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## Comment on: Bacteraemia with an MBL-producing *Klebsiella pneumoniae*: treatment and the potential role of cefiderocol heteroresistance

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We read with interest the Research Letter from Witt *et al.*,<sup>1</sup> describing a case report of *Klebsiella pneumoniae* NDM-5 bacteraemia with a subsequent recurrence in which the authors postulate a link to cefiderocol heteroresistance.

The bacterium isolated on the second episode at day 32 was phenotypically and genetically almost identical to the index pathogen, and the authors describe the recurrence in terms of clinical failure and attribute potential persistence of subpopulations that were heteroresistant to cefiderocol, a phenotype which they investigated by population analysis profiling (PAP). However, given the fact that the initial antibiotic regimens resulted in a negative blood culture sustained for 24 days, we would argue that the initial therapy should be considered clinically successful and application of Ockham's razor provides an alternative explanation for the recurrence, simply that the patient was colonized with the original *K. pneumoniae* NDM-5 isolate in a reservoir such as the gut and due to the patient's unresolved underlying co-morbidities and inadequate source control, they developed a reinfection, not a persistence of the original bacteraemia.

More importantly we would challenge the validity of assertions made by the authors of heteroresistance to cefiderocol defined by their use of the PAP method. Although PAP is well cited in the literature as a laboratory method for detecting heteroresistance, and has been accepted as a 'gold standard' method for quantifying the frequency of heteroresistance for vancomycin intermediate *Staphylococcus aureus* (hVISA),<sup>2</sup> the method is nevertheless not clinically validated and care needs to be taken

when translating that methodology to other bacterial pathogens and antibiotics in order to avoid misinterpreting methodological artefacts as evidence of heteroresistance.

Cefiderocol poses perhaps a unique challenge in this respect as it is a siderophore cephalosporin and requires low iron concentrations ( $\leq 0.03$  mg/L) in the culture medium, reflecting physiological conditions, for accurate determination of MICs. As a result, the only reference method recommended for MIC determination for cefiderocol is broth microdilution in an iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB). The PAP method by contrast is based on growth of bacterial cells at an inoculum density 1000-fold higher than the reference method and on standard Mueller-Hinton agar (MHA) plates in which the iron concentration is undefined and uncontrolled.

It has been previously established that MICs of cefiderocol for *K. pneumoniae* determined by CLSI agar dilution reference method using MHA plates do not correlate well with broth microdilution results using ID-CAMHB, with only 57% essential agreement and 60% categorical agreement, where most of the discordance was due to higher MICs on agar dilution plates compared with broth microdilution (21% major errors, 19% minor errors).<sup>3</sup>

According to the Supplementary data provided,<sup>1</sup> heteroresistance was recorded where there was a 6 log reduction in cfu observed on the 32 mg/L plate (in the case of cefiderocol corresponding to 2-fold the CLSI resistance breakpoint of 16 mg/L) compared with the drug-free control. However, as cefiderocol MICs by agar dilution are typically at least 1 dilution higher than broth microdilution, it is not valid to apply the broth dilution breakpoint of 16 mg/L for this purpose as the equivalent agar dilution breakpoint is likely in the region of 32 mg/L, meaning heteroresistance should be assessed on plates containing cefiderocol 64–128 mg/L. This is reinforced by the fact that the 'MIC' reported for both isolates from PAP plates was  $\geq 32$  mg/L despite the disc diffusion assay reporting an 18 mm zone diameter, which is classified as susceptible and equivalent to an MIC of  $\leq 4$  mg/L by broth microdilution in ID-CAMHB.

Therefore, it is our opinion that the results of the PAP analysis interpreted by Witt *et al.*<sup>1</sup> as evidence of cefiderocol-heteroresistant subpopulations are most likely a combination of methodological artefacts from the agar dilution-based method in non-iron-depleted medium plus the higher inoculum used. Future investigations of heteroresistance to cefiderocol or other antibiotics should be careful to ensure appropriate methods are used to minimize potential misinterpretation of *in vitro* observations, especially when seeking to postulate correlations to clinical outcomes.

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## Transparency declarations

All authors are employees of Shionogi, the marketing authorization holder for cefiderocol.

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## References

**1** Witt LS, Steed DB, Burd EM *et al.* Bacteraemia with an MBL-producing *Klebsiella pneumoniae*: treatment and the potential role of cefiderocol

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