**ABSTRACT (edited)**

**INTRODUCTION**

P. aeruginosa is an opportunistic pathogen involved in many chronic infections. In cystic fibrosis patients, it persists in the lung is associated with its capacity to form biofilms, which bacteria are embedded in a matrix and protected from the host immune system.

In this biofilm model, P. aeruginosa adopts specific phenotypes (metabolically less active, sessile or mucoid [overproducing exopolysacharides as alginate]) [1], which are much less responsive to antibiotic activity than planktonic bacteria.

To investigate the activity of commonly used antipseudomonal antibiotics against such biofilms, we developed an in vitro biofilm model in an Artificial Sputum Medium (ASM) mimicking the physico-chemical properties of the mucus of cystic fibrosis patients [2].

**AIMS OF THE STUDY**

- To compare the activity of antibiotics against biofilms grown in broth or in ASM.
- To test for the specific influence of ASM components on the antibiotic activity.
- To investigate the importance of P. aeruginosa phenotype on antibiotic activity.

**METHODS**

**Strains** reference PAO1 and stable mucoïd ATCC39324

**Media**: Mueller Hinton Broth (MHB), Artificial Sputum Medium (ASM) [2].

MHB: malt mucin (CAZ, CSE), DNA (CIP, AMK) or egg yolk (MEM) increased MHB to the values observed in ASM. Stronger biofilms were produced in ASM and for the mucoid strain (CV absorbance at day 12: approx. 2 and 4 for PAO1 and MHB and ASM respectively). In most cases, ABSs were less effective (lower EM middle and less potent (higher CD$_{50}$) on biomass than on viability and (i) in ASM than in MHB (except CIP, AMK, MEM [PAO1 only] equipotent for viability in both media), CIP, AMK and MEM (PAO1 only) were globally the most effective and potent drugs in both media but still remained unable to eradicate bacteria in this biofilm model (figures 4).

**MICs** were determined by microdilutions according to CLSI guidelines. MICs were determined as the concentration for which ≤99.9% reduction of the initial inoculum was observed after plating on Tryptic Soy Agar plates.

**Biofilms** were grown for up to 12 days in 96 well plates and then exposed to antibiotics. Quantification of biofilms was obtained by measurement of biomass via crystal violet staining and viability by fluorescein diacetate assay (FDA).

**RESULTS**

**Antibiotic activity** against **metabolic activity and biomass in mature biofilms**

In most of the cases, activity was similar against bacterial viability and biomass.

**Antibiotics** were less active in ASM compared to the standard medium (lower potencies [higher CD$_{50}$ see CD$_{50}$ in Table 3] and lower maximal efficacies against the mucoid strains for some drugs).

None of the tested antibiotics was able to completely eradicate mature biofilms.

**CONCLUSIONS**

- Antibiotic potency against P. aeruginosa growing in biofilms is markedly reduced in artificial sputum medium, possibly due to interactions with specific constituents which impair bactericidal activity.
- Poor bacterial responsiveness in these conditions may contribute to explain persistence of infection in cystic fibrosis patients.
- These data emphasize the importance of selecting appropriate media for testing antibiotic activity in vitro in order to get clinically-meaningful conclusions.

**REFERENCES**


**ANALYSIS**

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**Table 1: MIC/MBC against PAO1 and ATCC 39324 in standard medium and ASM**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
<th>MBC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MHB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIP</td>
<td>0.06</td>
<td>0.3</td>
</tr>
<tr>
<td>AMK</td>
<td>1.5</td>
<td>7</td>
</tr>
<tr>
<td>MEM</td>
<td>2</td>
<td>6.99</td>
</tr>
<tr>
<td><strong>ASM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIP</td>
<td>0.14</td>
<td>0.54</td>
</tr>
<tr>
<td>AMK</td>
<td>0.19</td>
<td>0.28</td>
</tr>
<tr>
<td>MEM</td>
<td>0.39</td>
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</table>

**Table 2: Antibiotic interactions with ASM components**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MHB</th>
<th>AMK</th>
<th>MBC</th>
<th>MHB</th>
<th>AMK</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC</td>
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<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>DNA</td>
<td>1.5</td>
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<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>EGG YOLK</td>
<td>&gt;3</td>
<td>&gt;3</td>
<td>&gt;3</td>
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</tbody>
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