A-052

ICAAC 2014 Washington, DC | September 5-9

Antibiotic (AB) activity against *Pseudomonas aeruginosa* (PA) with Normal or Mucoïd Phenotypes in an Artificial Sputum Medium (ASM) *in vitro* Biofilm Model

E. Bassères, P.M. Tulkens, F. Van Bambeke

Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain - Brussels - Belgium

Françoise Van Bambeke
Pharmacologie cellulaire et moléculaire
av. Mounier 73 B1.73.05
1200 Brussels – Belgium
francoise.vanbambeke@uclouvain.be

ABSTRACT (edited)

Background: PA is the main cause of respiratory failure in cystic fibrosis (CF) patients. Its persistence in the lung is associated with its capacity to form biofilms, against which ABs are considered poorly active. We set up an in vitro model allowing for pharmacodynamic comparison of AB activity against PA biofilms grown in MHB or in ASM (mimicking the physicochemical conditions prevailing in CF patients' lungs).
Methods: PAO1 and ATCC39324 (mucoid strain) were grown in MHB or ASM (see Table) in microplates for up to 12 days, with medium replacement every 48h. They were thereafter exposed to ABs during 24h, using a broad range of concentrations in order to obtain full concentration-response curves and calculate maximal efficacy (E_{max}) and relative potency (C_{50}) by fitting a Hill equation (sigmoid) to the data. Biofilm biomass and bacterial viability within biofilms were quantified using crystal violet (CV) and fluorescein diacetate (PMID: 15567221) assays. In parallel, MBC were determined on planktonic cultures and in both media by CFU enumeration.
Results: MBCs were 4-8 dilutions higher in ASM than in MHB except for MEM against the mucoïd strain. Supplementing MHB with mucin (CAZ, CST), DNA (CIP, AMK) or egg yolk (MEM) increased MBCs 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 6 and 10 for ATCC39324 in MHB and ASM respectively). In most cases, ABs were less effective (lower E_{max}) and less potent (higher C_{50}) (i) on biomass than on viability and (ii) 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 within biofilms (figure 4).
Conclusions: In this model, CIP, AMK and to some extent MEM appear the most active on PA biofilms. ASM constituents reduce bactericidal activity, which may contribute to explain biofilm persistence in CF patients.

INTRODUCTION

P. aeruginosa is an opportunistic pathogen involved in many chronic infections. In cystic fibrosis patients, it persists in the lung as biofilms, in which bacteria are embedded in a matrix and protected from the host immune system.
In these biofilms, *P. aeruginosa* adopts specific phenotypes (metabolically less active, sessile or mucoïd [overproducing exopolysaccharides 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 intrinsic activity of antibiotics.
- To iInvestigate the importance of *P. aeruginosa* phenotype on antibiotic activity.

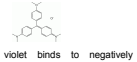
METHODS

Strains: reference PAO1 and stable mucoïd ATCC30324
Media: Mueller Hinton Broth (MHB); Artificial Sputum Medium (ASM) [2]. ASM (1L): mucin 10g, DNA 4g, diethylene triamine pentaacetic acid 5.9mg, casaminoacids 5g, NaCl 4g, KCl 2.2g, tris base 1.81g, egg yolk emulsion 5mL.

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

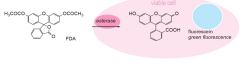
Biofilms were grown for up to 12 days in 96 well plates and then exposed for 24h to antibiotics. **Quantification of biofilms** was obtained by measurement of biomass via crystal violet staining and viability by fluorescein diacetate assay (FDA) [3]

Crystal violet (CV)



Crystal violet binds to negatively charged surface molecules and to polysaccharides in the extracellular matrix. It stains living and dead bacteria as well as the matrix allowing the quantification of the biofilm biomass.

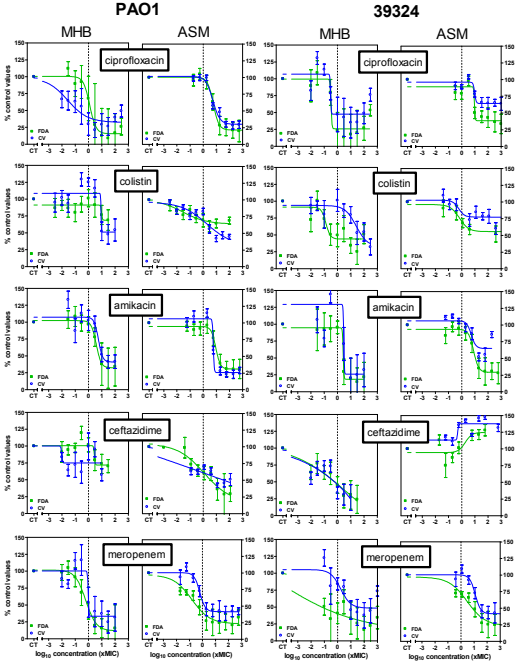
Fluorescein diacetate (FDA)



The quantification of biofilm viability was based on bacterial ability to convert non-fluorescent fluorescein diacetate into highly fluorescent fluorescein by non-specific esterases.

RESULTS

Antibiotic activity on 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 C_{50}; see C_{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.

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

MIC/MBC (mg/L)	MHB		ASM	
	PAO1	39324	PAO1	39324
ciprofloxacin	0.06/0.06	0.06/0.06	1/4	2/16
colistin	1/1	0.5/0.5	1/16	2/32
amikacin	1/1	1/1	2/64	2/16
ceftazidime	1/1	1/1	4/256	2/128
meropenem	0.5/0.5	0.125/0.125	1/8	0.5/0.5

MICs were similar or higher in ASM as compared to MHB

MBCs were markedly higher in ASM than in MHB

Table 2: Antibiotic interactions with ASM components

	PAO1 MBCs in MHB (mg/L)		
	+mucin	+DNA	+egg yolk
ciprofloxacin	0.06	**0.5**	**4**
colistin	**16**	1	1
amikacin	1	**64**	**64**
ceftazidime	**64**	1	1
meropenem	0.5	0.5	**>64**

Mucin, DNA and egg yolk contributed to decrease bactericidal activity.

Table 3: Concentrations (in mg/L) causing 50 % reduction in metabolic activity within biofilms (C_{50})

mg/L	PAO1		39324	
	MHB	ASM	MHB	ASM
ciprofloxacin	0.14	7.94	0.34	20
colistin	13.18	6.9	0.28	>2000
amikacin	5.89	25.76	5.51	21.43
ceftazidime	>30	148.61	2.47	>200
meropenem	0.51	1.47	0.06	3.62

In most of the cases, antibiotic relative potency against viability within biofilms was reduced (higher C_{50}) in ASM.

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

(1) Ciofu *et al.* Phenotypes selected during chronic lung infection in cystic fibrosis patients: implications for the treatment of Pseudomonas aeruginosa biofilm infections. FEMS Immunol Med Microbiol. 2012; 65:215-25
(2) Sriramulu *et al.* Microcolony formation: a novel model of Pseudomonas aeruginosa for the cystic fibrosis lung. J Med Microbiol. 2005; 54 (Pt7):667-76
(3) Wanandy *et al.* Optimisation of the fluorescein diacetate assay. J Microbiol Methods 2005; 60: 21-30

This poster will be made available for download after the meeting at : http://www.facm.ucl.ac.be/posters