EP115

Mutant Prevention Concentration (MPC) values for amikacin tested against clinical isolates of ESBL positive *Klebsiella pneumoniae*: Consideration of Amikacin Inhale pulmonary drug concentrations.

J.M. Blondeau, S.D. Shebelski
Department of Clinical Microbiology, Royal University Hospital and the University of Saskatchewan, Saskatoon, SK, Canada.

ABSTRACT

Objectives: Amikacin has been available for many years, however, Amikacin Inhale is a novel therapeutic approach (an integrated drug-device combination) in development for treatment of Gram-negative pneumonia in the intubated and mechanically ventilated patients. Amikacin epithelial lining fluid (ELF) drug concentrations after administration of Amikacin Inhale in ventilated patients are reported to be 976 mg/L. To date, MPC data for amikacin is lacking for key pathogens associated with ventilator associated infections including strains that are multidrug resistant. We tested amikacin minimum inhibitory concentration (MIC) and MPC of ESBL positive strains and compared these values to ELF concentrations.

Methods: Clinical isolates of ESBL *K. pneumoniae* (n=10) 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.

Results: The MIC_{50} (mg/L) , MIC_{90} (mg/L) and MIC range values were as follows: 1, 1, 0.5-4. The MPC_{50}, MPC_{90} and MPC range values were as follows: 8, 16, 8-64 mg/L. MPC/MIC ratio values ranged from 8-16. The highest MPC value (64) was seen with the strains having the highest MIC value. With an amikacin ELF of 976, ELF/MIC_{50}, ELF/MIC_{90} and $ELF/_{MPC50}$, ELF/MPC_{90} ratios would be 976, 976, and 122, 61 respectively.

Conclusion: MPC defines the drug concentration threshold blocking growth of the least susceptible cell present in high density bacterial populations – such as those seen during acute infection. This is the first report of MPC of amikacin tested against ESBL *K. pneumoniae* strains. ELF amikacin drug concentration after administration of Amikacin Inhale would be 61-976 times the MPC_{90} and MIC_{90} respectively. This suggests a reduced likelihood for resistance selection.

INTRODUCTION

- Amikacin – an aminoglycoside – has been in clinical use for decades.
- Aminoglycosides have been important agents for the treatment of serious Gram-negative infections.
- In many institutions, amikacin use was third after gentamicin and tobramycin and in some instances resistance to gentamicin and tobramycin lead to amikacin use.
- While resistance to aminoglycosides is well known, many Gram-negative bacteria resistant to other drug classes remain susceptible to aminoglycosides.
- Amikacin inhaled delivers high pulmonary drug concentrations in intubated patients but is not associated with system drug toxicity.
- We tested ESBL positive *Klebsiella pneumoniae* clinical isolates by minimum inhibitory and mutant prevention concentrations measurements and compared the MIC and MPC values to epithelial lining drug concentrations for amikacin inhaled.

MATERIALS AND MANAGEMENT

Bacterial Strains

- *Klebsiella pneumoniae* 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:

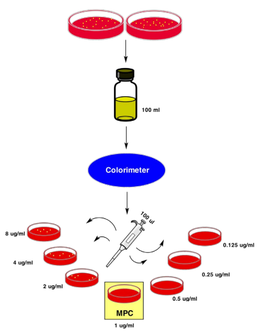
- Amikacin was provided by Bayer AG and used according to the manufacturers instructions.

Susceptibility Testing/PFGE

- 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).
- MPC testing as per Figure 2, 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 and read at 24 and 48 hours. The lowest drug concentration blocking 100% of growth was the MPC.

Figure 1 - MIC

Figure 2 - MPC



RESULTS

Table 1: MIC and MPC values for ESBL + strains of *K. pneumoniae* tested against amikacin.

	Klebsiella pneumoniae strains													
	1	2	3	4	5	6	7	8	9	10	MIC_{90}	MPC_{90}	$ELF/MIC_{50/90}$	$ELF/MPC_{50/90}$
MIC	4	0.5	0.5	1	1	1	1	4	0.25	1	1		976/976	
MPC	64	8	8	8	16	8	16	64	16	8		16		61/61
MPC/MIC_{Ratio}	16	16	16	8	16	8	16	16	64	8				

- For individual strains, ELF/MIC or ELF/MPC values ranged from 244-3904 and from 15-122 respectively.
- For 9/10 strains, MPC/MIC ratio values were 8-16.
- The breakpoint for amikacin susceptibility with *K. pneumoniae* was ≤16 µg/ml; 8/10 (80%) strains had MPC values ≤16 µg/ml.

CONCLUSION

- Amikacin inhaled delivers high ELF drug concentration (976 mg/L).
- 80% of ESBL + *K. pneumoniae* strains had MPC values ≤16 µg/ml.
- Amikacin ELF drug concentrations were 244-1952 X MIC values.
- Amikacin ELF drug concentrations were 15-122 X MPC values.
- High ELF/MPC ratios suggest amikacin inhale has a reduced likelihood for resistance selection.

REFERENCES

1. Stass H, Corkery K, Gribben D, Eldon MA. Pharmacokinetics and tolerability of BAY41-6551 in subjects with chronic kidney disease. Journal of Aerosol Medicine and Pulmonary Drug Delivery 2011;24(4):191-199.
2. Luyt CE, Clavel M, Guntupalli K, Johannigman J, Kennedy JI, Wood C, Corkery K, Gribben D, Chastre J. Pharmacokinetics and lung delivery of PDDS-aerosolized amikacin (NKTR-061) in intubated and mechanically ventilated patients with nosocomial pneumonia. Critical Care 2009;13(6):R200.
3. Luyt CE, Eldon MA, Stass H, Gribben D, Corkery K, Chastre J. Pharmacokinetics and tolerability of amikacin administered as BAY41-6551 aerosol in mechanically ventilated patients with gram-negative pneumonia and acute renal failure. Journal of Aerosol Medicine and Pulmonary Drug Delivery 2011;24(4):183-90.
4. Niederman MS, Chastre J, Corkery K, Fink JB, Luyt CE, Garcia MS. BAY41-6551 achieves bactericidal tracheal aspirate amikacin concentrations in mechanically ventilated patients with Gram-negative pneumonia. Intensive Care Medicine 2012;38(2):263-71.
5. Stass H, Willmann S, Wendl T. Risk assessment for amikacin inhale in ICU patients using whole-body physiologically-based PK models. Society of Critical Care Medicine. San Francisco, California, 2014.

This work was supported (in part) by a research grant from Bayer AG..

ECCMID 2015