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Lack of upward creep of glycopeptide MICs for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in the UK and Ireland 2001–07—authors' response

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Sir,

Lawes *et al.*¹ favour an alternative interpretation of our results,² suggesting that upward creep in vancomycin MIC occurred but was undetectable in the stored samples because its mechanism was unstable. The group's two published studies,^{3,4} cited to support this view, are difficult to assess because they lack control data and estimates of variability.

Among four susceptibility testing methods used by Edwards *et al.*,³ only Etest at the time of isolation gave any indication of creep, the mode MIC for 15 isolates collected in 2010 being half a doubling dilution higher than for the isolates from each of the preceding 3 years. Ludwig *et al.*,⁴ also using Etest, reported that the MICs of vancomycin for *Staphylococcus aureus* declined during storage at -70°C , and more rapidly for isolates with higher ($>1\text{ mg/L}$) MICs.

Edwards *et al.*³ acknowledge that their results are unusual insofar as they found higher MICs (for stored isolates) by broth microdilution than Etest, conflicting with the results of other published studies; broth microdilution MICs for stored isolates were not significantly different from the initial results using Etest. They did not use agar dilution, complicating any comparison with our work. Edwards *et al.*³ suggest that changes in the MICs of vancomycin took place within the first 6 months of storage; Lawes *et al.*¹ state that declines in MIC 'continued even at 9 months', but Ludwig *et al.*⁴ describe this later fall only for daptomycin, with declines in MICs of vancomycin occurring earlier (within 3 months), despite the fact that these two agents have closely related MICs for staphylococci.

The biggest problem is the lack of information about the variability of MIC measurements in the two studies. 'Careful quality

control checks to standardize methods and minimize inter-observer error' are noted by Lawes *et al.*,¹ but are supported only by evidence of good agreement in reading the Etest zones, with no evidence for the reproducibility of the actual MIC measurements; nor do the authors give MICs for control strains. Edwards *et al.*³ acknowledge that they 'did not seek to prove the reproducibility of MIC results for each of the testing methods, which may limit conclusions about intermethod differences'. This caveat equally limits conclusions about differences between MICs measured at different times, since the 'acceptable' range for control MICs—spanning at least two doubling dilutions in both the CLSI and EUCAST guidelines—greatly exceeds the MIC creep claimed by any group. Unless MICs are reproducible within a very much narrower tolerance, apparent variation over time may be experimental, not biological, as illustrated by our own experience.

Our original MICs were obtained by testing batches of isolates retrieved from frozen storage—mostly at least 6 months after collection and therefore after any postulated unstable resistance would have been lost. The original results nonetheless gave the impression of MICs rising significantly over time, but this was refuted when the isolates from different years were retested together. Lawes *et al.*¹ are mistaken in believing that, for the appearance of MIC creep in our original results to be explained by experimental factors confounded with the time of collection, there would have to be systematic error over time (not just a random error within and between laboratories) and that this would require a consistent change in experimental practice across 19 laboratories over 7 years. Our study quite simply did not use results measured by the 19 contributing laboratories: both the original and re-test MICs were measured at one central laboratory. Furthermore, it is easy to show by simulation that if even a small proportion of the total experimental variation is (randomly) associated with the year of collection, spuriously significant trends over time become common; the error does not have to be systematic over time.

A further difficulty with the hypothesis that upward creep in MIC has occurred, but is undetectable in stored samples, is its weakness in explaining our observations on teicoplanin. Teicoplanin and vancomycin share a mechanism of action, and their MICs are positively and strongly correlated. In our original results, the MICs of vancomycin appeared to rise whereas those of teicoplanin appeared to fall. It would be a most unusual resistance mechanism that had opposite effects on the MICs of two related antimicrobials. Such a discrepancy is, however, entirely consistent with experimental variation between years.

We would welcome greater research attention on, and understanding of, the issues of precision, accuracy, repeatability and reproducibility in MIC testing by various methods. Meanwhile, in this case, we remain unconvinced by our colleagues' critique of our findings.

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Members of the BSAC Extended Working Party on Resistance Surveillance

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Transparency declarations

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