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Colistin heteroresistance in *Klebsiella pneumoniae* and its association with slow-growing sub-populations within biofilms

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The emergence of multidrug-resistant strains of *Klebsiella pneumoniae* is a growing clinical concern that is leading to the re-introduction of the old and toxic colistin as a salvage therapy. However, several cases of heteroresistance to this antimicrobial have been recently reported in planktonic studies. Therefore, the understanding of the conditions that trigger heteroresistance is attracting considerable research interest. In this scope, this work aimed to more comprehensively study the response of *K. pneumoniae* biofilms to colistin and to inspect the occurrence of heteroresistance in biofilm-cells. *K. pneumoniae* presented susceptibility to colistin in its planktonic form, though biofilms presented an enhanced resistance. The population analysis profiles pointed out the existence of a slow-growing sub-population resistant to colistin within a *K. pneumoniae* strain that seemed to be exclusively associated with biofilms. This resistant sub-population is characterized by a small colony morphology (diameter around 5 mm), which remains unchangeable, and a completely different response to colistin compared to the observed in the wild-type morphotype. Colistin was ineffective in this small colony variant since it was never achieved any reduction in biofilm-cells viability. These findings suggest that heteroresistance is linked to biofilm formation and to a morphological distinct sub-population. Moreover, this is the first evidence that biofilm formation can trigger the emergence of heteroresistance from an apparently susceptible strain.