Biological features of *P. aeruginosa* biofilms: time to rethink the actual antimicrobial practices in clinical settings

Sousa, A.M., Rodrigues, A., Pereira, M.O.

IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho,Campus de Gualtar 4710-057, Braga, Portugal

E-mail: anamargaridasousa@deb.uminho.pt

Introduction

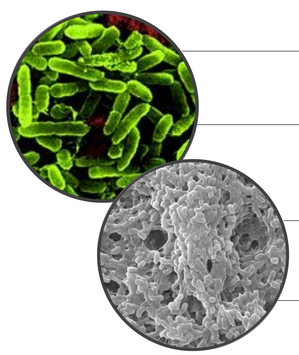
Pseudomonas aeruginosa is a well known opportunistic pathogen, responsible for high morbidity and mortality in several human infections. Such success is mainly due to its metabolic versatility, phenotypic plasticity and high degree of genomic flexibility. Furthermore, the infection accomplishment in human host is partly due to their growth within biofilms. Once within biofilms, *P. aeruginosa* can augment its pathogenicity and improve its ability to survive to stressful conditions, such host immune defenses and antibiotics action.

Although clinical guidelines have changed taking in account the presence of biofilms, they consider biofilm features identical to whole *P. aeruginosa* species. For instance, since *P. aeruginosa* biofilm-associated infections are diagnosed, clinical guidelines pointed out the administration of ciprofloxacin as the empirical or first line therapy and colistin the last-resort therapy option, regardless the strain and its biofilm pathogenicity traits.

Regarding the impact of *P. aeruginosa* biofilms in antibiotic resistance, it was considered crucial to deeply study biofilms formed by several strains, isolated from different human sites, and to know their responses to relevant antibiotics.

AIM: To deep characterize virulence and antibiotic resistance profiles of *P. aeruginosa* cells recovered from in vitro biofilms developed by several clinical strains.

Methods & Materials



Seven *P. aeruginosa* strains

PAO1, ATCC 10145, CECT 111, PA14, PAI1, PAI2, PAI3

Biofilm formation

96-well plates, TSB, 24 h, 37 ºC

Biofilm quantification

Crystal Violet, absorbance 570 nm

Characterization of biofilm-associated bacteria

Antibiotic susceptibility

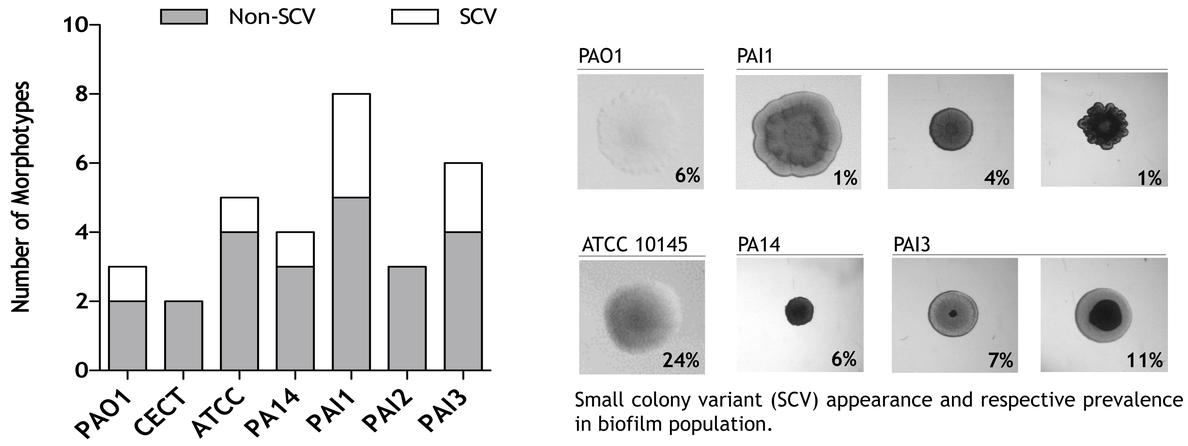
Virulence factors expression

Biofilm-population colony diversity

Results

- Virulence factors expression

P. aeruginosa strain	Biofilm Formation Ability	Hemolysin production	Motility			Slime Production
			Swimming	Swarming	Twitching	
PAO1	Good	+	1.19 ± 0.08	0.94 ± 0.12	0.77 ± 0.07	-
CECT 111	Moderate	-	1.12 ± 0.19	0.87 ± 0.07	0.89 ± 0.06	-
ATCC 10145	Moderate	+	0.86 ± 0.21	0.80 ± 0.10	0.60 ± 0.10	-
PA14	Weak	+	3.22 ± 0.46	1.28 ± 0.11	1.33 ± 0.15	+
PAI1	Weak	+	0.52 ± 0.08	0.82 ± 0.08	0.54 ± 0.05	+++
PAI2	Moderate	+	2.88 ± 0.59	1.94 ± 0.53	0.86 ± 0.05	+
PAI3	Moderate	+	1.39 ± 0.12	1.39 ± 0.16	0.79 ± 0.12	+



Small colony variant (SCV) appearance and respective prevalence in biofilm population.

- Antibiotic susceptibility

Strain	MIC (mg/L)				MBC (mg/L)		MBEC (mg/L)	
	Colistin		Ciprofloxacin		Colistin	Ciprofloxacin	Colistin	Ciprofloxacin
PAO1	2	S	4	R	16	8	>16	>16
CECT 111	2	S	4	R	16	8	>16	>16
ATCC 10145	2	S	4	R	8	8	>16	>16
PA14	2	S	4	R	16	8	>16	>16
PAI1	2	S	4	R	>16	16	>16	>16
PAI2	2	S	4	R	8	>16	>16	>16
PAI3	2	S	8	R	>16	>16	>16	>16

S - susceptible strain, R - resistant strain, according EUCAST clinical breakpoints.

Conclusions

- It was not observed a common *P. aeruginosa* biofilm profile as implicit in current clinical guidelines;
- Virulence factors expression was highly dependent of the genetic background of *P. aeruginosa* strain; administration of ciprofloxacin to control or eradicate *P. aeruginosa* biofilm-cells seemed obsolete;
- Medical decision about antibiotic administration should regard the virulence factors expressed by the strain isolated, the antibiotic resistance and diversity of biofilm-phenotypes including SCV.

Acknowledgments: The financial support from IBB-CEB and Fundação para a Ciência e Tecnologia (FCT) and European Community fund FEDER, trough Program COMPETE, in the ambit of the project PTDC/SAUSAP/113196/2009 / FCOMP-01-0124-FEDER-016012 and Ana Margarida Sousa PhD Grant (SFRH/BD/72551/2010) are gratefully acknowledged.