“A means to an end”? – Adaptation to critical care-related antibiotics does not influence biofilm formation by *Pseudomonas aeruginosa*

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INTRODUCTION:

Ventilator-associated pneumonia (VAP), affects between 8-28% of patients in Critical Care under ventilation[1].
Pseudomonas aeruginosa is one of the most commonly isolated gram-negative bacterial species isolated from VAP, and has a wide repertoire of AMR- and virulence-related characteristics, including biofilm production[2].
AMR is a major factor in treatment failure, and there is little knowledge on how the physiology of bacteria changes in response to sub-inhibitory concentrations of antibiotics.

We aimed to investigate whether physical characteristics change using 6 clinically prescribed antibiotics to examine whether such mutations occur and what differences there are between them.

METHODS:

A clinical *P. aeruginosa* strain was serially exposed (15 passages) to Meropenem, Imipenem, Doripenem, Ceftazidime/Avibactam, Ceftazidime and Colistin using disk diffusion test methodology.

Measurements of inhibition zones (IZ) were taken at each passage and compared to the EUCAST breakpoints[3].

Biofilm formation by adapted strains was measured using the crystal violet microplate assay[4]. Biofilms were grown for a total of 12 hours, before calculating biofilm formation units via recorded planktonic growth and biofilm production at OD600.

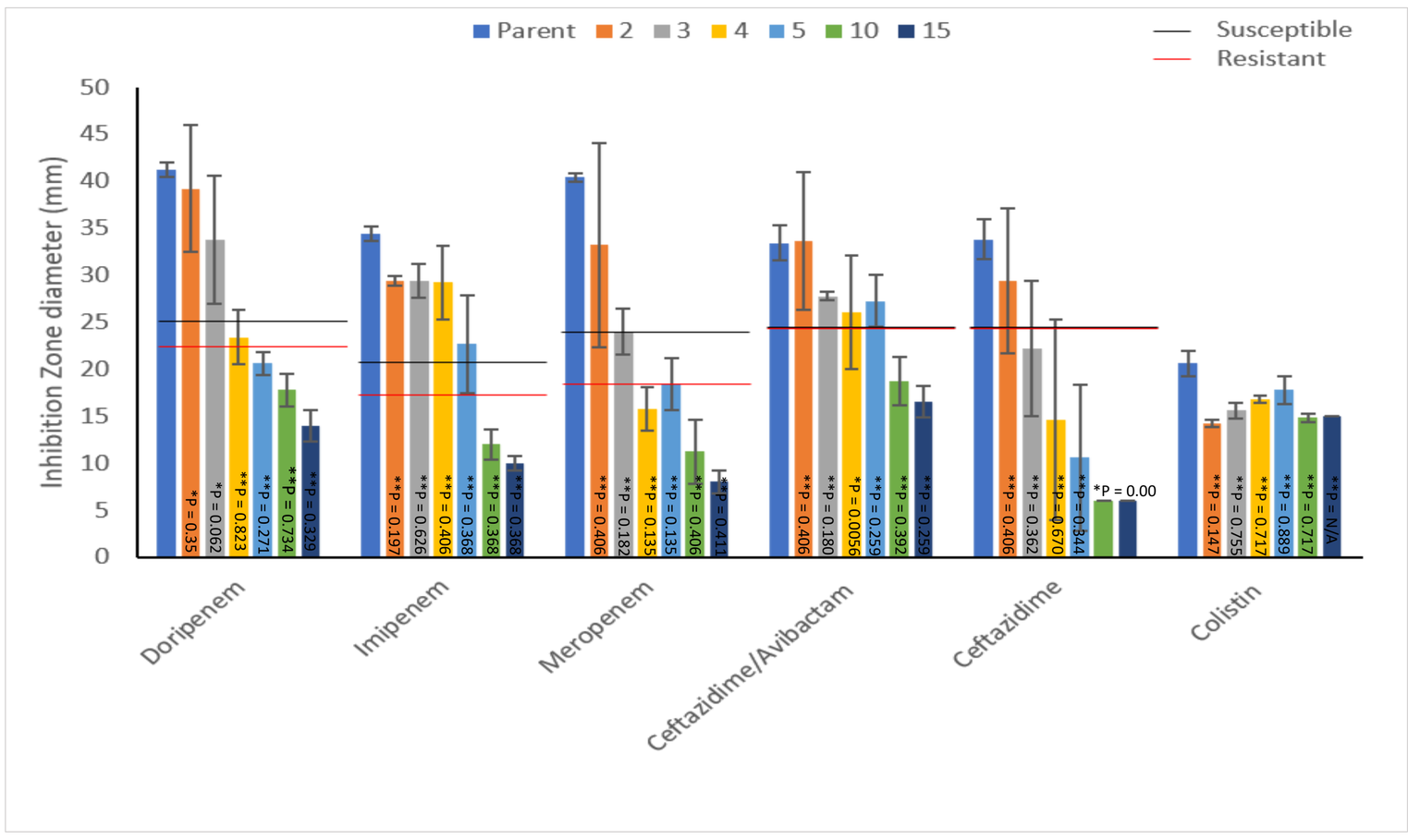

Figure 1: Sensitivity of antibiotic-adapted *P. aeruginosa*. EUCAST breakpoints are displayed as black (≥mm = susceptible) and red (≤ = resistant) lines. P-values were calculated using ANOVA* and Kruskal-Wallis** via Minitab 16.

RESULTS:

P. aeruginosa developed clinical resistance against all antibiotics except colistin (Figure 1). Isolates that were exposed to ceftazidime became resistant to the antibiotic in the shortest amount of time (Passage 3).

Adaptation to carbapenem antibiotics occurred in the following order: Meropenem > Doripenem > Imipenem.

No significant difference was detected, however, when comparing the adapted strains to the parent (Figure 1) and whether duration of exposure affected resistance.

No significant difference was found between overall antibiotic exposure and biofilm formation when comparing parent isolates to the adapted (Figure 2).

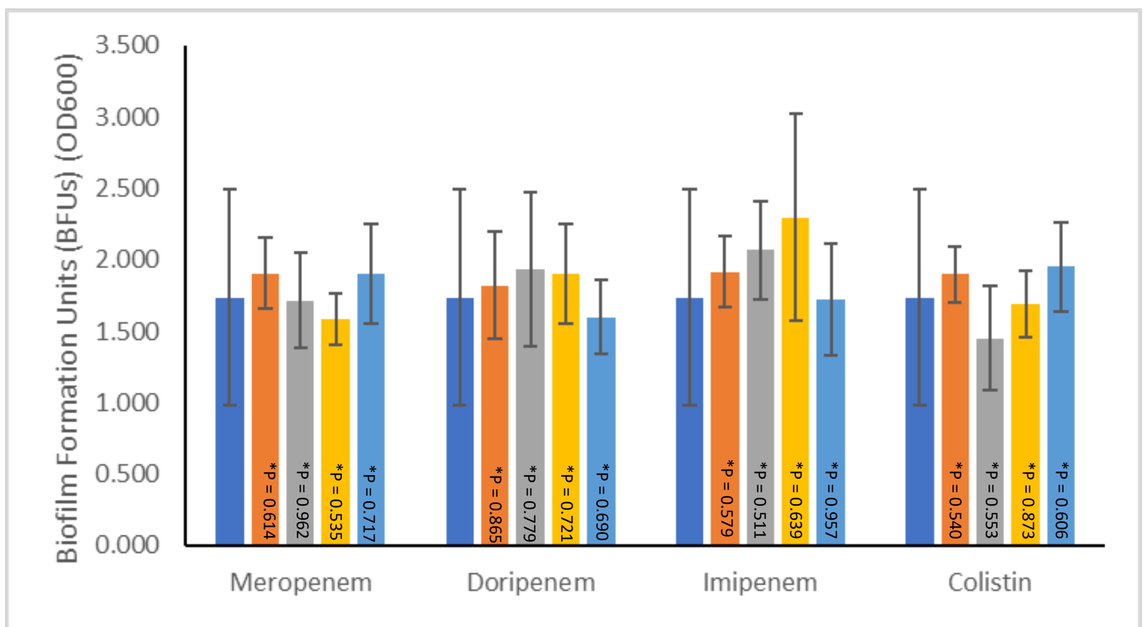

Figure 2: Biofilm Formation Units (BFUs) after 12hrs of growth. All stats were calculated using ANOVA* (Minitab 16)

CONCLUSIONS:

- *P. aeruginosa* developed resistance against critical care-related antibiotics, including carbapenems and cephalosporins – though such evidence suggests a weak relationship between antibiotic exposure and continuous prescription.
- No relationship was detected between biofilm production and antibiotic exposure – suggesting that resistance is not associated with costs to growth or virulence.
- Current work is underway to characterise further growth and virulence phenotypes, as well as whole genome sequencing to identify resistance-related mutations.

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