MANAGEMENT

Bacterial Strains

- **Clinical isolates were collected/tested through the clinical microbiology laboratory at Royal University Hospital, Saskatoon, Saskatchewan.**
- **Isolates were sub-cultured on plates containing sheep blood agar, stocked in skim milk and stored at -70°C.**

Antimicrobial Agents:

- **Amikacin was provided by Bayer AG and used according to the manufacturers instructions.**

Susceptibility Testing

- **MIC testing (Figure 1) was as per the recommended method by the Clinical & Laboratory Standard Institute (CLSI). In brief, 10^5 CFU/ml bacterial inoculum in Mueller-Hinton broth tested against doubling drug concentration following overnight incubation (18-24 hours) under ambient condition (O_2 or in CO_2 for *H. influenzae*.**
- **MPC testing (Figure 2) was based on 10^{10} cfu bacterial inoculum in Mueller-Hinton broth plated on trypticase soy agar containing 5% sheep red blood cells and 2 fold concentration increment of antibiotic. Plates were incubated in O_2 (or CO_2) and read at 24 and 48 hours. The lowest drug concentration blocking 100% of growth was the MPC.**
- **For kill experiments (Figure 3), all measurements were done in triplicate and the results averaged. Results were also averaged for each of the 3 strains tested such that each data point on the graphs represents 9 averaged measurements.**

Figure 1 - MIC

Figure 2 - MPC

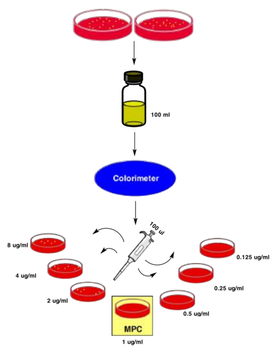
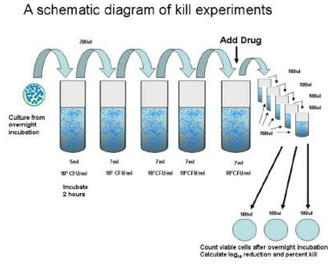


Figure 3 – Kill Experiments



RESULTS

Figure 1: Log Reduction of *E. coli* strains exposed to MIC, MPC and pulmonary drug concentrations of amikacin.

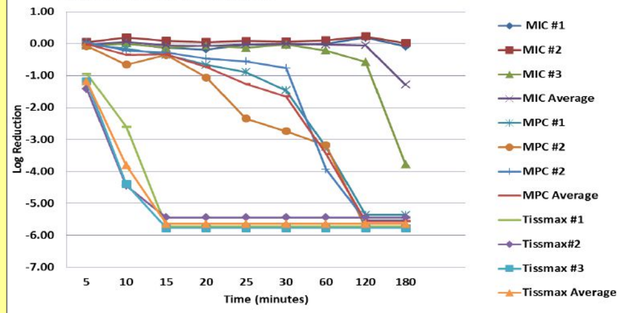


Figure 2: Percent Kill of *E. coli* strains exposed to MIC, MPC and pulmonary drug concentrations of amikacin.

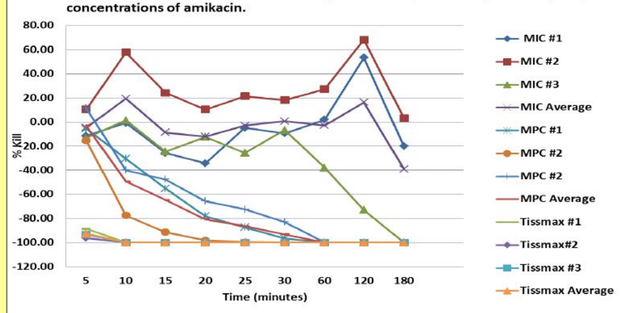


Figure 3: Log Reduction of *K. pneumoniae* strains exposed to MIC, MPC and pulmonary drug concentrations of amikacin.

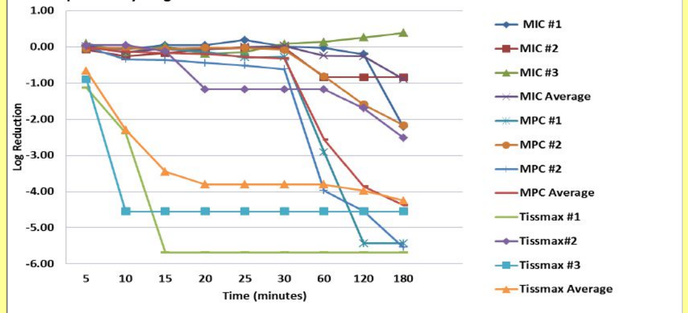
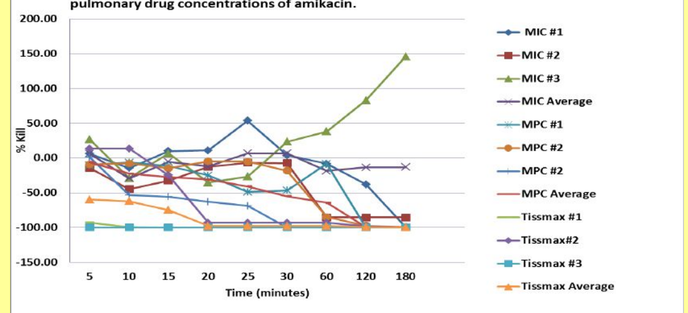


Figure 4: Percent Kill of *K. pneumoniae* strains exposed to MIC, MPC and pulmonary drug concentrations of amikacin.



- **MIC/MPC (µg/ml) values for the 3 *E. coli* strains were 2/64, 2/64, 2/32; for the 3 *K. pneumoniae* strains were 1/8, 4/64, 0.5/8.**
- **Bacterial cells were killed faster by the pulmonary drug concentration (976 µg/ml) then by the MPC (8-64 µg/ml) or MIC (0.5-4 µg/m) drug concentrations.**
- **99-100% kill of bacteria occurred at the MPC drug concentration after 30 minutes of drug exposure and by 120 minutes of drug exposure for all strains tested.**
- **>99% killing of the *K. pneumoniae* bacterial cells (all strains) were killed following 10 minutes of pulmonary drug concentration exposure.**
- **>93% killing of the *E. coli* bacterial cells (all strains) were killed following 20 minutes of pulmonary drug concentration exposure.**
- **Higher or lower MPC values did not appear to impact the killing by pulmonary drug concentrations of amikacin.**

CONCLUSION

- **Reported pulmonary drug concentrations of amikacin inhale rapidly killed ESBL+ *K. pneumoniae* and *E. coli* clinical isolates with MPC values below and above the susceptibility breakpoint.**
- **This data may have important implications for treatment of ventilated patients with multidrug resistant Gram-negative bacilli.**

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