Supplementary Methods

Isolate collection

B. cepacia complex (n=180) and *S. maltophila* (n=101) isolates were prospectively collected from sputum samples from CF patients. Antimicrobial susceptibility testing was performed on one isolate per patient.

B. cepacia complex isolates were collected from four study sites: Hospital for Sick Children (n=10), St. Michael's Hospital (n=36), 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). The collection of *B. cepacia* complex isolates in this study included the following species: *B. cenocepacia* (n=78), *B. multivorans* (n=41), *B. stabilis* (n=16), *B. vietnamiensis* (n=19) *B. dolosa* (n=14), *B. cepacia* (n=7) and unknown (n=5). *B. cepacia* complex genomovar typing was done by *recA* typing as previously described (1).

S. maltophilia isolates were obtained from CF pediatric patients at The Hospital for Sick Children, Toronto (n=67) and CF adult patients (n=34) at St. Michael's Hospital, Toronto.

Conventional susceptibility testing

Conventional antimicrobial susceptibility testing was performed on isolates grown planktonically by microbroth dilution, using methods as per Clinical Laboratory Standard Institute (CLSI) guidelines (2). Antibiotic plates were prepared containing tobramycin in cation-adjusted Mueller Hinton Broth (CAMHB) at concentrations of: 0, 10, 100, 200, 300, 800, 1600 and 3200 µg/ml. Bacterial inoculum was prepared by diluting 6 µl of 0.5 McFarland turbidity standard with 100 µL of double-distilled water. 10 µl of the bacterial inoculum was added to each well of the antibiotic plate to achieve an inoculum of approximately 5×10^5 CFU/mL,

which was then incubated in aerobic conditions at 37˚C for 24-48 hours. Purity of each isolate tested was checked by subculturing from an antibiotic-free well on the panel. Minimum inhibitory concentrations (MICs) were determined by visually assessing turbidity of the wells for signs of bacterial growth after 24 hours of incubation. In the instance where an isolate did not grow after 24 hours, it was incubated for another 24 hours, for a total of 48 hours. Repeat susceptibility was performed on isolates that failed to grow at 48 hours using CAMHB supplemented with 3% horse serum.

Biofilm susceptibility testing

Antimicrobial susceptibility testing was also performed on isolates grown as a biofilm, using a modified method of the Calgary biofilm technique (3-4). Bacterial suspension for biofilm generation was prepared by diluting 30 µl of 0.5 McFarland turbidity standard in 1.97 mL of Trypticase Soy Broth (TSB). 150 µl of the bacterial suspension was added into each well of the 96 well microtitre plate. The plates were then incubated in aerobic conditions at 37˚C on a shaker (Labnet Orbit 1000, Woodbridge, New Jersey) to obtain biofilm formation on the peg lid (Innovotech, Manitoba, Canada). An estimation of the inoculum was determined by measuring OD_{650nm} using the MRX Microplate Reader (Dynex Technologies, Chantilly, Virginia) of the broth in the well in which the biofilm peg was growing. Once an OD of approximately 0.062 (known to correspond to approximately 10^5 colony forming units (CFU)/ml) was achieved for the majority of the isolates on the plate, the biofilm-laden peg lid was placed into the antibiotic panel containing tobramycin at concentrations of 0, 10, 100, 200, 400, 800, 1600, and 3200 µg/ml. To confirm that the proper inoculum had been achieved, serial dilutions from a peg inoculated antibiotic-free well were plated onto solid media. Testing was repeated for isolates where the

48 inoculum did not fall between 10^4-10^6 CFU/ml. The plate was then incubated in aerobic
49 conditions at 37˚C for 24 hours. After 24 hours of incubation, the peg lid was placed into a 96
50 well plate with 200 µl of double distilled water to rinse of residual tobramycin from the pegs.
51 The peg lid was then placed into recovery plate containing only CAMHB. The plate was
52 incubated at 37˚C in aerobic conditions for 24 hours. The biofilm inhibitory concentration (BIC)
53 of each isolate was determined by visually assessing the turbidity of each well.
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References

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