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

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Objectives: There have been several reports of upward creep in vancomycin MICs for Staphylococcus aureus [predominantly methicillin-resistant S. aureus (MRSA)] over recent years, but only in single centres or using contemporaneous results. We aimed to test the hypothesis of MIC creep in a multicentre study, testing all the isolates concurrently.

Methods: Nineteen laboratories in the UK and Ireland contributed isolates from blood to the BSAC Bacteraemia Resistance Surveillance Programme every year between 2001 and 2007. MICs for 271 MRSA from these sites were re-measured at a single central laboratory during a single week by the BSAC agar dilution method, but with 2-fold instead of conventional 2-fold dilutions. Re-test results were compared with the original results obtained each year at the same central laboratory.

Results: The re-test results were much less variable than the original results and avoided the confounding of experimental variation with year of collection. They demonstrated statistically significant but very slow downward trends in MICs of vancomycin and teicoplanin, at 0.027 and 0.055 doubling dilutions/year, respectively. The original results had suggested more rapid trends in MICs, upward for vancomycin and downward for teicoplanin. The proportion of EMRSA-16 fell from 21% to 9% over the study period, while EMRSA-15 rose from 76% to 85%.

Conclusions: Historical data can give a misleading impression of trends in MIC values because of experimental variation between tests conducted at different times. There was no upward creep in glycopeptide MICs for MRSA in the UK and Ireland between 2001 and 2007.

Keywords: S. aureus, vancomycin, teicoplanin, MIC creep, bacteraemia, antimicrobial resistance surveillance

Introduction

Vancomycin MIC ‘creep’ or ‘shift’ in Staphylococcus aureus is a phenomenon whereby isolates with higher vancomycin MICs become more common over time, although the MICs still remain within the susceptible range for the drug (≤2 mg/L).1 Creep is predominantly claimed for methicillin-resistant S. aureus (MRSA), though perhaps only because these are more likely to be investigated than methicillin-susceptible S. aureus (MSSA) strains.

The recorded vancomycin MIC distribution for S. aureus ranges from 0.06 to >8 mg/L, but MICs for >99% of isolates lie in the range 0.25–2 mg/L, with a mode of 1 mg/L being found in nearly all studies.2 Therefore, in practice, ‘a vancomycin MIC shift’ for S. aureus tends to mean an increase in the proportion of isolates with MICs of 2 mg/L.

Vancomycin MIC creep for S. aureus has been reported from North America, Asia and Europe over the last 5 years3–7 and has special significance, as clinical data from several sources suggest poorer responses in vancomycin-treated patients if the MIC is (according to the particular study) >1, >1.5 or ≥2 mg/L.8 However, doubts remain about the significance of these observations, not least because MICs are ordinarily accepted as varying by one doubling dilution between experiments. Experimental variation between MICs measured at different times on different media batches could, therefore, give a false impression of creep,
with publication bias then likely to favour reporting of upward creep. The BSAC Bacteraemia Resistance Surveillance Programme has collected and tested S. aureus from blood from multiple centres in the UK and Ireland since 2001, and provides an ideal resource to test whether there has been any trend in MICs. At the same time, by re-examining historical isolates in a single experiment, it allows a test of the hypothesis that experimental variation between previous measurements made at different times might produce the illusion of MIC creep.

**Methods**

**Isolate collection and microbiological methods**

As previously described, 25 clinical laboratories across the UK and Ireland each contributed up to 10 clinically significant isolates of S. aureus from blood each year, excluding duplicates within 7 days of a previous blood culture positive for S. aureus. Isolates were frozen at −70°C in blood glycerol broth for storage at the single central laboratory (HPA, Colindale). On retrieval, they were subcultured twice before being tested against vancomycin and teicoplanin by the BSAC agar dilution MIC method in three or four batches each year. These contemporaneous annual tests produced the ‘original’ results. An oxacillin MIC >2 mg/L defined an isolate as MRSA; in addition, for isolates collected from 2005 onwards, the detection of mecA by PCR also defined an isolate as MRSA, irrespective of the oxacillin MIC. All MICs were recorded exactly, i.e. not censored and not reported as >x mg/L or ≤x mg/L.

Thirty laboratories contributed isolates at some time, while 19 contributed every year from 2001 to 2007 and were included in this study. The testing used 96-spot plates, and there were 5 control isolates in each run, so it was planned to re-test a multiple of 91 isolates. A prior power calculation showed that 273 isolates would be sufficient, but two were lost by human error. Thus, 271 MRSA isolates from the 19 centres were included in the study, randomly selected with stratification to give equal numbers (37–39) from each year. The isolates were revived from cryogenic storage in the same way as for the original MIC tests and re-tested in three batches on 96-point plates over the course of a single week. Isolates from different years were distributed evenly among the batches and sets of plates and the BSAC method was modified to measure the MIC more precisely by using √2-fold dilutions (0.35, 0.5, 0.71, 1, 1.41, 2 mg/L etc.) instead of the usual 2-fold dilution series (0.5, 1, 2 mg/L etc.). This modification substantially increased the study’s power to detect subtle changes in MIC distributions. Typing data were available for just over half the isolates included (n=145) and were based on a combination of multilocus sequence typing (MLST) and SCCmec typing.

**Statistical methods**

MICs were analysed for trend by interval regression for log(MIC). The underlying assumption of this method is that the true MICs are normally distributed on a log scale, and that the mean of this MIC distribution changes linearly over time whilst its variance remains constant (Figure 1a). Interval regression allows for the fact that concentration (and hence true MIC) is a continuous quantity, but the experimental method necessarily divides that continuum into intervals: an experimental MIC measurement only gives an upper limit for the minimum concentration required to inhibit growth, whereas the true MIC could be anywhere between that limit and the next lower concentration in the test series (so, for instance, a recorded MIC of 1 mg/L on a √2-fold dilution series means a true MIC between 0.71 and 1.00 mg/L, whereas a recorded MIC of 1 mg/L on a conventional 2-fold dilution series means a true MIC between 0.50 and 1.00 mg/L). Using more closely spaced test concentrations gives more information; doubling dilutions may give too little information to fit the models for narrow MIC distributions.

MICs were also described using geometric means, calculated in the conventional way using reported MICs on the √2-fold or 2-fold dilution series. These means are shown in the figures and, for consistency, trend lines on these figures were estimated by simple linear regression of log(MIC) on time, which gave very similar results to interval regression.

The re-test study was designed to have >90% power to detect a trend of 0.05 doubling dilutions/year (equivalent to doubling of vancomycin MICs every 20 years) with a 5% significance level. Power was estimated by simulation. First, an interval regression model with no time trend was fitted to the original vancomycin MICs for all the MRSA collected at the 19 centres from 2001 to 2007 so as to estimate the mean and variance of the underlying assumed log-normal MIC distribution. Then, MIC creep was simulated by generating MICs from a log-normal distribution with the same variance, but with the mean MIC increasing steadily over time. Finally, the simulated continuous MICs were rounded up to the next tested concentration to reflect the experimental situation, and analysed for trend by interval regression. The process was repeated a thousand times. The estimated power of the study was the proportion of simulations in which the analysis found a statistically significant trend. Figure 1(a) illustrates this model of idealized ‘smooth’ creep.

To test for robustness of the interval regression method, several other models of creep were simulated. The first (Figure 1b) had the same starting point as above, with MICs for isolates drawn from the same single log-normal distribution as above, and having the same mean (x mg/L) and variance estimated for the original observed data. It was then supposed that there could be distinct subgroups of isolates with mean MICs differing in doubling steps from the starting point (at 2x, 4x, 8x and so on) but having the same variance. Creep was simulated as a stepwise process in which isolates could, at any point, join the group with the next higher mean MIC (or, equivalently, be replaced by an isolate from the next higher group). This might apply, for example, if there were multiple clones and a constant process of clonal replacement over time, or if isolates accumulated resistance in discrete steps. In this model, the overall variance increased over time as the isolates spread out over the subgroups, violating the assumptions of the analysis method.

Re-testing the isolates provided a revised estimate of the variance in MICs after eliminating year-to-year experimental variation, and this was used to re-calculate power post hoc for both versions of the log-normal model.

In a further test of robustness, we used the observed (positively skewed) distribution of log re-test MICs directly instead of modelling it as a normal distribution. As before, in one model the geometric mean MIC increased smoothly over time with little change to the shape of the distribution; in the other model MICs increased stepwise (in this case, in units of √2-fold) so that variance increased over time. Bootstrap samples (with replacement) of the observed log MICs were taken to act as random samples for the initial distribution, with underlying continuous MICs assumed to be distributed log-uniformly between the measured concentrations.

Several rates of MIC progression were simulated for each model, between 0 (no creep) and 0.09 log₂ units per year (doubling of MIC in 11 years). In all models, the simulated continuous MICs were rounded up to experimentally measured concentrations and analysed by interval regression in 1000 replicates, as before. This method of analysis assumes that the underlying log MIC distribution is normal with constant variance at all times, which was true only in the first of the simulations of creep. The purpose of the additional simulations was to test whether the method was reasonably robust in other, perhaps more realistic, situations.

Finally, results from interval regression were compared with those from simple linear regression on log MICs for the models using empirical
Figure 1. Illustrations of ‘smooth’ and ‘stepwise’ models of MIC creep at 0.05 doubling dilutions/year. (a) Smooth increase in MIC with constant variance—a simplified representation of creep for modelling purposes. (b) Stepwise increase in MIC producing increasing variance, as might arise with incremental, stepwise acquisition of resistance, or replacement of current strains by increasingly resistant strains. Line = simulated underlying continuous MIC distribution. Shaded bars = corresponding conventional (doubling dilution) MIC. Note log scale. For illustrative purposes, isolates with simulated MICs > 16 mg/L (<1% of total even in year 20) are excluded from the graphs.
distributions. Simple linear regression assumes that the observed log MICs are normally distributed with constant variance at all times, which cannot be true when MIC values can only take a small number of discrete values. The purpose of this comparison was to assess whether the simpler method performed adequately despite being mis-specified.

Results

MIC distributions and trends

A total of 1693 isolates of S. aureus were collected between 2001 and 2007; 1297 were from the 19 centres that contributed every year, and 531 of these were MRSA. Both vancomycin and teicoplanin MICs had narrow unimodal distributions, with no evidence of distinct subgroups (Figure 2). According to the original results, one isolate (not included in the random selection for re-test) was resistant to vancomycin (MIC 4 mg/L) under current BSAC/EUCAST criteria, though not under those (resistant: MIC >4 mg/L) at the time when it was collected; eight were resistant to teicoplanin under current criteria (MICs 4–16 mg/L), of which four were randomly included in the re-test study. The original MICs (Table 1) showed much greater variation between years than did the re-test results (Figure 3).

The original MIC results showed an upward trend for vancomycin and downward trend for teicoplanin MICs (Figure 4): vancomycin MICs were estimated to have increased by 0.078 doubling dilutions per year, equivalent to doubling every

![Modelled underlying MIC: Geometric mean 0.82 mg/L SD 0.46 doubling dilutions](image)

![Modelled underlying MIC: Geometric mean 0.57 mg/L SD 0.71 doubling dilutions](image)

![Observed MIC distributions in original tests, and their estimated underlying log-normal continuous distributions, for 531 MRSA isolates from 19 centres contributing in all 7 years.](image)

![Geometric mean MIC by year of collection, and test series (original versus re-test). Each point represents the geometric mean MIC for one year, one antibiotic and one set of MIC tests. For clarity and to avoid overlapping, the points for the seven years in each antibiotic-method group are spread out slightly on the x-axis (2001 the leftmost and 2007 the rightmost). Note that when geometric means are calculated directly on measured MICs, as here, the original results are expected to be (and are) on average 0.25 doubling dilutions higher than re-test results using \(\sqrt{2}\)-fold dilutions. That is because the 2-fold scale rounds up and overestimates the true MIC by 0.5 doubling dilutions on average, whereas the \(\sqrt{2}\)-fold scale overestimates only by 0.25 doubling dilutions.](image)
13 years (95% CI 8–27 years, P<0.001), whereas teicoplanin MICs were estimated to have decreased by 0.065 doubling dilutions per year, equivalent to halving every 16 years (95% CI 10–42 years, P=0.002). The re-test results showed that there was, in fact, very little trend over time (Figure 5), and that those MIC trends that were found were consistently downwards: MICs of vancomycin and teicoplanin were estimated to fall by 0.027 and 0.055 doubling dilutions per year, equivalent to halving every 37 and 18 years, with P-values of 0.006 and 0.003, respectively. Among the 145 isolates with typing information, 118 belonged to EMRSA-15 (SCCmecIV) based on MLST and SCCmec type, and 27 to EMRSA-16 (SCCmecII); the geometric mean vancomycin MIC at re-test was 0.74 mg/L for the former group versus 0.81 mg/L for the latter.

Statistical simulations

The simulations showed that the analysis used to detect MIC creep was robust to reasonable departures from the strict assumptions of interval regression. All the models gave false positive error rates close to the design level, mistakenly identifying a significant (P<0.05) trend in MIC when there was none in ~5% of replicates, and all gave unbiased estimates of the simulated rate of change of MIC. The re-test experiment had greater power to detect change in MIC than was predicted because the re-test results were less variable than the original MICs, having eliminated several possible sources of experimental variation. On the other hand, power was lower for the observed, somewhat skewed, log MIC distributions than for the idealized log-normal distributions originally simulated to represent them. The power to detect changes in teicoplanin MICs was lower than in vancomycin MICs, because of the greater variance in teicoplanin MICs. The power to detect ‘stepwise’ creep (as Figure 1b), which increases the spread of MICs over time, was lower than for ‘smooth’ creep (as Figure 1a). As very little MIC creep was actually detected, it was not possible to distinguish which model (smooth or stepwise) better represented it. Taking the case with the least power—using actual MIC distributions and assuming stepwise change—the power of the re-test study to detect MIC creep at a rate of 0.05 log₂ units/year (doubling of MIC over 20 years) was at least 92% for vancomycin and 59% for teicoplanin.
MIC creep in MRSA

Discussion

Assessment of any asserted claims of vancomycin MIC creep needs to take account of several key factors and potential confounders. These include: (i) how representative the tested strains are of the sampled population; (ii) changes in MRSA or MSSA clones over time; (iii) use of historical MIC data; (iv) the potential for conventional 2-fold dilution scales to mask or exaggerate MIC differences of less than a full doubling dilution; (v) systematic differences between different testing methods; (vi) changes over time in medium composition; and (vii) the possible effect of isolate storage on the phenotypic expression of the mechanisms associated with raised vancomycin MICs. The publications reporting MIC creep have mostly been single-centre studies, albeit spread across the USA, Europe and the Far East, with MICs collected contemporaneously rather than by re-testing collected isolates. In contrast, two multicentre national surveillance programmes in the USA and Canada have failed to detect MIC creep.

Our re-test results show very clearly that there was hardly any difference in geometric mean vancomycin MIC between the isolates collected in different years in the UK and Ireland between 2001 and 2007, and no upward trend. Therefore, the differences between years seen in the original contemporaneous MICs do not, in fact, reveal real differences in the isolates but are due to year-to-year variation in the measurement, i.e. the effect of unidentified experimental factors that are confounded with the year of collection. Isolates were frozen and retrieved from cryogenic storage for both the original and repeated testing, so the effect of storage is not a plausible explanation for the difference between original and re-test results.

The absence of upward trend in MICs for either agent, shown by the concurrent re-testing of the isolates, is in agreement with results from the North American multicentre studies. The impression given by the original results of rising vancomycin MICs and falling teicoplanin MICs had little biological plausibility, as the two glycopeptides have similar modes of action and might reasonably be expected to be vulnerable to similar mechanisms of resistance, and is now shown to be an artefact.

This re-test study avoided possible artefacts by using isolates only from centres that contributed every year, re-testing them in a single week, and ensuring that isolates from each year were distributed across all of the test batches. The MICs were measured more precisely than in the traditional 2-fold dilution method and the method of analysis—interval regression—took into account the uncertainty inherent in using discrete concentrations for testing. While the assumptions of interval regression were not fully met, the method was shown to be sufficiently robust to accommodate realistic departures from these assumptions. Traditional analysis by linear regression of directly measured MICs also makes an assumption of normality, which is clearly violated when MICs can take only one of three or four values; however, it, too, was quite robust and gave similar results to those of interval regression.

There was much greater variation in the original results than in the re-test results, as might be expected, showing that there was year-to-year experimental variation over the narrow MIC ranges in which glycopeptide MICs for S. aureus are distributed. The reasons for this variation are unclear but may reflect a range of factors such as changes in media, staff performing the experiments or antibiotic potency. Published standards for MIC testing allow run-to-run variation of two doubling dilutions, as MICs from 0.5 to 2 mg/L are acceptable for ‘quality control’ strains. The BSAC agar dilution method is a consistent, well-established method of MIC measurement and we have shown previously that, when bacteraemia isolates were re-tested in different years at this central laboratory, 95% of MICs agreed within ±1 doubling dilution (across a wide range of organisms and antibacterial agents). The level of MIC variation between years noted in this study is consistent with that degree of reproducibility and should not cause surprise: there is variability in MIC determination by any method, and the issues this raises should be more widely recognized.

One notable shift that has occurred among MRSA over the period of the study is the relative decline of EMRSA-16 and the increasing dominance of EMRSA-15. In 2001, EMRSA-16 accounted for 21.4% of MRSA collected in the BSAC surveillance programme and EMRSA-15 for 75.7%. These proportions changed progressively to reach 9.0% and 85.4%, respectively, by 2007. Typing data were available for just over half of the isolates included here and the geometric mean vancomycin MIC, based on the re-test values with 2-fold dilutions, was 0.74 mg/L for EMRSA-15 and 0.81 mg/L for EMRSA-16. A shift from EMRSA-16 to EMRSA-15 would naturally lead to slightly lower vancomycin MICs over time; however, this effect is estimated to be too small to explain the results seen here. There was no evidence of rising MICs within either EMRSA-15 or EMRSA-16. The high degree of clonality among MRSA in the UK and Ireland encourages speculation that these particular clones may lack the capacity to become non-susceptible to vancomycin, but a similar absence of upward creep has been reported by multicentre studies in other regions, where other clones are prevalent.

In conclusion, we were unable to detect upward teicoplanin or vancomycin MIC creep in MRSA strains isolated from bloodstream infections in the UK and Ireland between 2001 and 2007. Rather, we have shown that the use of historical data can give a misleading impression of trends in MIC values over time because of experimental variation between tests conducted in different years. We recommend that claims of vancomycin MIC creep should not be made without careful re-testing of isolates using a single set of experiments over a short time.

Acknowledgements

Collecting laboratories
- England: William Harvey Hospital, Ashford; Birmingham City Hospital, Birmingham; Bristol Royal Infirmary Hospital, Bristol; Countess of Chester Hospital, Chester; Coventry and Warwickshire Hospital, Coventry; St Mary’s Hospital, London; University College Hospital, London; Wythenshawe Hospital, Manchester; Freeman Hospital, Newcastle; Northern General Hospital, Sheffield; Southampton General Hospital, Southampton; Royal Shrewsbury Hospital, Shrewsbury; and Royal Cornwall Hospital, Truro.
- Ireland: Cork University Hospital, Cork; and Beaumont Hospital, Dublin.
- Northern Ireland: Belfast City Hospital, Belfast; and Altnagelvin Area Hospital, Londonderry.
- Wales: Ysbyty Gwynedd, Bangor; and University Hospital Wales, Cardiff.
Members of the BSAC Extended Working Party on Resistance Surveillance

Working Party members, May 2012: A. P. MacGowan (North Bristol NHS Trust, Chair), M. Allen (Novartis Pharmaceuticals), D. Brown (EUCAST Scientific Secretary), H. Grundmann (RIVM, Netherlands), M. Heginbotham (Public Health Wales), A. Kidney (Quotient Bioresearch), D. M. Livermore (University of East Anglia; HPA), V. Martin (North Bristol NHS Trust), J. Morrison (Quotient Bioresearch), S. Mushtaq (HPA), C. Pick (Janssen Pharmaceutica), J. Porter (Pfizer), R. Reynolds (North Bristol NHS Trust), A. Shah (Astellas Pharma), J. Steenbergen (Cubist Pharmaceuticals), C. Thomson (Astellas Pharma) and A. White (tranScrip partners).

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Transparency declarations

D. M. L. has shareholdings in AstraZeneca, Dechra, Eco Animal Health, Merck, Pfizer and AstraZeneca, has accepted grants, speaking invitations and conference invitations from Pfizer, Novartis, AstraZeneca and Astellas, and has advisory or consultancy relationships with Achaogen, AstraZeneca, Basilea, Cubist, GlaxoSmithKline, Kolixid, McKinsey, Meiji, Tetraphase and Theravance. All other authors: none to declare.

Author contributions


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