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Biofilm Compared to Conventional Antimicrobial Susceptibility of *Stenotrophomonas maltophilia* Isolates from Cystic Fibrosis Patients

Kitty Wu,[a] Yvonne C. W. Yau,[b] Larissa Matukas,[c] Valerie Waters[a]

Division of Infectious Diseases, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada[a]; Division of Microbiology, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada[b]; Division of Microbiology, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada[c]

***Stenotrophomonas maltophilia* is a multidrug-resistant organism increasingly isolated from the lungs of cystic fibrosis (CF) patients. One hundred twenty-five *S. maltophilia* isolates from 85 CF patients underwent planktonic and biofilm susceptibility testing against 9 different antibiotics, alone and in double antibiotic combinations. When *S. maltophilia* isolates were grown as a biofilm, 4 of the 10 most effective antibiotic combinations included high-dose levofloxacin and 7 of the 10 combinations included colistin at doses achievable by aerosolization.**

Stenotrophomonas maltophilia is one of the most common multidrug-resistant pathogens infecting the airways of cystic fibrosis (CF) patients (1–3). Antibiotics to treat CF pulmonary infections are chosen based on conventional antimicrobial susceptibility testing of organisms grown planktonically ("free-floating") in liquid. However, it is known that organisms such as *S. maltophilia* actually grow as biofilms (communities of bacteria) on airway epithelial cells, suggesting that antibiotics chosen based on biofilm susceptibility testing may be more effective in CF (4, 5).

The objectives of this study were to compare biofilm antimicrobial susceptibility to conventional, planktonic antimicrobial susceptibility (as is currently done in clinical microbiology laboratories) for *S. maltophilia*, highlight the differences in antibiotic combinations derived using the two methods, and identify potentially more effective choices for inhibiting biofilm growth of *S. maltophilia* in the CF lung.

A total of 125 CF *S. maltophilia* isolates from sputum and bronchoalveolar lavage were prospectively collected from the microbiology laboratories at the Hospital for Sick Children (74 isolates from 51 CF patients; maximum of 2 isolates per patient) and St. Michael's Hospital (51 isolates from 34 CF patients; maximum of 2 isolates per patient) in Toronto, Canada, between January 2011 and July 2012. Planktonic susceptibility testing of *S. maltophilia* isolates was performed by broth microdilution according to CLSI guidelines (6). Isolates were also grown as biofilms using a modification of the Calgary biofilm technique (7). The following antibiotics were tested alone and in double combination: ceftazidime, ticarcillin-clavulanate, tobramycin, levofloxacin, moxifloxacin, trimethoprim-sulfamethoxazole, doxycycline, colistin, and azithromycin. Tobramycin (100 mg/liter and 200 mg/liter) (8) and colistin (100 mg/liter and 200 mg/liter) (9) were tested at concentrations achievable in CF sputum by aerosolization. Levofloxacin was tested at both high concentrations (50 mg/liter and 100 mg/liter, corresponding to achievable sputum levels by aerosolization) (10, 11) and low concentrations (2 mg/liter and 4 mg/liter, corresponding to achievable serum levels).

Biofilm inocula of the 125 *S. maltophilia* isolates tested fell between 2.5×10^4 and 4.6×10^6 CFU/ml (median, 5.5×10^5 CFU/ml), requiring a range of 4.5 h to over 24 h (median, 6.5 h) for biofilm generation. When tested against individual antibiotics, significantly fewer *S. maltophilia* isolates were susceptible to fluoroquinolones, colistin, tobramycin, doxycycline, trimethoprim-sulfamethoxazole, and β-lactams when grown as biofilms than when grown planktonically (Fig. 1). High-dose levofloxacin was the most effective antibiotic against *S. maltophilia* in both the planktonic and biofilm forms. *S. maltophilia* isolates were then tested against double combinations of antibiotics grown as a biofilm and planktonically. When *S. maltophilia* isolates were grown planktonically, 6 of the 10 most effective antibiotic combinations included high-dose (achievable by aerosolization) levofloxacin and 5 of the 10 most effective antibiotic combinations included colistin at doses achievable by aerosolization (Tables 1 and 2; see also the supplemental material for complete results). In contrast, only 4 of the 10 most effective antibiotic combinations included high-dose (achievable by aerosolization) levofloxacin and 7 of the 10 most effective antibiotic combinations included colistin at doses achievable by aerosolization when isolates were grown as a biofilm.

This study is the first to examine the antimicrobial susceptibility of a large collection of predominantly CF *S. maltophilia* isolates grown both planktonically and in a biofilm. In a biofilm environment, traditional antibiotics used to treat CF patients, β-lactams and aminoglycosides, are not very effective, as β-lactams target rapidly dividing bacteria and aminoglycosides act on aerobically growing organisms (12, 13). Our study confirmed that *S. maltophilia* growing as a biofilm is very rarely susceptible to β-lactams and aminoglycosides (to which it is intrinsically resistant) (14), with fewer than 10% of isolates being susceptible to ceftazidime and ticarcillin-clavulanate and only 20% of isolates being susceptible to high-dose tobramycin which correlates with levels achiev-

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Address correspondence to Valerie Waters, valerie.waters@sickkids.ca.
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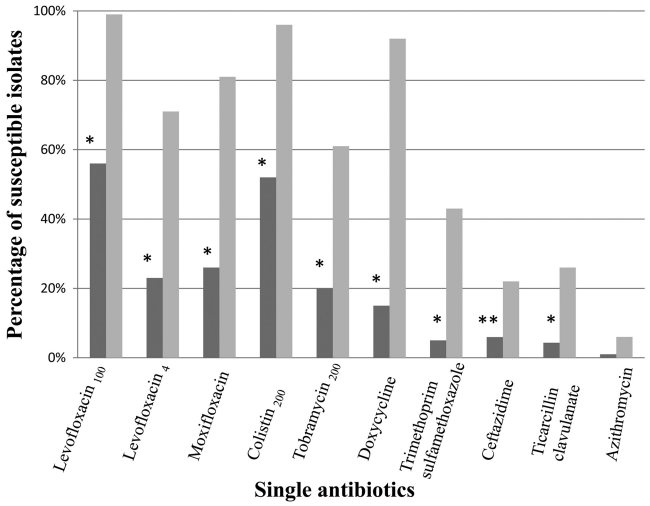

FIG 1 Percentage of *S. maltophilia* isolates susceptible to single antibiotics when grown as a biofilm (dark gray) compared to planktonic (light gray) (*, $P < 0.0001$; **, $P < 0.05$, by Fisher's exact test). Levofloxacin$_{100}$, levofloxacin tested at a maximum concentration of 100 mg/liter achievable in sputum after aerosolization; levofloxacin$_4$, levofloxacin tested at a maximum concentration of 4 mg/liter achievable in serum; colistin$_{200}$, colistin tested at a maximum concentration of 200 mg/liter achievable in sputum after aerosolization; and tobramycin$_{200}$, tobramycin tested at a maximum concentration of 200 mg/liter achievable in sputum after aerosolization.

able by aerosolization. Trimethoprim-sulfamethoxazole is often considered the drug of choice in the treatment of *S. maltophilia* infections; however, *S. maltophilia* resistance to trimethoprim-sulfamethoxazole has been increasingly described (15). In our assays, only half of *S. maltophilia* isolates were susceptible to trimethoprim-sulfamethoxazole alone using planktonic susceptibility testing; fewer still (less than 10%) were susceptible when grown as a biofilm.

In our study, colistin was included in many of the most effective double antibiotic combinations, and the majority of *S. maltophilia* isolates were susceptible to colistin when grown planktonically or as a biofilm. It is important to note, however, that very high concentrations of colistin (to approximately the levels achievable by aerosolization) were used in this assay based on previous *in vitro* susceptibility reports (9) and high lung concentrations achieved in animal models (16–18). However, the pulmonary concentration of colistin that can be achieved through inhalation is limited by several factors, including significant bronchospasm and hypersensitivity pneumonitis (19–21). Colistin may thus be less effective *in vivo* with lower achievable pulmonary concentrations (22, 23) than has been demonstrated *in vitro* against *S. maltophilia*.

TABLE 1 Most effective antibiotic combinations against planktonically grown *S. maltophilia* isolates

Antibiotic combination	% susceptible isolates (no.)
Levofloxacin$_{100}$-azithromycin	99 (124)
Levofloxacin$_{100}$–trimethoprim-sulfamethoxazole	99 (124)
Levofloxacin$_{100}$–ticarcillin-clavulanate	99 (124)
Levofloxacin$_{100}$-colistin$_{200}$	99 (124)
Doxycycline-colistin$_{200}$	98 (123)
Levofloxacin$_{100}$-ceftazidime	98 (123)
Colistin$_{200}$–trimethoprim-sulfamethoxazole	98 (123)
Tobramycin-levofloxacin$_{100}$	98 (123)
Levofloxacin$_4$-colistin$_{200}$	98 (122)
Moxifloxacin-colistin$_{200}$	98 (122)

TABLE 2 Most effective antibiotic combinations against biofilm-grown *S. maltophilia* isolates

Antibiotic combination	% susceptible isolates (no.)
Ceftazidime-colistin$_{200}$	65 (81)
Levofloxacin$_{100}$–ticarcillin-clavulanate	62 (78)
Colistin$_{200}$–trimethoprim-sulfamethoxazole	62 (78)
Moxifloxacin-colistin$_{200}$	61 (76)
Doxycycline-colistin$_{200}$	60 (75)
Levofloxacin$_{100}$-ceftazidime	59 (74)
Levofloxacin$_{100}$-azithromycin	58 (73)
Levofloxacin$_{100}$-colistin$_{200}$	58 (72)
Levofloxacin$_4$-colistin$_{200}$	58 (72)
Ticarcillin-clavulanate–colistin$_{200}$	58 (72)

The most effective antibiotic tested alone against planktonic and biofilm-grown *S. maltophilia* isolates in our study was high-dose levofloxacin. Previous *in vitro* studies have demonstrated that fluoroquinolones, such as levofloxacin, can disrupt *S. maltophilia* biofilms and significantly reduce *S. maltophilia* biofilm mass (24, 25). In addition, high lung concentrations of aerosolized levofloxacin can be achieved in mouse models of lung infection (10) and in CF patients (11, 26). Inhaled levofloxacin may thus represent a potentially effective suppressive antimicrobial therapy for patients chronically infected with *S. maltophilia*, although antimicrobial resistance may develop with long-term use.

This study has several limitations. Based on current clinical practice, double, not triple, antibiotic combinations known to have *in vitro* activity against *S. maltophilia* were tested (9, 27, 28). However, current practices are losing efficacy, and different solutions may be required. Results may also be biased toward patients with repeated samples, although the majority of susceptibility results of isolates from the same patient were different in our study.

In conclusion, both colistin and levofloxacin, at levels achievable by inhalation, were effective at inhibiting the growth of CF *S. maltophilia* isolates under biofilm conditions. Further prospective studies are needed to determine whether aerosolized levofloxacin treatment can significantly decrease the pulmonary burden of *S. maltophilia* and improve clinical outcomes in CF patients.

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We have no conflicts of interest to declare.

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