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In Vitro Efficacy of High-Dose Tobramycin against *Burkholderia cepacia* Complex and *Stenotrophomonas maltophilia* Isolates from Cystic Fibrosis Patients

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***Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* infections are associated with poor clinical outcomes in persons with cystic fibrosis (CF). The MIC_{50} based on planktonic growth and the biofilm concentration at which 50% of the isolates tested are inhibited (BIC_{50}) of tobramycin were measured for 180 *B. cepacia* complex and 101 *S. maltophilia* CF isolates and were 100 μg/ml for both species. New inhalation devices that deliver high tobramycin levels to the lung may be able to exceed these MICs.**

As individuals with cystic fibrosis (CF) age, they are increasingly infected in their lungs with multidrug-resistant Gram-negative organisms such as *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia*, which are associated with poor clinical outcomes (1–5). Treatment of these infections is difficult (6–9) due to their numerous mechanisms of antimicrobial resistance, including efflux pumps, chromosomally encoded β-lactamases, decreased outer membrane permeability, intracellular survival, and biofilm formation (10–12). However, newer inhalational antibiotic therapies have the ability to deliver very high concentrations of drug to the lung, which may be able to overcome some of these mechanisms. One of the new inhalational antibiotics available is tobramycin inhalation powder (TIP), delivered by the Podhaler device, which can achieve up to 1.5- to 2-fold higher sputum tobramycin concentrations (up to 2,000 μg/g) than tobramycin inhalation solution (TIS) (13). It is not known whether these higher tobramycin concentrations can overwhelm the efficient efflux pumps known to be present in *B. cepacia* complex and *S. maltophilia* (14–16).

In order to determine whether the known pulmonary concentrations of inhaled high-dose tobramycin powder can overcome these inhibitory concentrations, the aim of this study was to measure the inhibitory concentrations of tobramycin for a large collection of *B. cepacia* complex and *S. maltophilia* isolates, grown planktonically and in a biofilm, from pediatric and adult CF patients.

B. cepacia complex isolates ($n = 180$) were prospectively collected from sputum samples from CF patients from four study sites, The Hospital for Sick Children ($n = 10$), St. Michael's Hospital ($n = 36$), the Cystic Fibrosis Foundation *Burkholderia cepacia* Research Repository at the University of Michigan ($n = 16$), and the Canadian *Burkholderia cepacia* Complex Research and Referral Repository at the University of British Columbia, Vancouver ($n = 118$). *S. maltophilia* isolates ($n = 101$) were obtained from pediatric CF patients at The Hospital for Sick Children in Toronto ($n = 67$) and from adult CF patients ($n = 34$) at St. Michael's Hospital in Toronto. All the isolates used in this study were independent strains (1 isolate/patient). Antimicrobial susceptibility testing was performed on isolates grown planktonically by broth microdilution using Clinical and Laboratory Standards Institute (CLSI) guidelines (17). Antimicrobial susceptibility testing was also performed on isolates grown as a biofilm using a modified form of the Calgary biofilm technique (18, 19). The antibiotic panels contained tobramycin at concentrations of 0, 10, 100, 200, 400, 800, 1,600, and 3,200 μg/ml. The MIC based on planktonic growth and the biofilm inhibitory concentration (BIC) of tobramycin for each isolate were determined by visually assessing the turbidity of each well (see Supplementary Methods in the supplemental material for more detail).

The tobramycin MIC_{50} and BIC_{50} (the BIC at which 50% of isolates were susceptible) were 100 μg/ml for a large collection of CF *B. cepacia* complex isolates (Table 1), largely consistent across most species of the *B. cepacia* complex. *Burkholderia vietnamiensis*, previously shown to be more susceptible to aminoglycosides (15), had an MIC_{50} of 10 μg/ml. *Burkholderia dolosa* isolates, responsible for an outbreak at a U.S. CF care center (20), demonstrated a higher MIC_{50} of 200 μg/ml. Similarly, the tobramycin MIC_{50} and BIC_{50} for *S. maltophilia* were 100 μg/ml. The distribu-

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TABLE 1 Tobramycin MICs and BICs for *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* CF isolates

Organism (no. of isolates)	MIC_{50} (μg/ml)	BIC_{50} (μg/ml)	MIC_{90} (μg/ml)	BIC_{90} (μg/ml)
B. cepacia complex (180)	100	100	400	400
B. cenocepacia (83)	100	100	800	800
B. multivorans (41)	100	100	400	400
B. stabilis (16)	100	100	100	400
B. vietnamiensis (19)	10	10	100	100
B. dolosa (14)	200	200	200	400
B. cepacia (7)	100	100	800	400
S. maltophilia (101)	100	100	1,600	3,200

tion of the tobramycin MICs and BICs for *B. cepacia* complex and *S. maltophilia* is shown in Fig. 1. A significant proportion of *B. cepacia* complex isolates had tobramycin MICs ($n = 20/180$, 11%) and BICs ($n = 32/180$, 18%) that were ≤10 μg/ml, as did *S. maltophilia* isolates, with 34% ($n = 34/101$) of MICs and 29% of BICs ($n = 29/101$) that were ≤10 μg/ml. Conversely, the MIC_{90} and BIC_{90} for *B. cepacia* complex isolates were 400 μg/ml and for *S. maltophilia* isolates were 1,600 μg/ml and 3,200 μg/ml (Table 1), respectively, suggesting that in these cases, TIP administration may not be capable of exceeding these high inhibitory concentrations.

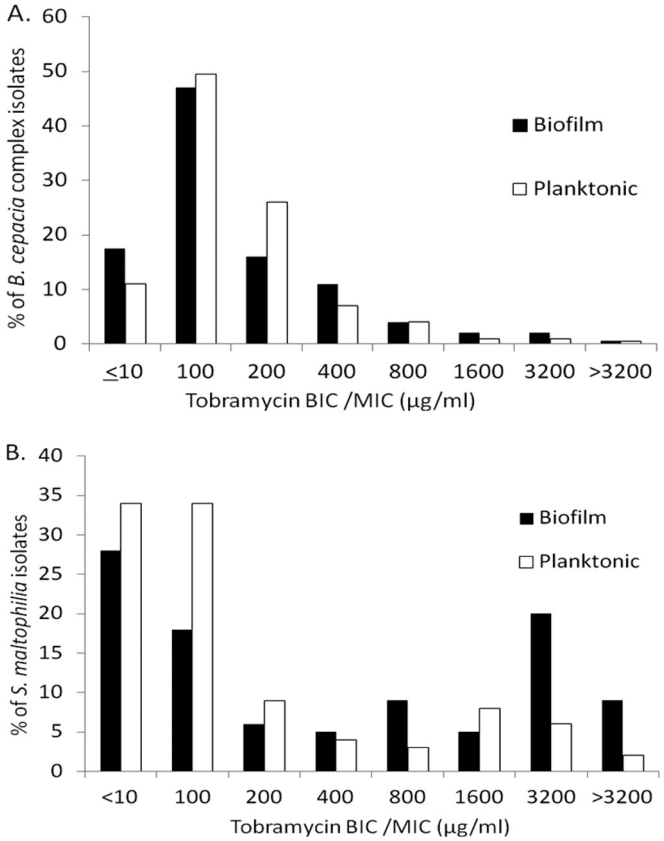

FIG 1 Distribution of tobramycin biofilm inhibitory concentrations (BICs) measured by biofilm antimicrobial susceptibility testing and MICs measured by planktonic antimicrobial susceptibility testing for *Burkholderia cepacia* complex (A) and *Stenotrophomonas maltophilia* (B) cystic fibrosis isolates.

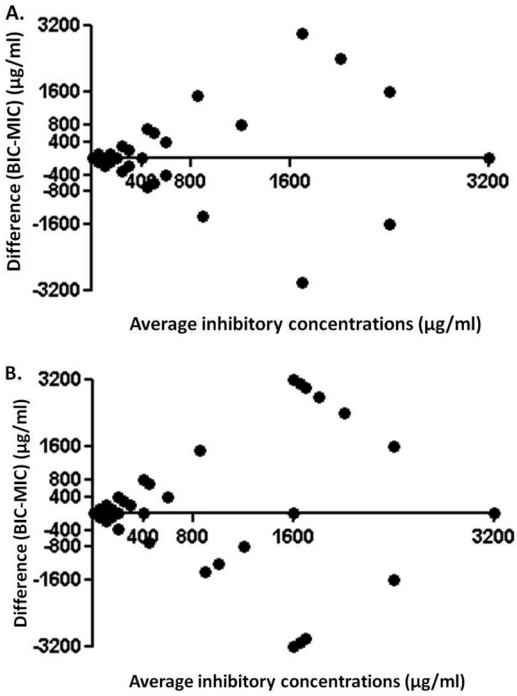

FIG 2 Bland-Altman plots of average inhibitory concentrations versus difference between biofilm inhibitory concentrations (BICs) and MICs for *Burkholderia cepacia* complex (A) and *Stenotrophomonas maltophilia* (B) cystic fibrosis isolates, with points on the *x* axis ($y = 0$) indicating complete agreement.

The correlations between the two methods (planktonic and biofilm) of antimicrobial susceptibility testing was calculated using the Spearman correlation coefficient and were found to be statistically significant for *B. cepacia* complex isolates ($r = 0.5549$, $P < 0.0001$) and for *S. maltophilia* isolates ($r = 0.3638$, $P = 0.0002$), suggesting that tobramycin can function well against organisms grown in a biofilm state, as expected in the CF lung. The agreement between the two methods of antimicrobial susceptibility testing is illustrated in a Bland-Altman plot for *B. cepacia* complex (Fig. 2A) and *S. maltophilia* (Fig. 2B) isolates.

To date, this is the largest *in vitro* study of a contemporary collection of clinical CF isolates to determine the tobramycin concentrations required to inhibit the planktonic and biofilm growth of *B. cepacia* complex and *S. maltophilia*. Although traditionally considered to be intrinsically resistant to systemically attainable aminoglycoside concentrations based on CLSI breakpoints (17), our data suggest that TIP treatment can achieve a maximal drug concentration (C_{max})/MIC ratio of up to 20-fold for the majority of *B. cepacia* complex and *S. maltophilia* isolates from CF patients. It is unknown what C_{max}/MIC ratio is required to successfully suppress bacterial growth in the CF lung, but there is a relationship between the C_{max} and the MIC required to inhibit *Pseudomonas aeruginosa* growth, with higher ratios associated with greater reduction in bacterial density (21).

We also demonstrated that tobramycin inhibitory concentrations were similar regardless of whether the organisms were grown planktonically or as a biofilm, suggesting that at these high levels, tobramycin may be effective in the CF lung environment. Different classes of antimicrobials have various degrees of efficacy against dense slow-growing matrix-enveloped bacterial commu-

nities based on their ability to penetrate biofilms and their mechanism of action (22). Aztreonam, for example, is not as effective as tobramycin at reducing *P. aeruginosa* biofilm mass on airway epithelial cells, and tolerance to aztreonam may develop secondary to biofilm exopolysaccharide production (23). In our study, however, high-dose tobramycin overcame mechanisms of biofilm resistance and inhibited bacterial protein synthesis in stationary-phase organisms.

Despite these results, however, *in vitro* susceptibility testing, whether by the planktonic or biofilm method of growth, does not necessarily predict clinical response in CF patients, and it is unclear whether TIP, which delivers a sputum tobramycin concentration 1.5- to 2-fold higher than TIS, will translate into improved efficacy in the treatment of these infections. Clinical trials of TIP therapy in this patient population are under way to assess this question (ClinicalTrials.gov identifier NCT02212587).

In conclusion, TIP administration may deliver pulmonary drug concentrations in excess of what is required to inhibit the majority of *B. cepacia* complex and *S. maltophilia* CF isolates, even when grown as a biofilm. This offers a potential therapeutic option to a CF population for whom there is no effective chronic suppressive antimicrobial treatment.

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