Biofilm compared to conventional antimicrobial susceptibility for *Stenotrophomonas maltophilia* isolates from cystic fibrosis patients

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Running title: *S. maltophilia* and biofilm testing

Keywords: *Stenotrophomonas maltophilia*, cystic fibrosis, biofilm

Word count: 999 words

All of the authors have no conflict of interest to declare.

The study was performed at The Hospital for Sick Children, Toronto, Canada.
Abstract

Stenotrophomonas maltophilia is a multi-drug 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 antibiotics combinations. When S. maltophilia 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 multi-drug 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 2 methods, and identify potentially more effective choices at 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, azithromycin, Tobramycin (100 mg/L and 200 mg/L) (8) and colistin (100 mg/L and 200 mg/L) (9) were tested at concentrations achievable in CF sputum by aerosolization. Levofloxacin was
tested at both high concentrations (50 mg/L and 100 mg/L—corresponding to achievable sputum levels by aerosolization) (10-11) and low concentrations (2 mg/L and 4 mg/L—corresponding to achievable serum levels).

Biofilm inocula of the 125 *S. maltophilia* tested fell between $2.5 \times 10^4$ to $4.6 \times 10^6$ colony forming units (CFU)/ml (median $5.5 \times 10^5$ CFU/ml), requiring a range of 4.5 hrs to over 24 hours (median 6.5 hrs) 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 compared to when grown planktonically (Figure 1). High-dose levofloxacin was the most effective antibiotic against *S. maltophilia* in both the planktonic and biofilm form. *S. maltophilia* isolates were then tested against double combinations of antibiotics grown as a biofilm and planktonically. When grown planktonically, six of the ten most effective antibiotic combinations included high-dose (achievable by aerosolization) levofloxacin and five of the ten most effective antibiotic combinations included colistin at doses achievable by aerosolization (Table 1; for complete results see supplement). In contrast, only four of the ten most effective antibiotic combinations included high-dose (achievable by aerosolization) levofloxacin and seven of the ten 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 as well as 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 susceptible to high-dose tobramycin which correlates with levels achievable 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 both planktonically or as a biofilm. It is important to note, however, that very high concentrations of colistin (to approximate 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*.

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 both mouse models of lung infection (10) as well as 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 towards 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.
Acknowledgments

The authors would gratefully like to acknowledge the assistance of Ms. Danuta Kovach in the development of biofilm assay for *S. maltophilia* and Ms. Patricia Schneider for her help with data entry. This work was supported by the Canadian Foundation for Infectious Diseases.
References


8. Dales L, Ferris W, Vandemheen K, Aaron SD. 2009. Combination antibiotic susceptibility of biofilm-grown *Burkholderia cepacia* and *Pseudomonas aeruginosa* isolated...


Figure Legends

Figure 1. Percentage of *S. maltophilia* isolates susceptible to single antibiotics when grown as a biofilm (dark grey) compared to planktonic (light grey) (* p<0.0001, ** p<0.05 by Fisher’s exact test). 

Levofloxacin<sub>100</sub>=levofloxacin tested at a maximum concentration of 100 mg/L achievable in sputum after aerosolization; Levofloxacin<sub>4</sub>=levofloxacin tested at a maximum concentration of 4 mg/L achievable in serum; Colistin<sub>200</sub>=colistin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization; Tobramycin<sub>200</sub>=tobramycin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization.
Table 1.

A. Most effective antibiotic combinations against planktonically-grown *S. maltophilia* isolates

<table>
<thead>
<tr>
<th>Antibiotic combination</th>
<th>% (n) of susceptible isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin&lt;sub&gt;100&lt;/sub&gt;/azithromycin</td>
<td>99 (124)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sub&gt;100&lt;/sub&gt;/trimethoprim-sulfamethoxazole</td>
<td>99 (124)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sub&gt;100&lt;/sub&gt;/ticarcillin-clavulanate</td>
<td>99 (124)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sub&gt;100&lt;/sub&gt;/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
<td>99 (124)</td>
</tr>
<tr>
<td>Doxycycline/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
<td>98 (123)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sub&gt;100&lt;/sub&gt;/ceftazidime</td>
<td>98 (123)</td>
</tr>
<tr>
<td>Colistin&lt;sub&gt;200&lt;/sub&gt;/trimethoprim-sulfamethoxazole</td>
<td>98 (123)</td>
</tr>
<tr>
<td>Tobramycin/levofloxacin&lt;sub&gt;100&lt;/sub&gt;</td>
<td>98 (123)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sub&gt;4&lt;/sub&gt;/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
<td>98 (122)</td>
</tr>
<tr>
<td>Moxifloxacin/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
<td>98 (122)</td>
</tr>
</tbody>
</table>

Levofloxacin<sub>100</sub>=levofloxacin tested at a maximum concentration of 100 mg/L achievable in sputum after aerosolization

Levofloxacin<sub>4</sub>=levofloxacin tested at a maximum concentration of 4 mg/L achievable in serum

Colistin<sub>200</sub>=colistin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization

Tobramycin<sub>200</sub>=tobramycin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization

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

<table>
<thead>
<tr>
<th>Antibiotic combination</th>
<th>% (n) of susceptible isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
<td>65 (81)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sub&gt;100&lt;/sub&gt;/ticarcillin-clavulanate</td>
<td>62 (78)</td>
</tr>
<tr>
<td>Colistin&lt;sub&gt;200&lt;/sub&gt;/trimethoprim-sulfamethoxazole</td>
<td>62 (78)</td>
</tr>
<tr>
<td>Moxifloxacin/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
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</tr>
<tr>
<td>Doxycycline/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
<td>60 (75)</td>
</tr>
<tr>
<td>Levofloxacin&lt;sub&gt;100&lt;/sub&gt;/ceftazidime</td>
<td>59 (74)</td>
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</tr>
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<td>58 (72)</td>
</tr>
<tr>
<td>Ticarcillin-clavulanate/colistin&lt;sub&gt;200&lt;/sub&gt;</td>
<td>58 (72)</td>
</tr>
</tbody>
</table>

Levofloxacin<sub>100</sub>=levofloxacin tested at a maximum concentration of 100 mg/L achievable in sputum after aerosolization

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Colistin<sub>200</sub>=colistin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization

Tobramycin<sub>200</sub>=tobramycin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization
Figure 1. Percentage of *S. maltophilia* isolates susceptible to single antibiotics when grown as a biofilm (dark grey) compared to planktonically (light grey). * p<0.0001, ** p<0.05 by Fisher’s exact test. Levofloxacin<sub>100</sub>=levofloxacin tested at a maximum concentration of 100 mg/L achievable in sputum after aerosolization; Levofloxacin<sub>4</sub>=levofloxacin tested at a maximum concentration of 4 mg/L achievable in serum; Colistin<sub>200</sub>=colistin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization; Tobramycin<sub>200</sub>=tobramycin tested at a maximum concentration of 200 mg/L achievable in sputum after aerosolization.