Biofilm Production as a Potential Risk Factor of *Pseudomonas aeruginosa* Bacteremia in Patients with Hematologic Malignancies

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Abstract

Pseudomonas aeruginosa is a major cause of bloodstream infection (BSI) and a high risk of End-Organ disease in patients with hematologic malignancies. Treatment of BSIs caused by *P. aeruginosa* is very challenging with mortality rates that range from 18% to 62%. The increasing incidence of multidrug-resistant *P. aeruginosa* is a global problem and a major risk of BSIs. The persistence of chronic *P. aeruginosa* infections is due in many cases to biofilm-growing strains. Biofilms produced by P. aeruginosa are highly tolerant to environmental and chemical agents, including antibiotics and therefore, difficult to eradicate. Despite its importance, microbial biofilm it is not assessed in clinical microbiology. Further, antibiotic–resistance tests are performed on planktonic cells and do not take into account biofilm-growing microorganisms. Thus, the resulting antibiotic susceptibility profiles might not be representative of the bacterial drug susceptibility *in vivo*.

Objectives

The project aims at defining risk factors associated with *P. aeruginosa* BSI in patients with hematological malignancies and to evaluate the clinical outcome at 30 days after the onset of bacteremia. To this purpose, specific objectives include:

1. The assessment of the role of biofilm production and antibiotic tolerance at sustaining *P. aeruginosa* invasion and persistence in patients with hematologic malignancies
2. The assessment of the contribution of different bacterial virulence factors including alkaline protease, elastase A, elastase B, haemolytic phospholipase C, non-haemolytic phospho-lipase C, exotoxin A and alginate and motility

Preliminary data

Ongoing studies in 23 *P. aeruginosa* strains, isolated from patients with pulmonary diseases, medical device-associated infections and surgical site infections (SSI) revealed that more than 90% of these strains are moderate/high biofilm producers (Fig. 1). In addition, the efficacy of antimicrobial agents against two strains of *P. aeruginosa*, isolated from patients with catheter-related blood stream infections, classified as high biofilm-producers were assessed in both planktonic (antibiogram) and biofilm (Anti-Biofilm Test - ABT) growth. The results, expressed as minimum inhibitory concentration (MIC) and biofilm MIC (BMIC), respectively, showed that biofilm promoted a consistent reduction of the effective antibiotic options (Fig. 2).

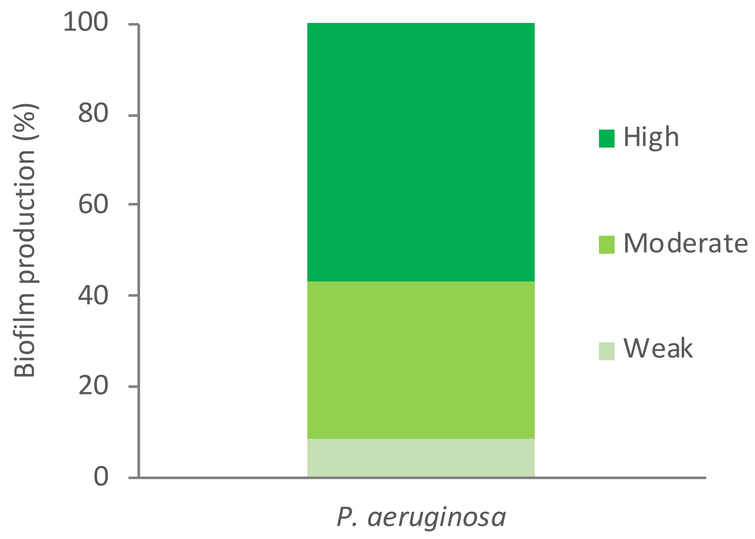

Figure 1. Biofilm production assessed on 23 *Pseudomonas aeruginosa* strain isolated from clinical isolates were classified as weak, moderate and high biofilm producers by the cBRT. Data are expressed as percentage.

High Biofilm Producer				
Antimicrobials	**MIC (mg/l)**	**INT**	**BMIC (mg/l)**	**INT**
Amikacin	≤ 2	S	≤ 4	S
AMC	-	-	-	-
Cefepime	4	S	> 32	R
Ceftazidime	4	S	32	R
Ciprofloxacin	≤ 0.25	S	1	R
Colistin	≤ 0.5	S	> 8	R
Gentamicin	≤ 1	S	2	S
Imipenem	1	S	> 16	R
PIT	8	S	> 128	R
TMP/SMX	-	-	-	-

High Biofilm Producer				
Antimicrobials	**MIC (mg/l)**	**INT**	**BMIC (mg/l)**	**INT**
Amikacin	≤ 2	S	≤ 4	S
AMC	-	-	-	-
Cefepime	≤ 1	S	> 32	R
Ceftazidime	2	S	> 4	R
Ciprofloxacin	≤ 0.25	S	0.125	S
Colistin	≤ 0.5	S	> 8	R
Gentamicin	≤ 1	S	2	S
Imipenem	1	S	> 16	R
PIT	≤ 4	S	> 128	R
TMP/SMX	-	-	-	-

Figure 2. Representative susceptibility profiles of *Pseudomonas aeruginosa* strains isolated from patients with bloodstream infections as determined by minimum inhibitory concentration (MIC) and biofilm MIC (BMIC). Both strains were classified by the cBRT as high biofilm-producers. Results are expressed as a concentration (mg/l). Susceptible (S) and resistant (R) strains to different antimicrobials as according to the European Committee on Antimicrobial Susceptibility Testing clinical breakpoint, table v 8.1. INT = Interpretation; AMC = Amoxicillin/clavulanic acid; PIT = Piperacillin/Tazobactam; TXP/SMX = Trimethoprim/Sulfamethoxazole.

Future research

The timely recognition of biofilm production as well as other specific factors, presenting a risk of death among patients with *P. aeruginosa* infection, may lead to a more effective decision-making in patient management by the medical team, with earlier and better-targeted interventions