EV0395

Killing of *Enterobacter cloacae* (Ecl), *Escherichia coli* (Ec) and *Klebsiella pneumoniae* (kp) by pulmonary drug concentration of Ciprofloxacin dry powder for inhalation (Cdpi).

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ABSTRACT

Background: Ciprofloxacin has broad spectrum activity against Gram-negative bacilli and Cdpi delivers high pulmonary drug concentrations of 35->409 µg/ml. Bacterial eradication impacts symptom resolution and clinical cure. We determined the rate and extent of killing Ecl, Ec and Kp clinical strains by the Cdpi pulmonary drug concentration over a range of bacterial densities (10^6-10^9 colony forming units/millilitre [cfu/ml]).

Materials/Methods: MIC and MPC values were measured for ciprofloxacin against clinical strains of Ecl, Ec and Kp. MIC testing used 10^5 cfu/ml in appropriate media with doubling drug dilutions with incubation under optimal conditions (temperature and atmosphere) and MPC testing utilized 10^{10} CFUs on agar media containing drug in doubling dilutions with incubation under ideal conditions. The lowest drug concentration blocking growth was the MIC or MPC, depending on method. For kill measurements 10^6-10^9 cfu/ml were exposed to pulmonary drug concentrations and the percent kill ($\log_{10}$ reduction) in viable cells measured at 30 minutes, 1, 2, 3, 4, 6, 12 and 24 hours.

Results: 3 strains each were used and all measurements were in triplicate resulting in 9 independent measurements per time point; results were averaged. For the Ecl 10^6-10^9 cfu/ml, 63-77% (0.46-0.65 $\log_{10}$) were killed by 1 hr of drug exposure and this increased to 65-95% kill (0.5-1.3 $\log_{10}$) by 6 hr and 98-99% (0.63-5.9 $\log_{10}$) kill by 12-24 hr. For EC 10^6-10^9 cfu/ml,75-81% (0.7-0.8 $\log_{10}$) by 1 hr, 75-95% kill (0.7-2.4 $\log_{10}$) by 6 hr and75-100% kill (0.66-6.4 $\log_{10}$) by 12-24 hr. For Kp (10^6-10^9 cfu/ml) 77-92% kill (0.64-1.28 $\log_{10}$) by 1 hr, 84-99% kill (0.82-2.75 $\log_{10}$) by 6 hr and 84-100% kill (0.85-7.49 $\log_{10}$) by 12-24 hr.

Conclusion: Cdpi was rapidly bactericidal against Ecl, Ec and Kp strains over low to high density bacterial burdens –burdens likely present during infection. Such findings are likely important for use of this formulation.

INTRODUCTION

- Ciprofloxacin, a fluoroquinolone, has a long history of successful clinical use in both inpatient and outpatient populations.
- The drug is intrinsically active against Gram-negative and Gram-positive pathogens, is bactericidal and exhibits concentration dependent killing.
- Against susceptible strains of fastidious Gram-negative bacilli (i.e. *Haemophilus* spp.) and *Enterobacteriaceae*, mutant prevention concentration (MPC) values are below the susceptibility breakpoints as set for minimum inhibitory concentration (MIC) testing.
- In our 3 acute care hospitals in Saskatoon (Royal University, St. Paul's and Saskatoon City Hospitals) and from community collected specimens susceptibility of methicillin-susceptible strains of *Staphylococcus aureus* remain between 91-93% (2015 data: n=619-845 clinical isolates).
- Ciprofloxacin dry power inhale (dpi) reportedly deliver high pulmonary drug concentrations ranging from 34-409 µg/ml; drug concentration values well in excess of MIC and MPC values for various pathogens including those with elevated MIC or MPC values to ciprofloxacin.
- We measured the killing of clinical isolates of *E. cloacae*, *E.coli* and *K. pneumoniae* with various MIC and MPC values by ciprofloxacin using the 409 µg/ml pulmonary drug concentration.

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:

- Ciprofloxacin 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

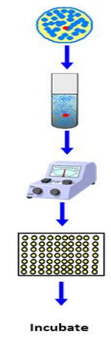


Figure 2 - MPC

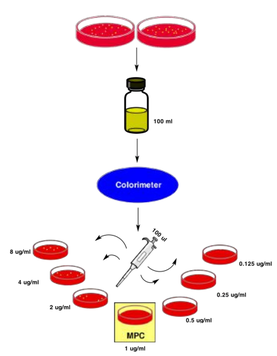
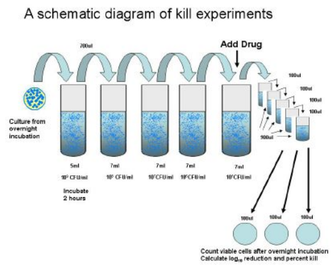
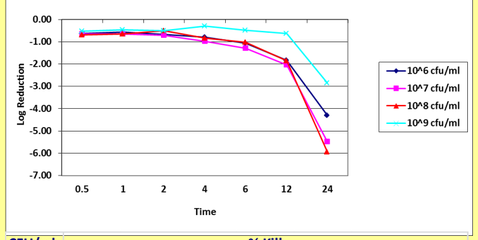


Figure 3 – Kill Experiments



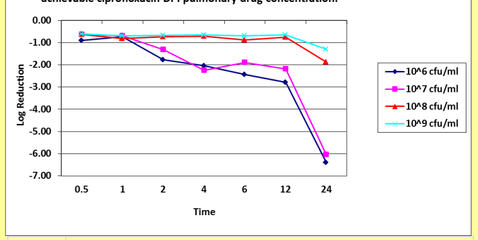
RESULTS/DISCUSSION

Figure 1: Killing ($\log_{10}$) of clinical isolates of *E. cloacae* by ciprofloxacin at the achievable ciprofloxacin DPI pulmonary drug concentration.



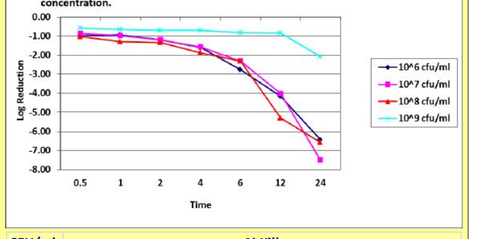
CFU/ml	% Kill						
	0.5	1	2	4	6	12	24
10^6	76	71	78	84	91	97	>99
10^7	77	78	81	89	95	99	>99
10^8	79	76	57	84	88	98	>99
10^9	68	63	67	49	65	73	>99

Figure 2: Killing ($\log_{10}$) of clinical isolates of *E. coli* by ciprofloxacin at the achievable ciprofloxacin DPI pulmonary drug concentration.



CFU/ml	% Kill						
	0.5	1	2	4	6	12	24
10^6	84	78	85	89	94	98	100
10^7	66	75	81	85	89	97	100
10^8	75	82	78	76	82	80	98
10^9	75	75	74	71	74	75	97

Figure 3: Killing ($\log_{10}$) of clinical isolates of *K. pneumoniae* by ciprofloxacin at the achievable ciprofloxacin DPI pulmonary drug concentration.



CFU/ml	% Kill						
	0.5	1	2	4	6	12	24
10^6	88	86	92	95	99	100	100
10^7	85	86	90	95	99	100	100
10^8	89	92	93	96	97	100	100
10^9	74	77	80	80	84	84	99

- MIC/MCP (µg/ml) values for the 3 *E. cloacae* strains were 0.016/0.25, 0.008/0.125, 0.031/0.125; for the 3 *E. coli* strains were 0.008/0.125, 0.008/0.063, 0.008/0.031; for the 3 *K. pneumoniae* strains were 0.031/2, 0.016/0.5, 0.016/0.5.
- 3 strains each of *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumoniae* were used and all measurements were done in triplicate and results averaged; each data point represents 9 independent measurements.
- Rapid killing occurred against all strains tested with 68-89% of bacterial cells killed following 30 minutes of drug exposure regardless of inoculum (i.e. 10^6-10^9 cfu/ml).
- 97-100% of bacterial cells were killed following 24 hours of drug exposure.
- Bacterial killing increased with increasing time of drug exposure. 65-99% of bacterial cells were killed following 6 hours of drug exposure.
- Bacterial eradication is essential for clinical cure.
- Rapid killing of bacteria likely accelerates clinical recovery from infection and reduces the likelihood for resistance selection.

CONCLUSION

- Ciprofloxacin dry power inhalation pulmonary drug concentration rapidly killed clinically significant Gram-negative bacilli over high and low bacterial density ranges likely present during infection.

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