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

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Background: 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* (PF) and *P. monteilii* (PM) 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.

Material/methods: Thirteen PF strains isolated from the oropharynx (n=7) and stool (n=6) of 13 patients in Belgium (four patients on carbapenem treatment) and four PM isolated from stool of a patient in Germany, were analyzed. Pulsed-Field Gel Electrophoresis (PFGE) and Minimum Inhibitory Concentrations (MICs, determined by E-test) were performed. *Caenorhabditis elegans* killing assay was made using *P. aeruginosa*-PAO1 (PA) and *E. coli*-OP50 as control strains. Biofilm production was quantified after 48h incubation at 37ºC, followed by crystal violet staining. Minimum Biofilm Inhibitory Concentration (MBIC) and Minimum Biofilm Eradication (MBEC) assays were performed after 24h of imipenem challenge.

Results: PF isolates belonged to the same clonal lineage and the PM 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 PF and PM, respectively). The lifespan of *C. elegans* showed that only PA control strain was significantly more virulent when compared with the avirulent *E. coli* (LT50 3.5 days vs 7.5 days) while PM and PF were not. All strains formed biofilm and PF produced almost as much biofilm as PA (3.1% less, while PM 46.4% less). After imipenem challenge, PF biofilm inhibition was similar to the MIC (MBIC range 0.25-0.5 mg/L), while it was much higher for PM (range 8-128mg/L) and MBEC were high for both species (range 32->128mg/L). Both MBIC and MBEC values were independent of sample origin or carbapenem treatment.

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