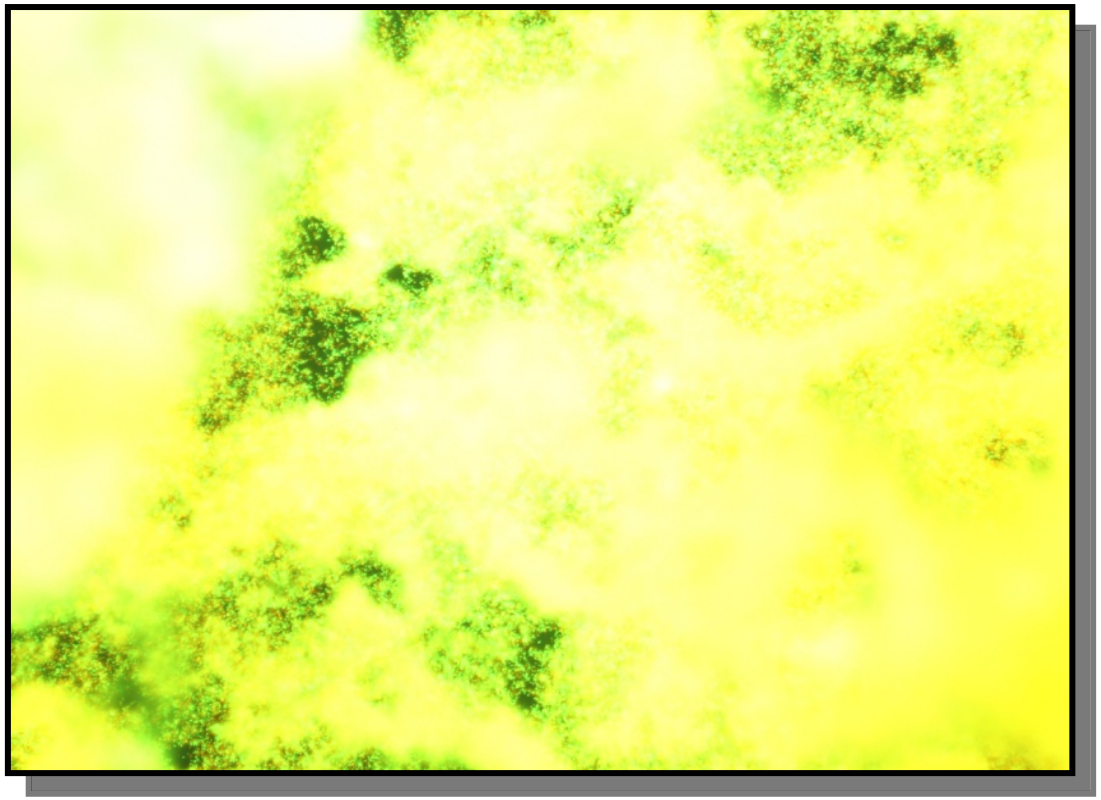
Nathalie M. Vandevelde
Cellular and Molecular Pharmacology

Activity of antibiotics in models of naïve and induced biofilms of *Streptococcus pneumoniae*.

N.M. Vandevelde, P.M. Tulkens, F. Van Bambeke



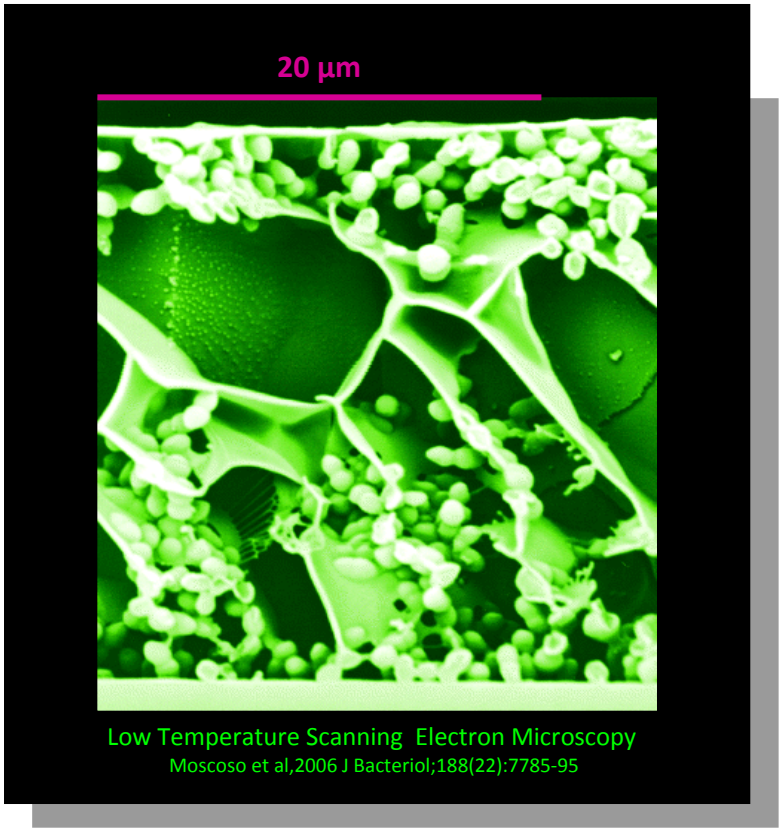
BIBR SYMPOSIUM

Louvain-la-Neuve

20 - 12 - 2013

Biofilms

ex: *S. pneumoniae* - strain R6



Bacterial <u>organized</u> communities :

Bacteria + **Extracellular matrix**

H_2O

Polysaccharides

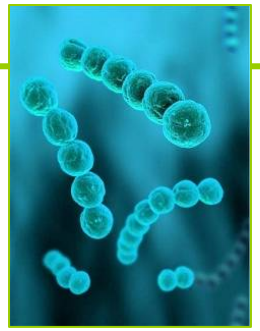
Proteins

Extracellular DNA

on inert or living surfaces
in pathological or healthy situations

Sanctuaries : **protection** ↔ IS + antibiotics

Streptococcus pneumoniae



Gram positive bacterium

Sepsis,
Meningitis,
Pneumonia,…

Sinusitis,
Otitis media,
Acute exacerbations of chronic bronchitis

Over 60% of bacterial infections (and up to 80% of chronic infections) are currently considered to involve microbial growth in biofilms[a]

[a] Moscoso et al, Int Microbiol. 2009 Jun;12(2):77-85.

Pharmacodynamic studies of antibiotic activity

Are non antibiotic drugs responsible of changes in antibiotic activity against S. pneumoniae?

S. pneumoniae Biofilms

Antibiotics

Interactions

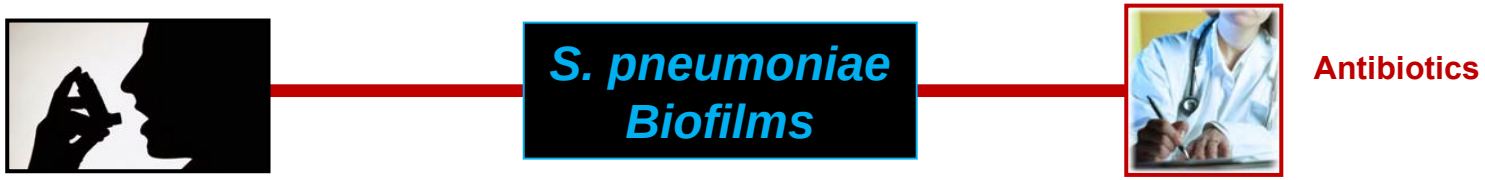
MOXIFLOXACIN

Fluoroquinolone >< DNA replication

Naïve and induced biofilms

Pharmacodynamic studies of antibiotic activity

Are non antibiotic drugs responsible of changes in antibiotic activity against S. pneumoniae?



IPRATROPIUM

Bronchodilator muscarinic antagonist

MOXIFLOXACIN

Fluoroquinolone >< DNA replication

Naïve and induced biofilms

First part:

Set up of naive and induced biofilm models

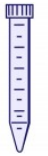
& First pharmacodynamic studies

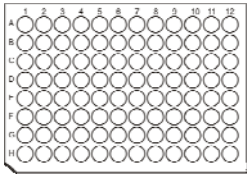
Methodology - biofilm formation and characterization

S. pneumoniae
ATCC 49619

Bacterial suspension

+ Medium
caMHB + 5% LHB

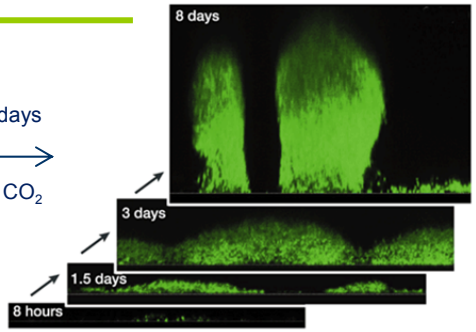




+ Medium
caMHB + 5% LHB + 2% Glucose

up to 11 days

37°C; 5% CO_2



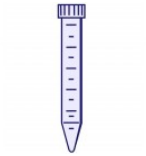
Fuqua and Greenberg, Nat Rev Mol Cell Biol. 2002;3(9):685-95

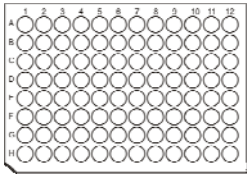
Methodology - biofilm formation and characterization

S. pneumoniae
ATCC 49619

Bacterial suspension

+ Medium
caMHB + 5% LHB

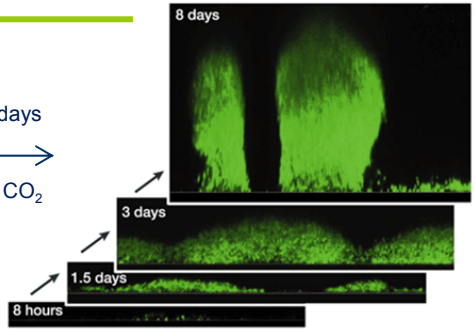




+ Medium
caMHB + 5% LHB + 2% Glucose

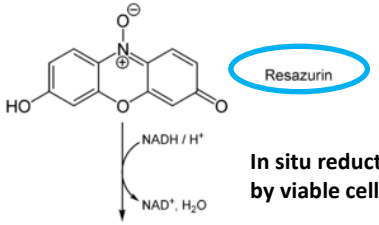
up to 11 days

37°C; 5% CO_2



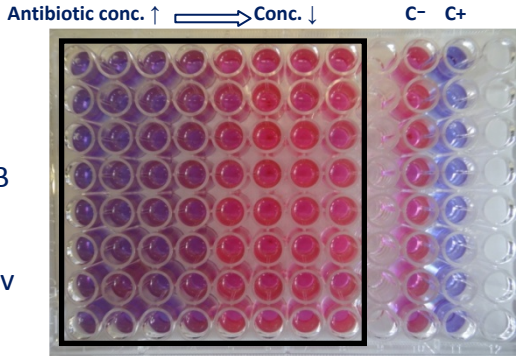
Fuqua and Greenberg, Nat Rev Mol Cell Biol. 2002;3(9):685-95

Bacterial viability within the matrix



In situ reduction by viable cells

Measure of the RF fluorescence
(λexc 560nm; λem 590nm)



C- :
Medium
without AB

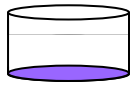
C+:
SDS 1%m/v

Live
↕
Dead

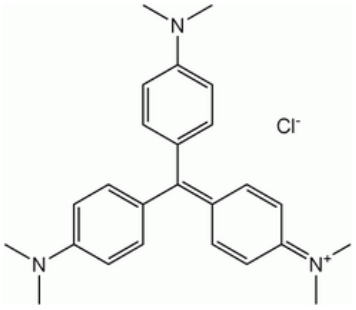
Biofilm thickness

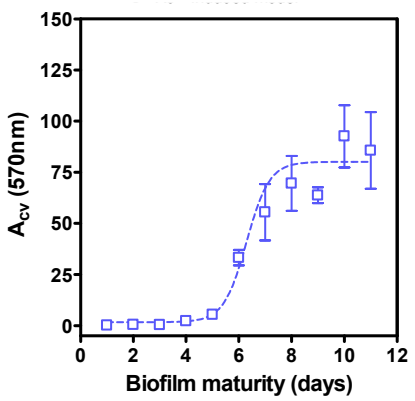
Biofilm staining with **Crystal violet**

Measure of the CV Absorbance



Bacteria
+
Matrix



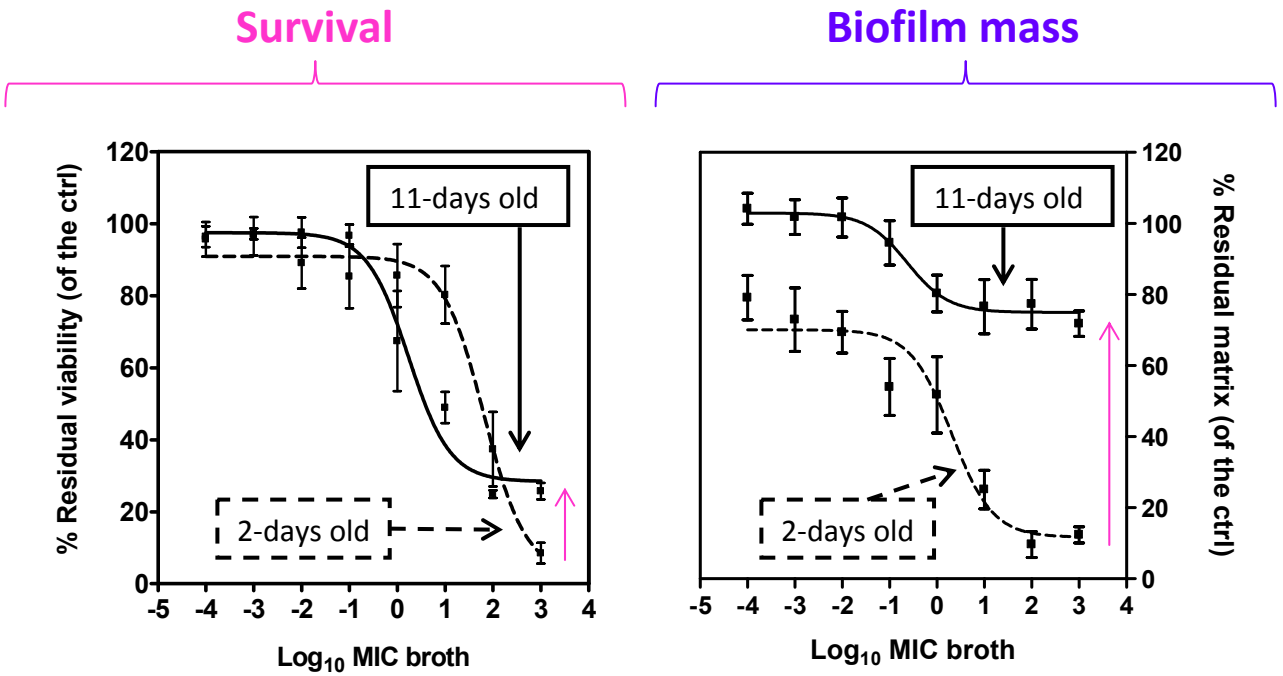


Results expressed in % of the Ctrl

Results : First moxifloxacin pharmacodynamic studies

Ex: ATCC 49619 biofilms
- - - : 2 days-old
— : 11 days-old

Moxifloxacin



Data were used to fit a sigmoid function (Hill equation, slope factor set to 1) by non-linear regression

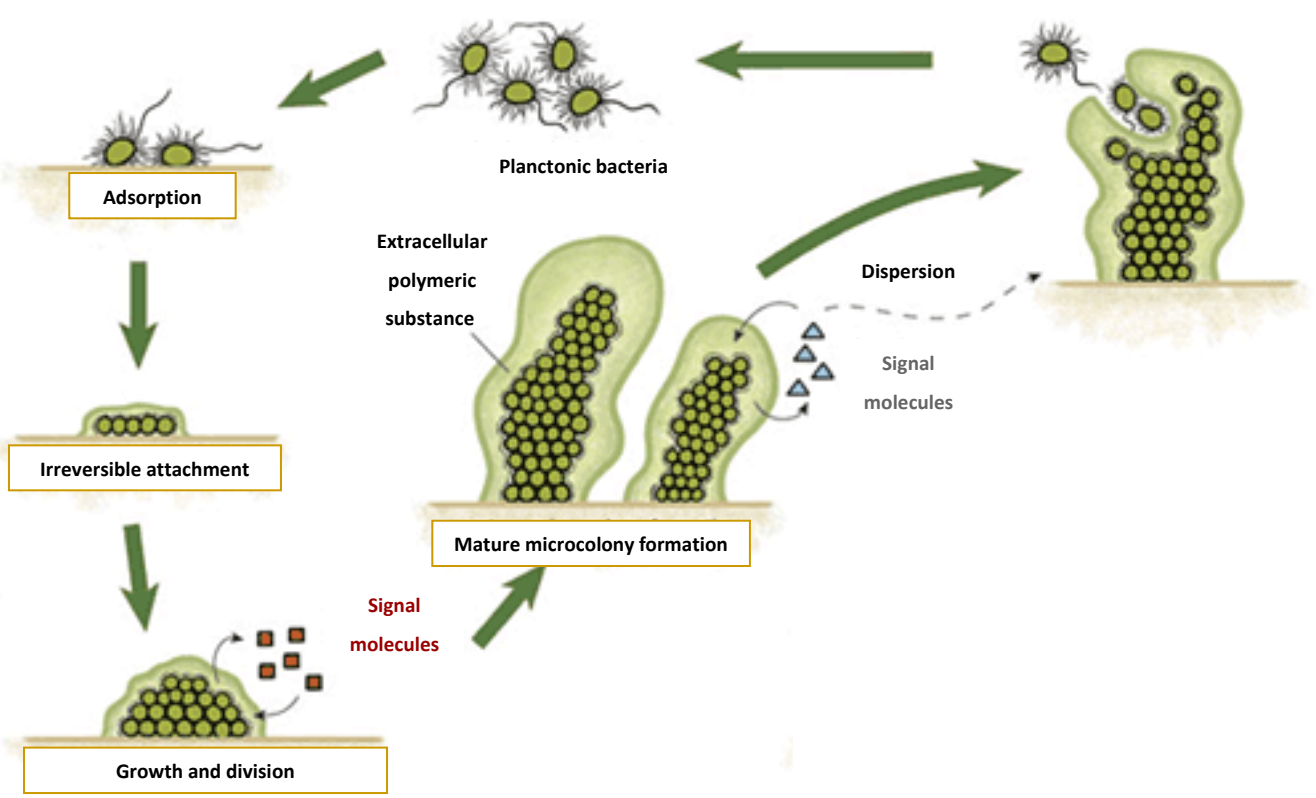
Biofilm model	Effect on bacterial survival		Effect on biofilm thickness	
	E_{max} (% loss of viability with 95% CI)	EC_{50} Concentration (X MIC [mg/L])	E_{max} (% loss of matrix with 95% CI)	EC_{50} Concentration (X MIC [mg/L])
2 days naïve	74.07 (65.42 to 82.72) /A	56,23/A	81.25 (70.63 to 91.87) /A	0.1 /A
11 days naïve	**42.18** (36.11 to 48.25) /B	3,78/B	**20.87** (13.59 to 28.15) /B	**>10^4** /B

Stat. analysis: unpaired, two-tailed t-test for comparisons between maturity stages, values with different letters are significantly different from each other (P<0.05)

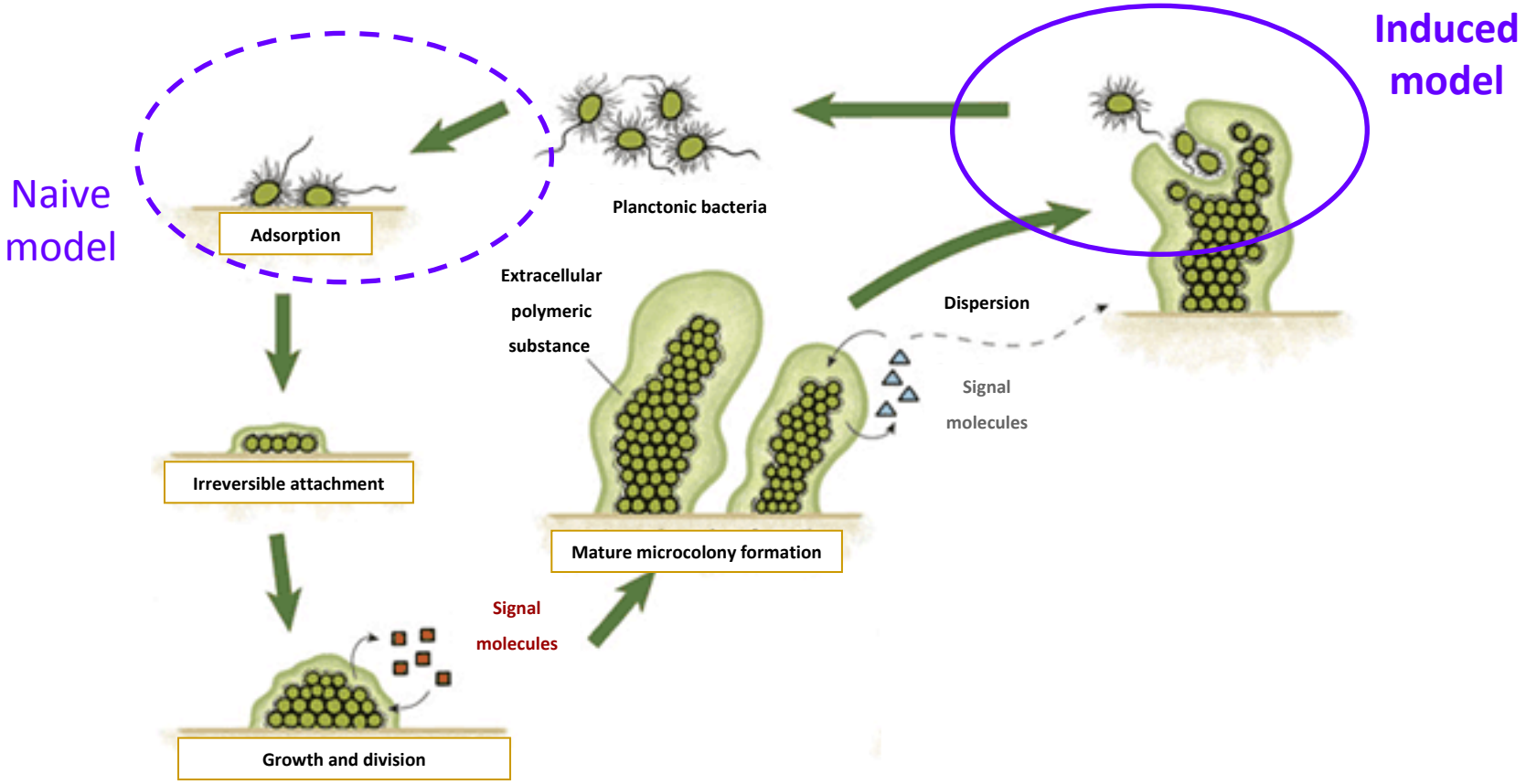
Matrix effect : ↓ Efficacy & Potency

Vandevelde N.M. *et al*, AAC, 2014, in press.

Methodology - 2 biofilm models



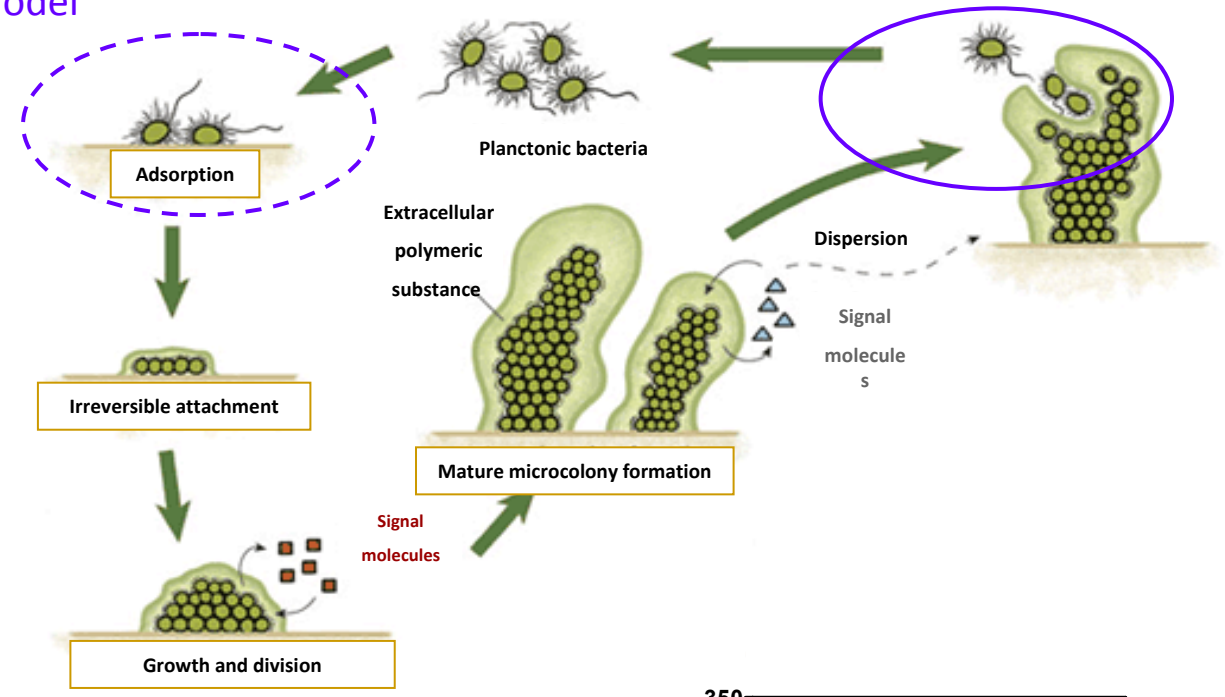
Methodology - 2 biofilm models

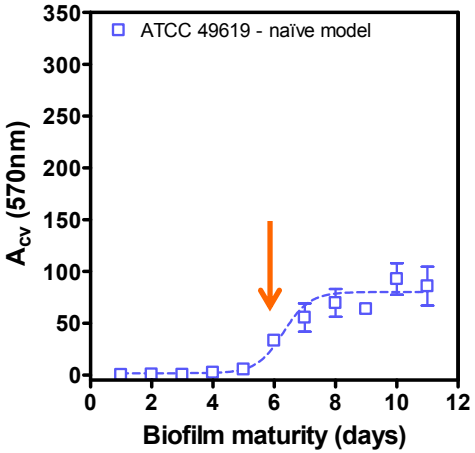


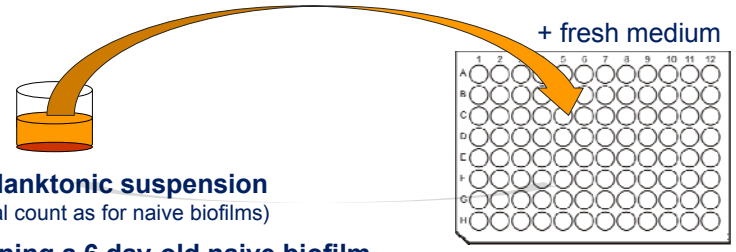
Methodology - 2 biofilm models

Naive model

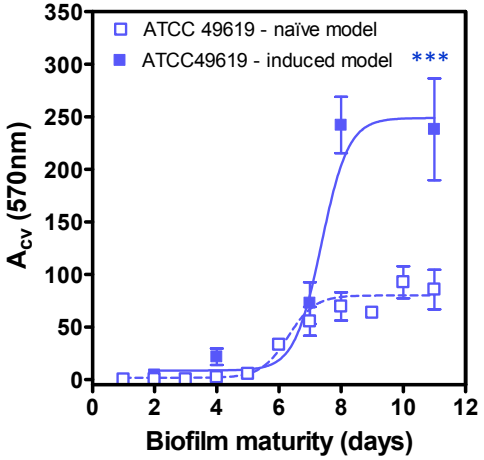
Induced model







bacterial planktonic suspension
(same bacterial count as for naive biofilms)
from a plate containing a 6 day-old naive biofilm



Results : Moxifloxacin pharmacodynamic studies

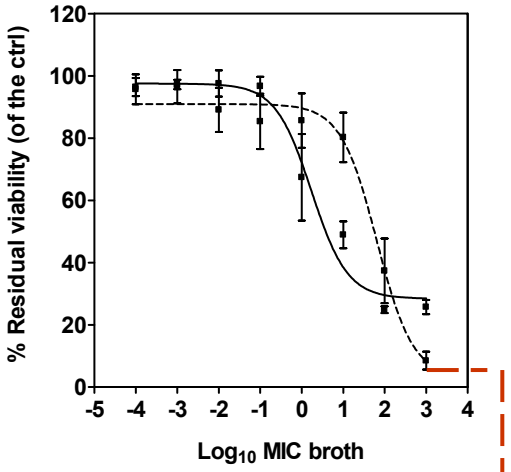
Ex: ATCC 49619 biofilms
- - - : 2 days-old
— : 11 days-old

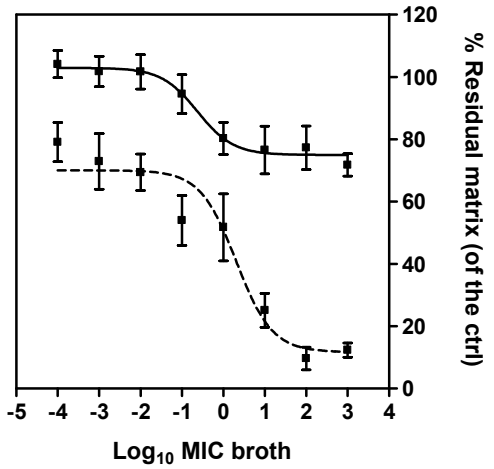
Survival | **Biofilm thickness**

Naive model

Bacterial induction leads to :

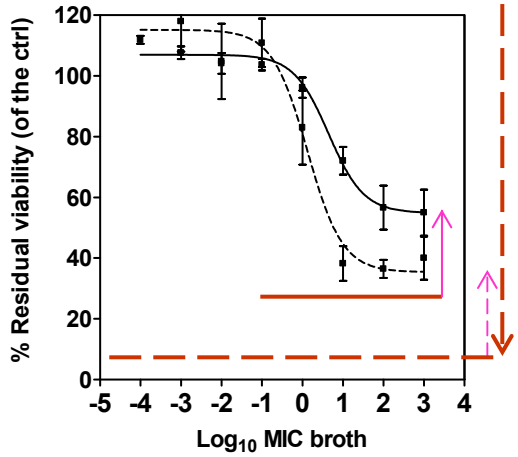
↓ Efficacy (E_{max}) on survival for 2 and 11-days old BF

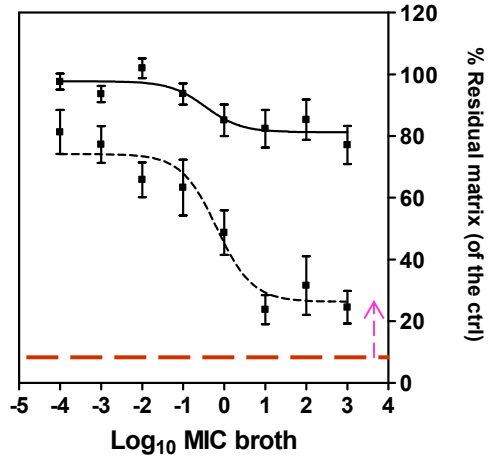




Induced model

and for 2-days old BF against biofim mass





Stat. analysis: unpaired, two-tailed t-test

Vandevelde N.M. *et al*, AAC, 2014, in press.

Second part:

Modulation of moxifloxacin activity against pneumococcal biofilms by bronchodilators

Combinations with Bronchodilators

Are non antibiotic drugs responsible of changes in antibiotic activity against S.pneumoniae biofilms?

BRONCHODILATORS (BD)

Interactions

S. pneumoniae **BIOFILMS**

Interactions

ANTIBIOTICS

1 **Biofilm growth in different media**

Ref. medium "m" : caMHB + 5%LHB + 2% Glc

m + IPRATROPIUM 1.45 ng/ml* (Muscarinic Antag.)

2 **Biofilm treatment in absence of BD**

Ex: MOXIFLOXACIN

Fluoroquinolone >< DNA replication

(*) Concentration in the Bronchoalveolar lavage (recovering bronchial epithelial cells)[a]

PHARMACODYNAMIC STUDIES

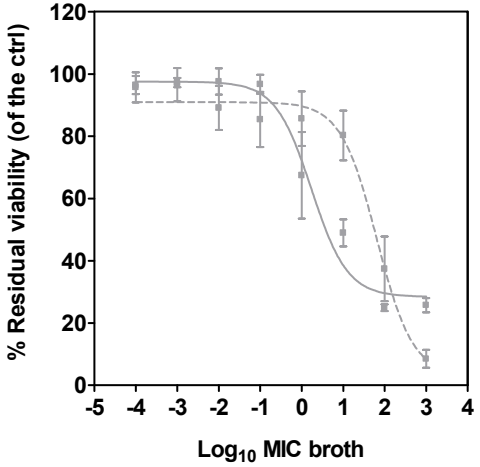
[a] Drug Information Handbook 18th Edition

Results - Combinations with Ipratropium : PD studies of MXF activity

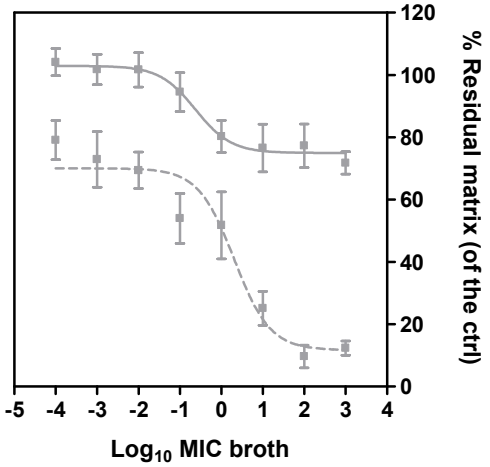
Ex: ATCC 49619 biofilms
- - - : 2 days-old , m
— : 11 days-old , m

Naive model

Survival



Biofilm thickness

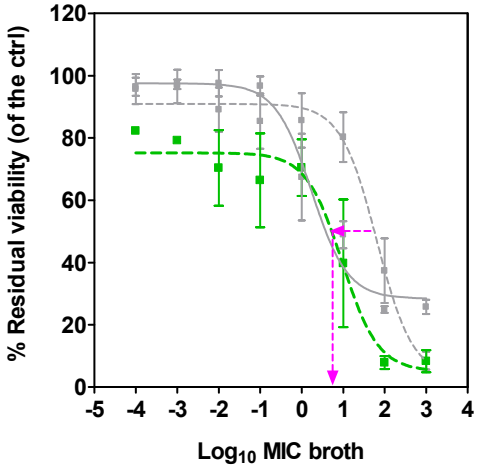


Results - Combinations with Ipratropium : PD studies of MXF activity

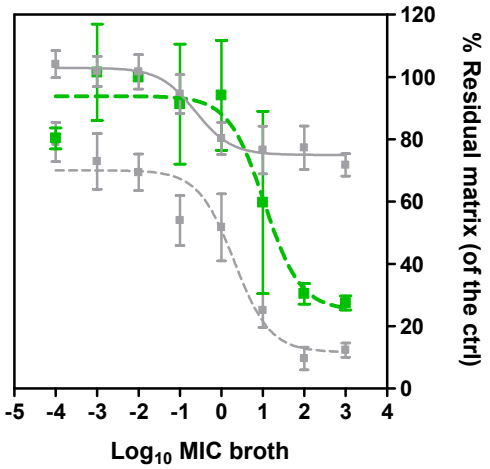
Ex: ATCC 49619 biofilms
- - : 2 days-old , m
— : 11 days-old , m
- - : 2 days-old , m + IPR

Naive model

Survival



Biofilm thickness



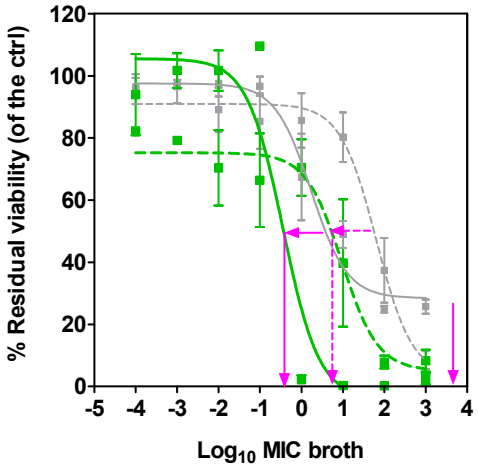
Results - Combinations with Ipratropium : PD studies of MXF activity

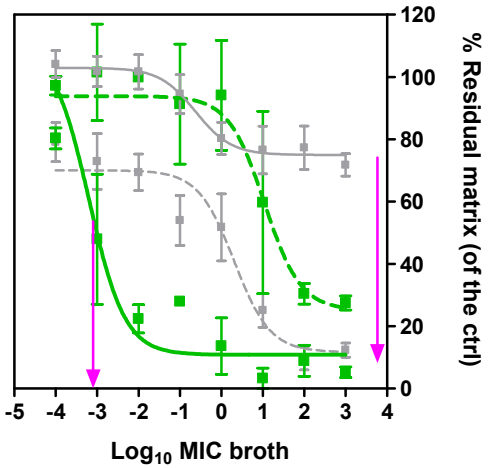
Ex: ATCC 49619 biofilms
- - - : 2 days-old , m
— : 11 days-old , m
- - - : 2 days-old , m + IPR
— : 11 days-old , m + IPR

Survival

Biofilm thickness

Naive model





↑ Efficacy (E_{max}) & Potency (EC_{50})

on survival & biofilm mass

11-days old BF > 2-days old BF

Results - Combinations with Ipratropium : PD studies of MXF activity

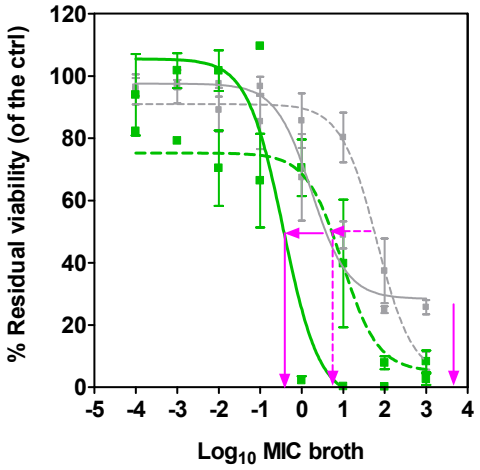
Ex: ATCC 49619 biofilms
- - : 2 days-old , m
— : 11 days-old , m
- - : 2 days-old , m + IPR
— : 11 days-old , m + IPR

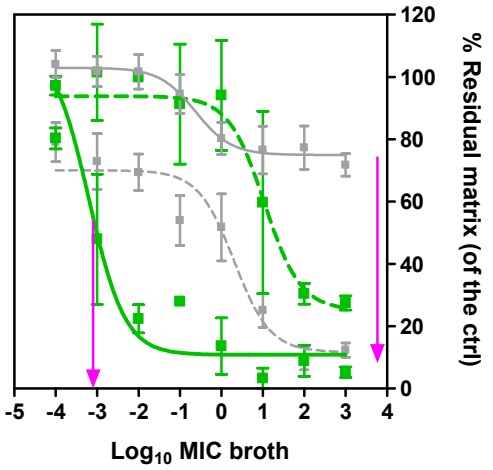
Survival | **Biofilm thickness**

Naive model

↑ Efficacy (E_{max}) & Potency (EC_{50})

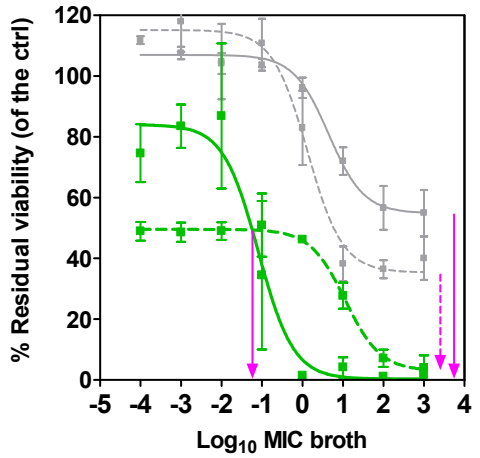
on survival & biofilm mass

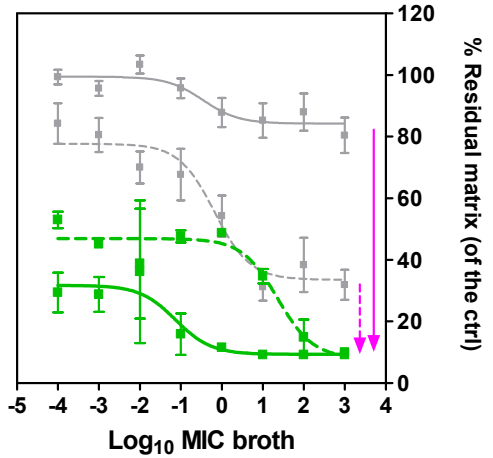




11-days old BF > 2-days old BF

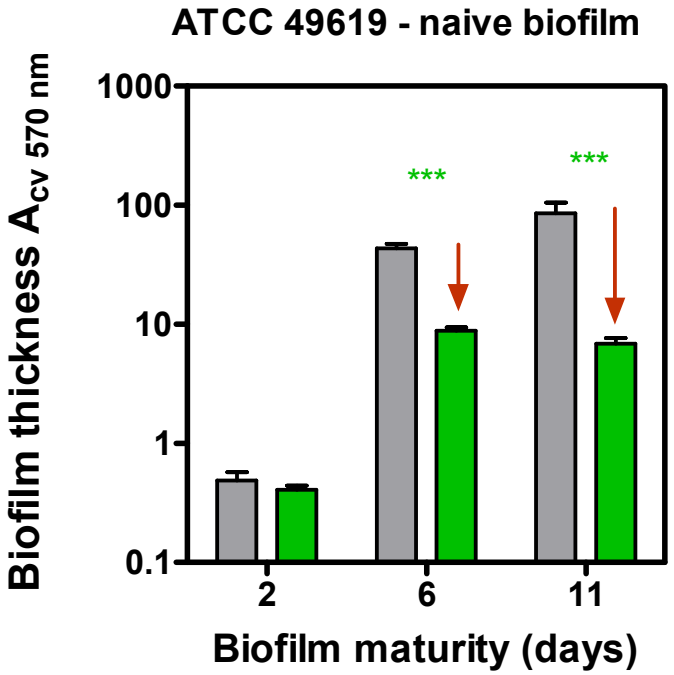
Induced model



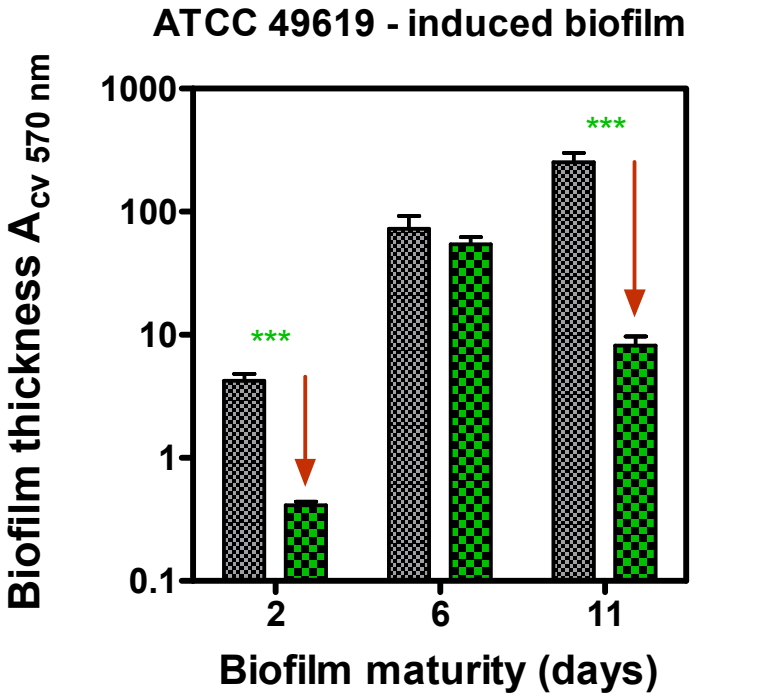


Results - Combinations with Ipratropium : PD studies of MXF activity

: reference medium
: reference medium + IPRATROPIUM

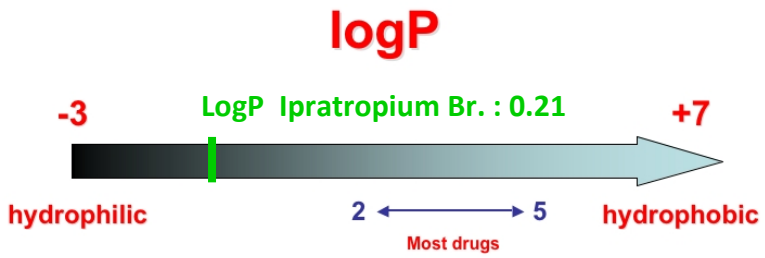


: reference medium
: reference medium + IPRATROPIUM



IPRATROPIUM discards biofilm

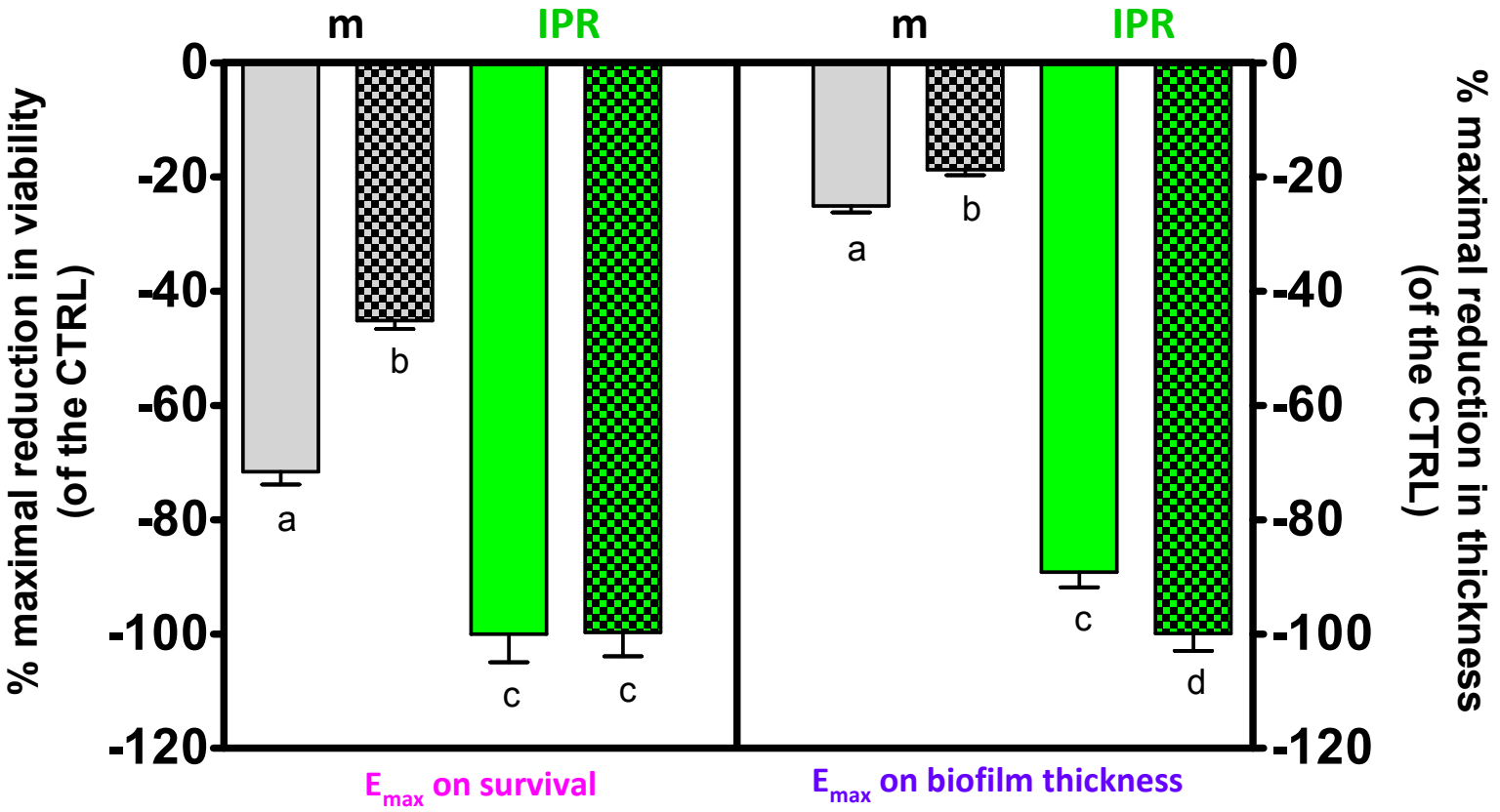
from day 6 in the naive model
from day 2 in the induced model



Stat. analysis: unpaired, two-tailed t-test

Summary

Moxifloxacin maximal efficacies on bacterial survival and matrix thickness for 11-days old naive (open bars**) and induced (**squared bars**) biofilms**



Stat. analysis: One way Anova with Tuckey post test

Take home message

Matrix effect reducing antibiotic activity

Old biofilm maturity stages and bacterial induction

Ipratropium, by targeting biofilm mass, highly increases antibiotic activity on survival and matrix of residual structure

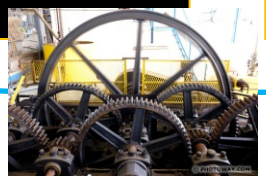
***In vivo* Model for Polytherapies**

Appropriate Co-medications

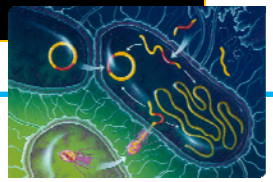
Last word to you…

Belgian Interdisciplinary Biofilm Research

How biofilms form



How resistance works

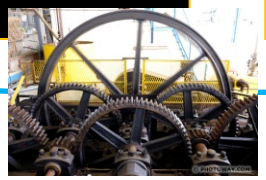


How resistance spreads

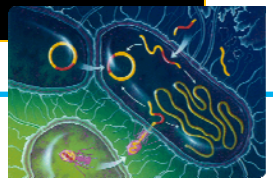
Last word to you…

Belgian Interdisciplinary Biofilm Research

How biofilms form



How resistance works



How resistance spreads

Together, let's start the March towards Biofilms!

Acknowlegment

All the Cellular and Molecular Pharmacology Research Group members and especially,

Pr. Françoise Van Bambeke (promotor) & Pr. Paul M. Tulkens

UCL Université catholique de Louvain

LDRI LOUVAIN DRUG RESEARCH INSTITUTE

Belgian Interdisciplinary Biofilm Research

fnrs FREEDOM TO RESEARCH

FRIA


Thank you !!!