Persistence likely associated to biofilm forming Pseudomonas spp. isolated from patients on carbapenem treatment

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Objectives: Infections caused by *Pseudomonas* spp. are often found in hospital patients, being carbapenems (such as imipenem and meropenem) usually used as treatment. *Pseudomonas P. fulva* and *P. monteilii* species are uncommon in patients and few data is available regarding its virulence. To assess the potential clinical concern of these species we selected isolates recovered from ICUs and evaluated its virulence and biofilm persistence in the human host.

Methods: *P. fulva* and *P. monteilii* isolated in 2012 were analyzed (**Table 1**). Pulsed-Field Gel Electrophoresis (PFGE) and Minimum Inhibitory Concentrations (MICs) were performed. *Caenorhabditis elegans* killing assay was made using *P. aeruginosa* (PAO1) and *E. coli* (OP50) as controls. Biofilm was quantified after 48h incubation, followed by crystal violet staining. Minimum Biofilm Inhibitory Concentration (MBIC) and Minimum Biofilm Eradication (MBEC) assays were performed after 24h of imipenem/meropenem challenge.

Table 1. Clinical data from patients with *P. fulva* and *P. monteiilii* isolates.

		P. fulva (n=13)	*P. monteilii* (n=4)
No. ICU patients		13	1
Previous carbapenem treatment			
	Meropenem	4	0
	Unknown	1	4
Gender			
	Male	7	0
	Female	5	0
	Unknown	1	4
Age			
	35-49 years	1	0
	50-65 years	6	0
	>65 years	6	0
	Unknown	0	4
ICU Country of origin			
	Belgium	13	0
	Germany	0	4
Sample origin			
	Oropharynx	7	0
	Stool	6	4

Results: *P. fulva* isolates belonged to the same clonal lineage and the *P. monteilii* isolates were also clonal. All strains were susceptible to imipenem (MIC range of 0.5-1mg/L) and presented reduced susceptibility to meropenem (ranging 2-3mg/L and 1.5-3mg/L in *P. fulva* and *P. monteilii*, respectively).

The lifespan of *C. elegans* showed that only *P. aeruginosa* PAO1 control strain was significantly more virulent when compared with the avirulent OP50 *E. coli* (LT50 3.5 days vs 7.5 days) while *P. monteilii* and *P. fulva* were not (**Figure 1**).

All strains formed biofilm and *P. fulva* produced almost as much biofilm as *P. aeruginosa* control strain (3.1% less, while *P. monteilii* 46.4% less). After imipenem/meropenem challenge, *P. fulva and P. monteilii* biofilm inhibition was similar to the MIC, while the MBEC were much higher for both species. Both MBIC and MBEC values were independent of sample origin or carbapenem treatment (**Table 2**).

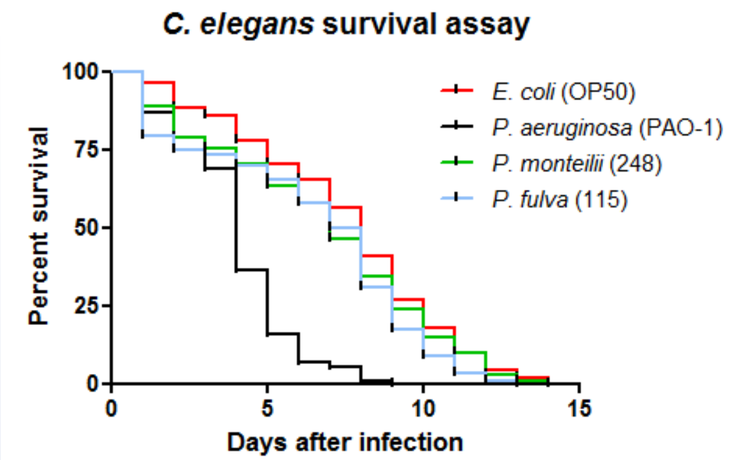

Figure 1. *C. elegans* survival curves.

Table 2. Minimum Biofilm Inhibitory Concentration (MBIC) and Minimum Biofilm Eradication (MBEC) of *P. fulva* and *P. monteiilii selected* isolates.

					Imipenem			Meropenem		
Strain	**Patient Id**	**Previous Carbapenem treatment**	**Sample origin**	**PFGE Pattern**	**MIC mg/L**	**MBIC mg/L**	**MBEC mg/L**	**MIC mg/L**	**MBIC mg/L**	**MBEC mg/L**
***P. fulva* 116**	A	No	Stool	A	0.5	0.5	64	2	2	>128
***P. fulva* 120**	B	Yes	Stool	A	0.5	0.25	128	3	2	>128
***P. fulva* 128**	C	Yes	Stool	A	0.5	0.25	>128	2	2	>128
***P. fulva* 115**	D	Yes	Oropharynx	A1	0.75	0.5	>128	2	2	>128
***P. fulva* 119**	E	No	Oropharynx	A2	0.5	0.5	>128	2	2	>128
***P. fulva* 145**	F	No	Oropharynx	A3	0.5	0.25	64	2	2	8
***P. monteilii* 247**	G	-	Stool	B	1	128	>128	2	2	>128
***P. monteilii* 248**	G	-	Stool	B	1.5	8	32	1.5	1	64
***P. aeruginosa* PAO1**	-	-	-	-	1	1	>128	0.25	1	>128

Conclusion: Although *P. fulva* or *P. monteilii* isolated in ICUs may not be virulent, they form biofilms which require very high carbapenem concentrations to be eradicated, explaining why these microorganisms may persist in the intestinal tract in patients who have taken carbapenems.

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