Comparative study of biofilm formation, pigment production, elastase activity, motility and antimicrobial resistance among *Pseudomonas aeruginosa* isolates recovered from different origins

ysaenz@riojasalud.es

Yolanda Sáenz[1]; Eugénie Bassères[2]; Hussein Chalhoub[2]; Ahalieyah Anantharajah[2]; Cristina Casado[1,3]; Vanesa Estepa[3]; Beatriz Rojo-Bezares[1]; Françoise Van Bambeke[2].

[1] Área de Microbiología Molecular, Centro de Investigación Biomédica de La Rioja (CIBIR), Logroño, Spain. [2] Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium. [3] Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain.

INTRODUCTION

Pseudomonas aeruginosa is an opportunist pathogen responsible for worldwide nosocomial infections, with great metabolic versatility and extraordinary ability to grow in varied and daily environments (such as soil, water, food, animal tissues, so on), from which it is easily transmissible[1]. *P. aeruginosa* presents high intrinsic resistance to several antibiotics, multiple virulence factors, and an extraordinary capacity to acquire new resistance mechanisms[1,2]. In addition, the dissemination of certain multidrug-resistant epidemiological clones has been previously reported[3-5]. The mucoid morphology, the ability to form biofilms in most surfaces, the quorum-sensing response among bacteria, and the ability of adherence on the host cells, altogether provide advantages to *P. aeruginosa* to colonize and multiply in different environments, to increase their levels of antibiotic resistance, and to protect from environmental pressures. *P. aeruginosa* pathogenicity is clearly multifactorial.[1,6,7]

OBJETIVES. To evaluate biofilm formation, pigment production, elastase activity, motility and antimicrobial resistance in *P. aeruginosa* isolates from clinical, healthy human, food and environmental samples.

MATERIAL AND METHODS

Bacterial isolates. A total of 44 *P. aeruginosa* isolates from clinical samples (20), faecal samples of healthy volunteers (9), foods (5) and environmental samples (10), as well as *P. aeruginosa* PAO1 reference strain were characterised.

Susceptibility testing to 15 antipseudomonal agents was performed by disc diffusion method.[8]

Biofilm production. Biofilm biomass and bacterial viability were quantified using crystal violet and FDA hydrolysis assays.

Pigment production, elastase activity and motility assays. Pyocyanin, pyoverdine and pyorubin production were quantified. Elastase activity was determined using Elastin Congo Red. Swimming and swarming motility was evaluated using LB plates with 0.3% and 0.5% agar, respectively.

Virulence and quorum-sensing markers (*exoU, exoS, exoY, exoT, exoA, lasA, lasB, lasR, lasI, aprA, rhlAB, rhlI, rhlR*) were studied by PCR.[9]

Molecular typing was determined by *SpeI*-PFGE[10] and MLST (http://pubmlst.org/paeruginosa/).

Data analysis. R commander and JMP software were used for statistical analysis. Variables were compared by Student t and Wilcoxon tests.

RESULTS

Sixteen isolates were multidrug resistant (MDR) (15 clinical, 1 environmental), whereas nineteen isolates (9 from healthy humans, 1 from meat food, 6 environmental and 3 clinical) were susceptible to all antibiotics tested (Figure 1).

Thirty-eight different PFGE profiles and 24 sequence types (including new STs [1059, 1455, 1456 and 2068]) were identified (Figure 2). The high risk clone ST175 was found in environmental and clinical isolates, ST253 among clinical and healthy humans, and ST111 and ST235 only among clinical isolates.

A high diversity of virulence markers was detected, with 11 isolates expressing *exoU* (8 clinical and 3 from healthy humans). Five isolates had an insertion sequence in the *lasR* gene. (Table 1)

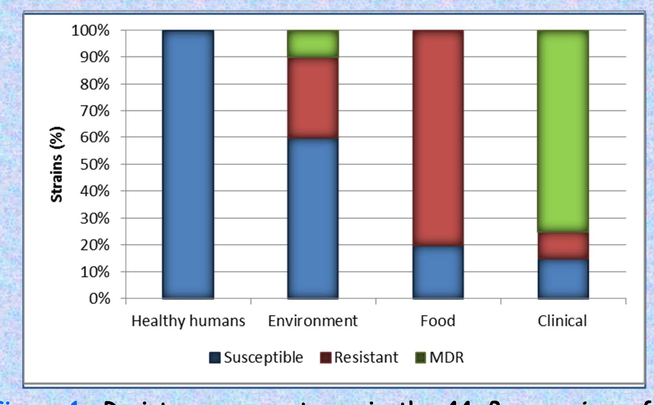


Figure 1: Resistance percentages in the 44 *P. aeruginosa* from different origins.
Resistant: non-susceptible to ≥1 agent in < 3 categories.
MDR[11]: non-susceptible to ≥1 agent in ≥ 3 categories.

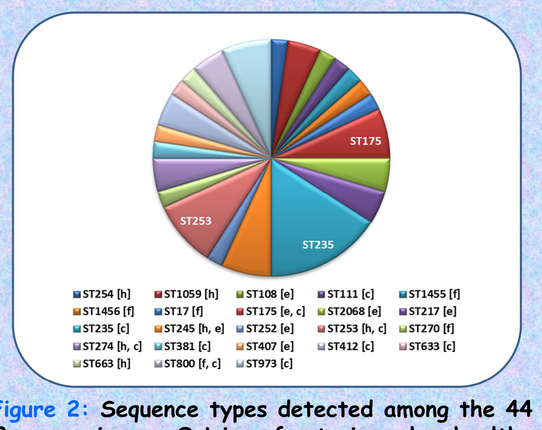


Figure 2: Sequence types detected among the 44 *P. aeruginosa*. Origin of strains: h, healthy volunteers; f, foods; e, environmental; and c, clinical samples.

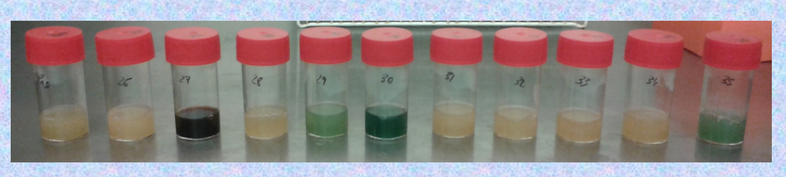
Table 1. Virulence and quorum-sensing markers.

No. strains	Virulence Pattern	Virulence genes												
		exoU	*exoS*	*exoY*	*exoT*	*exoA*	*lasA*	*lasB*	*aprA*	*rhlAB*	*rhlI*	*rhlR*	*lasI*	*lasR*
22	1	-	+	+	+	+	+	+	+	+	+	+	+	+
2	2	-	+	+	+	+	+	+	+	+	+	+	+	[a]-
4	3	-	+	+	+	+	+	+	+	+	+	+	+	[b]-
3	4	-	+	+	+	+	+	+	+	+	+	+	+	-
2	5	-	+	+	+	+	+	+	+	-	-	-	+	+
3	6	+	-	-	+	+	+	+	+	+	+	+	+	+
7	7	+	-	+	+	+	+	+	+	+	+	+	+	+
1	8	+	-	+	+	+	+	+	+	+	+	+	+	[b]-

[a], deletion of nucleotides in the *lasR* promoter region; [b], insertion sequence disrupting *lasR* gene.

Twenty-nine (66%) isolates were higher biofilm producers than PAO1 (environmental isolates: 80%; clinical isolates: 70%; food: 60%; healthy humans: 44%) (Figure 3A). MDR and ExoU-positive isolates showed significantly higher viability within biofilms (Figures 3B and 3C). Non-clinical isolates showed significantly higher elastase activity, growth rate and swimming and swarming motility (Figures 3D-3G).

Pyoverdin production was higher in environmental isolates but no impact of isolate origin was observed for pyocyanin and pyorubin production.



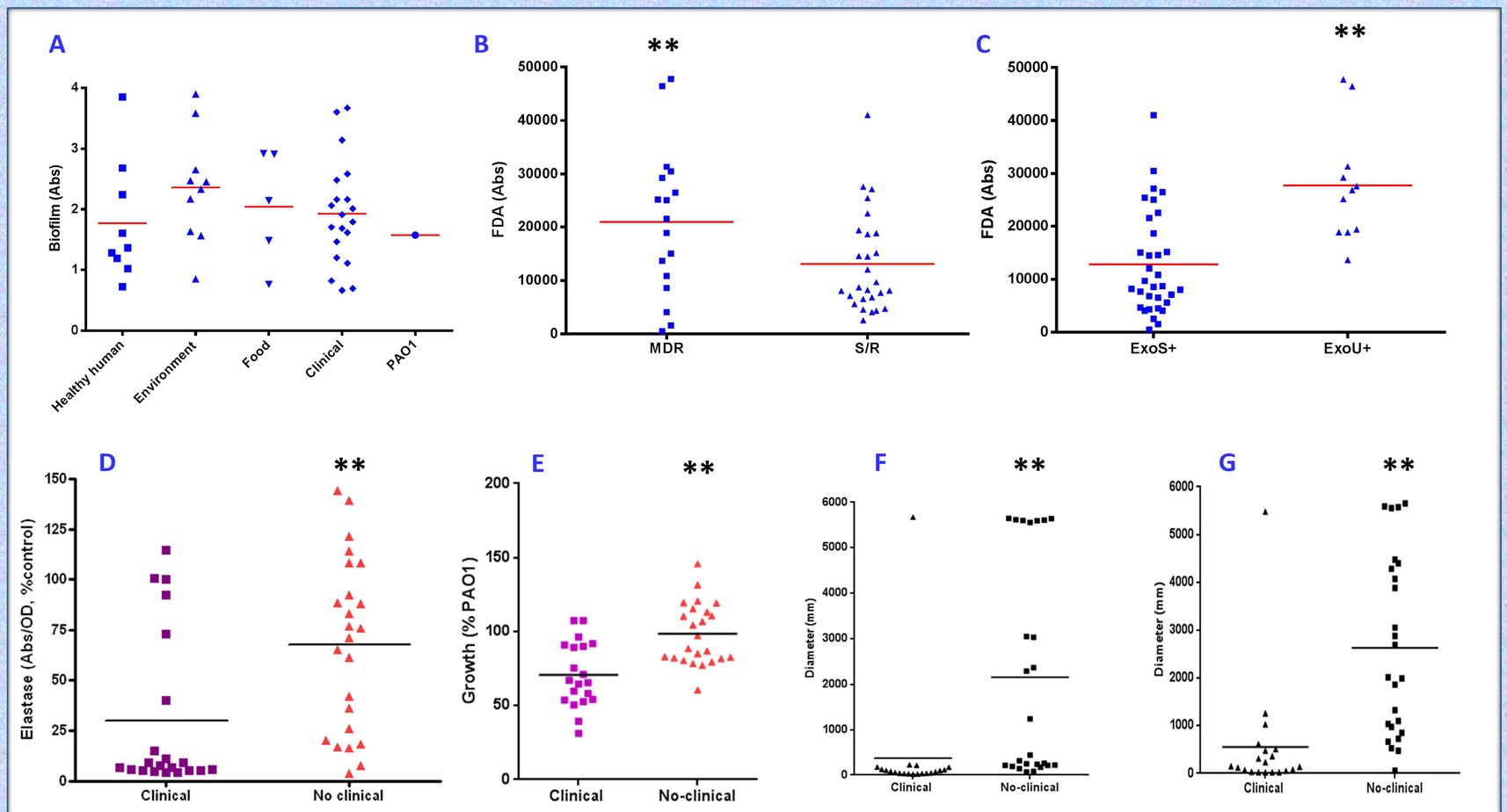


Figure 3: Results of biomass biofilm production classified by origins (A); metabolic activity in biofilm comparing MDR and Susceptible/moderate resistant strains (B); metabolic activity in biofilm comparing *exoU* and *exoS*-harbouring strains (C); comparative analysis of elastase activity (D), growth rate (E); swimming (F) and swarming (G) motility in clinical and no-clinical strains. **, p<0.01.

CONCLUSIONS

Most of environmental and clinical *P. aeruginosa* are high biofilm producers. Non-clinical isolates from different origins show high clonal diversity and variety of genetic lineages, increased elastase activity, growth rate and motility, but low antimicrobial resistance percentages. These data underline the variable capacity of *P. aeruginosa* to adapt to different environments.

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