Relationship between antibiotic susceptibility to β-lactams (BL), clarithromycin (CLR), and fluoroquinolones (FQ) of *Streptococcus pneumoniae (Sp)* isolated from COPD patients and the ability to produce mature biofilm in an *in vitro* model.

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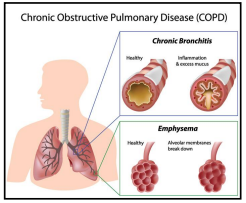
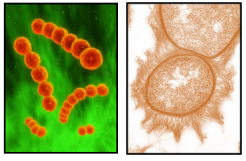




Background

COPD patients suffer from recurrent infections of the respiratory tract (**1,5**), with *Streptococcus pneumoniae* being one of the major causative organisms (**2**). Isolates from COPD patients show often reduced susceptibility to antibiotics (**3,6**). Yet, little is known about their propensity of these resistant strains to form biofilms, a specific lifestyle associated with persistent infections, as compared to fully susceptible strains.

Aim of the study

We examined the propensity of clinical strains isolated from COPD patients to form biofilm, in relationship with their susceptibility to three β-lactams, one macrolide and two fluoroquinolones.

Methods

- Strains: 38 non-duplicate clinical isolates from patients with clinically confirmed COPD.
- Susceptibility testing: MICs determined by microdilution (CLSI method).
- Biofilms: 10 days stationary culture in 96-well plates, with biomass quantified by crystal violet (CV) staining[4] (OD_{570nm}) , in comparison with ATCC49619 (susceptible reference strain).
- Recursive partitioning analysis (RPA) of biomass vs. MIC performed using JMP software (version 9).

Results

- As illustrated for the reference strain ATCC 49619 in Figure 1, biofilm mass started to increase after 5 days of culture to reach a maximum after 8 days.
- As shown in the Table, MIC values ranged was from highly susceptible to fully resistant isolates (EUCAST breakpoints) for all antibiotics except ceftriaxone.
- Strains with low MICs for β-lactams or macrolides produced more biofilm (see Figure 2), with optimal MIC split value separating high and low producers being systematically lower than the "S" EUCAST breakpoint (see Table for values) except for clarithromycin. In contrast, there was no significant difference in biomass vs. lower and higher MIC for fluoroquinolones.

Conclusion

- In vitro biofilm formation and decreased susceptibility to β-lactams or macrolides were inversely correlated in this collection of *S. pneumoniae* from COPD patients.
- These data indicate that the 2 properties do not combine to explain the recurrent character of infections in patients receiving these antibiotics. They also suggest physiologic changes in *S. pneumoniae*, reducing its capacity to form biofilm when becoming less susceptible β-lactams or macrolides, but not to fluoroquinolones.

References

[1]Anthonisen et al, Ann Intern Med 1987 106: 196-204; [2]Eller et al, Chest 1998 113: 1542-8 ; [3]Moscoso *et* al, Int Microbiol. 2009 Jun;12(2):77-85; [4]Roveta et al, Int J Antimicrob Agents. 2007 Nov;30(5):415-21; [5]Sin et al, 2006 Eur Respir J 28: 1245-57; [6]Simoes, Curr Med Chem. 2011;18(14):2129-45

Figure 1: Biofilm production over time by strain ATCC 49619, as evaluated by CV absorbance. Data are mean ± SD of 6 values.

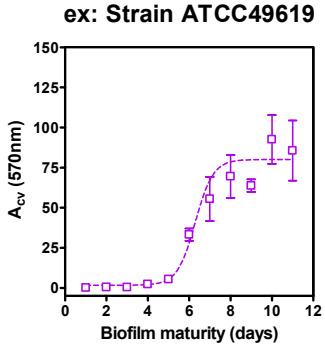
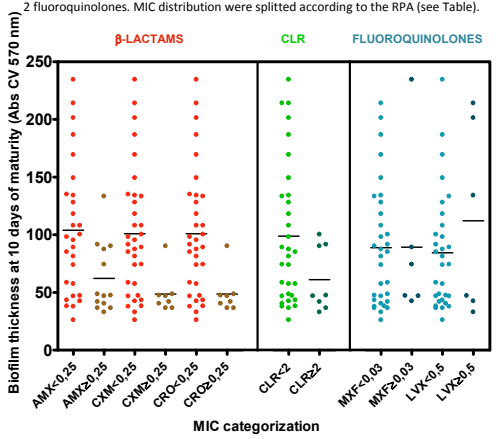


Figure 2: Comparison of biofilm production after 10 days of culture for *S. pneumoniae* strains from COPD patients in relation with their MICs to 3 β-lactams, a macrolide (clarithromycin) and 2 fluoroquinolones. MIC distribution were splitted according to the RPA (see Table).



Recursive partitioning analysis of biofilm formation (10 days) by *S. pneumoniae* strains with increasing MIC (n= 38)

ANTIBIOTICS	MIC range (mg/L)	Optimal MIC split value (mg/L) [a]	Logworth value [b]	BIOFILM MASS [c] below / above MIC split value (n)	p value [d]
Amoxicillin (AMX)	0.016 to >16	< 0.25 / ≥ 0.25	0.697	102.9 ± 60.7 (25) / 62.3 ± 30.5 (13)	0.0045 *
Cefuroxime (CXM)	0.002 to > 4	< 0.25 / ≥ 0.25	0.936	99.7 ± 57.3 (30) / 48.7 ± 17.5 (8)	0.0002 *
Ceftriaxone (CRO)	0.002 to > 0.5	< 0.25 / ≥ 0.25	0.936	99.7 ± 57.3 (30) / 48.7 ± 17.5 (8)	0.0002 *
Clarithromycin (CLR)	0.004 to > 4	< 2 / ≥ 2	0.239	96.4 ± 58.8 (30) / 61.1 ± 27.9 (8)	0.0234 *
Levofloxacin (LVX)	0.25 to > 16	< 0.5 / ≥ 0.5	0.150	84.4 ± 50.2 (31) / 109 ± 75.9 (7)	0.4408 (NS)
Moxifloxacin (MXF)	0.016 to 2	< 0.03/ ≥ 0.03	0.091	88.9 ± 52.9 (32) / 89.2 ± 73.6 (6)	0.9934 (NS)

[a] values of MIC separating data sets in 2 categories based on minimization of the sum of squared errors across the whole data as a function of the MIC (further splitting was unsuccessful because of the limited number of independent values) EUCAST S and R breakpoints (mg/L) are = or < 0.5 and > 2 for AMX, CXM [i.v.], and CRO, = or < 0.25 and > 0.5 for CLR), and > 2 for LVX, and > 0.5 for MXF (no I value for these fluoroquinolones).

[b] node splitting is based on the LogWorth statistic (higher values denote a higher significance in the split decision tree)

[c] quantification by crystal violet staining (triplicates) and expressed in % (mean ± SD) of the value of the reference strain ATCC49619

[d] unpaired t test, Welch corrected, to compare biofilm mass values below and above the MIC split value

These results are only applicable to the tested strains

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