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In vitro analysis of antimicrobial activity on the biofilm formed by Klebsiella pneumoniae

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Background: *Klebsiella pneumoniae* is one of the most important bacteria for the development of infections associated with invasive devices in critical care units. The objective of the present study is to evaluate *in vitro* the ability of various antimicrobials to remove the biofilm formed by *K pneumoniae*

Material/methods: From a strain of *K pneumoniae* isolated in an intensive care unit, biofilm formation was reproduced in vitro. Bacterial strain was cultured at a concentration of 10µl/ml in minimal mucin-containing medium at 37ºC. On a 96-well plate, 100 µL of culture medium with bacteria was inoculated into each well, to which 100 µL of various antimicrobials (amikacin, meropenem, tigecycline, fosomycin, colistin, ciprofloxacin and ceftriaxone) were added to a final concentration equivalent to the cut-off point of the MIC established by EUCAST, as well as dilution above and below it. The absorbance at 600 nm of the antibiotic solution added at 0h, 6h and 24h were compared to the absorbance of the antibiotic solution incubated without bacteria, after staining with violet crystal and reading in a spectrophotometer. The experiment was replicated in

three different days. The comparison of absorbance between samples was analysed using a Mann-Whitney U test.

Results: In the non-antibiotic sample, biofilm formation was observed at 6h [A600 nm: 0.905 (SD: 0.092) vs 0.155 (0.078); p<0.001)] and at 24h [1.191 (0.553) vs 0.175 (0.080); p<0.01)] of inoculation. For all concentrations analysed at 6 and 24 hours, all antimicrobials had a significant reduction of bacterial eradication capacity compared to the control sample. On the biofilm formed at 6h, at a dilution above the MIC and compared to the control group [A: 0.905 (0.223)] amikacin presented a greater eradication capacity [0.580 (0.189); p=0.019], followed by meropenem [0.611 (0.409); p=0.046], ciprofloxacin [0.636 (0.118); p=0.045] and fosfomycin [0.664 (0.405); p=0.044]. Ceftriaxone, [1,113 (0,340); p=0,325) showed no activity against the biofilm formed at 6h. On biofilm formed at 24 hours [A: 1.165 (0.615)] at concentrations above bacterial MIC, none of the selected antibiotics had a significant effect on biofilm, being fosfomycin [0.988 (0.477); p=0.071] and meropenem [0.953 (0.634); p=0.065] the most active antibiotics [tigecycline: 1.252 (0.500), ceftriaxone: 1.318 (0.568), ciprofloxacin: 1.431 (0.905), colistin: 1.052 (0.634)]. None of the antimicrobials achieved differences in biofilm eradication from the control group at concentrations equal to or lower than the MIC.

Conclusions: *K pneumoniae* present a great ability of biofilm formation, observing its formation in the first 6 hours and significantly reducing antimicrobial activity. It is necessary to establish new pK/pD cutoff points for an adequate management of infections associated with biofilm formation.