Journal of Antimicrobial Chemotherapy (2007) **60**, 788–794
doi:10.1093/jac/dkm258
Advance Access publication 10 July 2007

JAC

Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001–05

Gregory Steinkraus[1]*, Roger White[2] and Lawrence Friedrich[3]

[1]New Hanover Regional Medical Center, 2131 South 17th Street, Wilmington, NC 28402, USA; [2]Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, 280 Calhoun Street, Room QF 219, PO Box 250140, Charleston, SC 29425, USA; [3]Cubist Pharmaceuticals, 804 Prince Ferry Lane, Mt. Pleasant, SC 29464, USA

Received 19 April 2007; returned 23 May 2007; revised 14 June 2007; accepted 20 June 2007

***Objectives*: To assess whether methicillin-resistant *Staphylococcus aureus* (MRSA) vancomycin MIC shifts (MIC creep) at a tertiary care institution occurred that may have gone undetected using traditional susceptibility markers (percentage susceptible, MIC_{50}, MIC_{90}) over a 5 year period. Additionally, MIC trends were evaluated for oxacillin, linezolid and daptomycin.**

***Methods*: Etest MICs were performed on MRSA blood culture isolates (January 2001–December 2005). Only one isolate per patient was studied. The reported Etest MIC result was used and not rounded upward. MIC_{50}, MIC_{90}, median and geometric mean MIC, percentage susceptible and percentage resistant were calculated for each drug in each year. Non-parametric methods (linear correlation and Mantel–Haenszel χ^2) were used to assess MIC trends over time and the association of vancomycin, linezolid and daptomycin MICs with oxacillin MICs.**

***Results*: All isolates were susceptible to vancomycin, linezolid and daptomycin and resistant to oxacillin. MICs increased for vancomycin, linezolid and oxacillin ($P < 0.0001$); however, daptomycin MICs decreased slightly ($P = 0.0386$). For vancomycin, linezolid and oxacillin, there were significant increases ($P < 0.0001$) in the percentage of isolates with MICs that were higher than the respective 2001 median MIC, but not for daptomycin ($P = 0.1361$). Oxacillin MICs were associated with MICs of linezolid ($r = 0.364$, $P < 0.0001$), vancomycin ($r = 0.353$, $P < 0.0001$) and daptomycin ($r = 0.106$, $P = 0.0063$).**

***Conclusions*: Oxacillin, vancomycin and linezolid MICs increased over time. For vancomycin and linezolid, these MIC increases were not reliably detected by percentage susceptibility as they occurred below the susceptibility breakpoint. Although the MICs of all agents appeared to be associated with increasing oxacillin MICs, the strongest associations were noted for vancomycin and linezolid.**

Keywords: daptomycin, linezolid, susceptibility

Introduction

Staphylococci are among the most common organisms in hospital-acquired infections. For decades, therapies for methicillin-resistant *Staphylococcus aureus* (MRSA) have been limited primarily to vancomycin. Thus, the association of vancomycin treatment failures with increased vancomycin MICs is concerning, especially as these MICs are within what is considered the susceptible range.[1–4] Because susceptibility information is typically provided as categorical data (usually as the percentage of susceptible isolates), breakpoints may allow for shifts in MIC populations to go unrecognized unless there is a change in the categorical interpretation. This increase in MICs over time has been referred to as 'MIC creep'. Although percentile MIC markers (e.g. MIC_{50} and MIC_{90}) are more quantitative than categorical data, they may mask important changes in MIC distributions as well. The geometric mean MIC is a more sensitive marker and may more accurately reflect changes in MIC

*Corresponding author. +1-910-343-7078; Fax: +1-901-343-7829; Email: gregory.steinkraus@nhhn.org

distributions when compared with traditional percentile calculations such as the MIC_{50} or MIC_{90}.[5,6] Although the use of MICs rather than categorical data would be an improvement, the traditional 2-fold dilutions used in MIC testing may also obscure these changes.[6] MIC testing with the Etest, which incorporates intermediate as well as traditional dilutions, would seem to be correct for this problem. By utilizing both a more sensitive testing method (Etest) and a more sensitive susceptibility marker (geometric mean), important MIC changes over time will more likely be detected.[6] Other studies have not been able to detect changes in MICs using the traditional susceptibility markers such as the MIC_{50} or MIC_{90} (see Discussion). Thus, the primary objective of this study was to evaluate MIC trends for clinical MRSA blood isolates to vancomycin over a 5 year period (2001–05) using the Etest and the geometric mean MIC. Secondarily, MIC trends of these isolates to linezolid, oxacillin and daptomycin were also characterized.

Materials and methods

Microorganisms

Clinical MRSA isolates from blood cultures were collected from sequential individual patients at New Hanover Regional Medical Center in Wilmington, NC, USA from January 2001 through December 2005. Only one isolate per patient was included in this analysis. For those patients with more than one isolate, only the first isolate was tested. All isolates were identified as *Staphylococcus aureus* according to standard methods.[7] Initial susceptibility testing for oxacillin resistance was performed using the Microscan Pos BP Combo Type 20 Panel. Individual isolates were then stored in Microbank vials (Pro-Lab Diagnostics, Austin, TX, USA) at −70°C until MIC testing was performed. No thawing or subculturing of isolates was performed between initial storage and MIC testing.

Drugs evaluated

MICs of vancomycin, linezolid, oxacillin and daptomycin were determined by the Etest. Oxacillin testing was performed to confirm oxacillin resistance. Etest strips for each drug were obtained from AB BioDisk, Solna, Sweden.

MIC testing methods

Prior to MIC testing for each isolate, a single bead was aseptically removed from the Microbank vial and spread onto the surface of trypticase soy agar plates supplemented with 5% sheep blood. These plates were then incubated overnight (18–24 h) at 35°C in ambient air. Each isolate was subcultured and incubated overnight for a second time under the same conditions. From these plates, portions of three to five individual colonies were inoculated into 5 mL of tryptic soy broth and incubated for 18 h. A 0.5 McFarland turbidity standard was used to streak the inoculum onto the surface of a 150 mm Mueller–Hinton II agar plate (supplemented with 2% NaCl for oxacillin only) to create a confluent lawn of microbial growth. The surface of the plate was allowed to dry for 15 min prior to Etest strip application.

MIC testing was performed using the Etest method, following manufacturer's guidelines. The four antibiotic Etest strips were applied to the agar surface using an Etest applicator and were not moved following application. MICs were read in accordance with the manufacturer's guidelines. MIC testing of the organisms was performed over a period of 4 weeks in a single laboratory. All MICs were read by a single observer.

Data analysis

The MIC_{50}, MIC_{90}, MIC range, modal MIC, median MIC, geometric mean MIC, percentage susceptible (%S) and resistant (%R) were evaluated. Per cent susceptible and resistant were determined using the most recent CLSI (formerly NCCLS) breakpoints.[8] All calculations were performed for each year in the study using only those isolates collected in that calendar year. The susceptibility breakpoints were $\leq$2 mg/L for vancomycin, $\leq$4 mg/L for linezolid and $\leq$1 mg/L for daptomycin. The breakpoint for oxacillin resistance was $\geq$4 mg/L. Actual Etest MIC values were used for all calculations and analyses and not rounded up to the next highest traditional 2-fold MIC value. However, for the calculation of the geometric mean, values reported as 'greater than' (e.g. >256 mg/L for oxacillin) were rounded up to the next intermediate dilution (e.g. 384 mg/L) as absolute values are required for this calculation.

Assessment of MIC creep

Each of the previously described susceptibility markers was calculated in each year and plotted over time to visually inspect for trends. The MIC population distribution in each year for each agent was also plotted for visual inspection of changes in the distribution over time. Additionally, individual MICs were plotted against time to evaluate changes over time. Finally, the percentage of isolates with a median MIC less than or equal to the 2001 median MIC for each individual agent was calculated for each year. This was carried out by calculating the percentage of isolates in each year for each drug with an MIC less than or equal to the median MIC for the index year (2001).

Statistical analysis

MIC trends over the 5 years were assessed using non-parametric methods. Statistical significance was defined *a priori* as $P < 0.05$. For the analysis of MIC trends over time, non-parametric correlation (Spearman's ρ) was used. The Mantel–Haenszel χ^2 test was used to assess trends in the proportion of MICs less than or equal to the 2001 median MIC for each individual agent over the study period. Non-parametric correlation was also used to assess the association between MICs of oxacillin and those of vancomycin, linezolid and daptomycin.

Results

A total of 662 isolates were collected over the 5 year period and included in this analysis. The number of isolates tested in each year were 2001, $n = 108$; 2002, $n = 126$; 2003, $n = 143$; 2004, $n = 154$ and 2005, $n = 131$.

All MICs were evaluable on the Etest strips for all agents tested except oxacillin. A total of 71 oxacillin MICs were off-scale (>256 mg/L) over the study period. The percentage of off-scale MICs in each year was 8% (2001), 5% (2002), 6% (2003), 12% (2004) and 22% (2005). The susceptibility markers (e.g. MIC_{50} and MIC_{90}) evaluated for each agent are displayed in Table 1. The geometric mean MIC increased over the study period for vancomycin (1.5-fold), linezolid (1.4-fold) and oxacillin (1.4-fold) when compared with the baseline in 2001. The

Table 1. MRSA MIC (mg/L) statistics and susceptibility 2001–05

Drug	Year	Geometric mean MIC	Modal MIC	MIC range	MIC_{50}	MIC_{90}	%S/%R
Vancomycin	2001	0.62	0.75	0.25–1.00	0.75	1.00	100/0
	2002	0.70	0.75	0.38–1.00	0.75	1.00	100/0
	2003	0.86	1.00	0.50–1.50	1.00	1.00	100/0
	2004	0.92	1.00	0.50–1.50	1.00	1.00	100/0
	2005	0.94	1.00	0.50–2.00	1.00	1.00	100/0
Linezolid	2001	0.46	0.50	0.25–1.00	0.50	0.75	100/0
	2002	0.43	0.50	0.25–1.00	0.50	0.75	100/0
	2003	0.46	0.50	0.25–1.00	0.50	0.75	100/0
	2004	0.56	0.50	0.25–1.50	0.50	1.00	100/0
	2005	0.64	1.00	0.25–4.00	0.75	1.00	100/0
Daptomycin	2001	0.31	0.25	0.19–0.50	0.25	0.50	100/0
	2002	0.31	0.25	0.19–0.50	0.25	0.50	100/0
	2003	0.32	0.25	0.19–0.50	0.25	0.50	100/0
	2004	0.32	0.25	0.19–0.50	0.25	0.50	100/0
	2005	0.28	0.25	0.19–0.75	0.25	0.50	100/0
Oxacillin[a]	2001	117	128	16 to >256	128	256	0/100
	2002	150	256	32 to >256	192	256	0/100
	2003	173	256	48 to >256	192	256	0/100
	2004	198	256	48 to >256	256	>256	0/100
	2005	162	256	4 to >256	256	>256	0/100

[a]MICs > 256 mg/L were rounded up to 384 mg/L for the geometric mean calculation.

oxacillin geometric mean increase occurred despite a decrease between 2004 and 2005. Although the percentage of off-scale MICs for oxacillin was higher in 2005, this was offset by a similar percentage of isolates with MICs lower than those in 2004, resulting in the decreased geometric mean. The daptomycin geometric mean remained unchanged over time and actually declined slightly in 2005 (0.9-fold) from that in 2001. All isolates were categorized as susceptible to vancomycin, linezolid and daptomycin, whereas all isolates were resistant to oxacillin using current CLSI guidelines.

The MIC population distributions for each drug are displayed in Figures 1–4. For purposes of visual clarity, only data from 2001, 2003 and 2005 are shown in the Figures. The most noticeable population shifts were seen with vancomycin and linezolid (Figures 1 and 2). The shifts in vancomycin MICs occurred primarily as a result of a decrease in the percentage of isolates with an MIC $\leq$ 0.5 mg/L (46% in 2001 to 5% in 2005) and an increase in the percentage of isolates with an MIC = 1.0 mg/L (16% in 2001 to 69% in 2005) which began in 2002 (Figure 5). By 2005, vancomycin MICs > 1.0 mg/L comprised 7% of the overall MIC population, which was an increase from 0% in 2001. There were no isolates with a vancomycin MIC > 2 mg/L. For linezolid, MIC shifts occurred primarily with a decrease in isolates with MICs $\leq$ 0.5 mg/L (72% in 2001 to 41% in 2005) and an increase in isolates with an MIC = 1.0 mg/L (7% to 27%). This trend began in 2002 and was consistent from that time onwards. Isolates with linezolid MICs $\geq$ 2.0 mg/L were not noted until 2005 (4% of total MIC population).

Overall, MICs increased for vancomycin, linezolid and oxacillin ($P < 0.0001$) and declined slightly for daptomycin ($P = 0.0386$) over the study period. The MIC trends appeared to either plateau or decline between 2004 and 2005 for oxacillin, vancomycin and daptomycin, but continued to increase for linezolid. Results from the analysis of the MIC trends based on

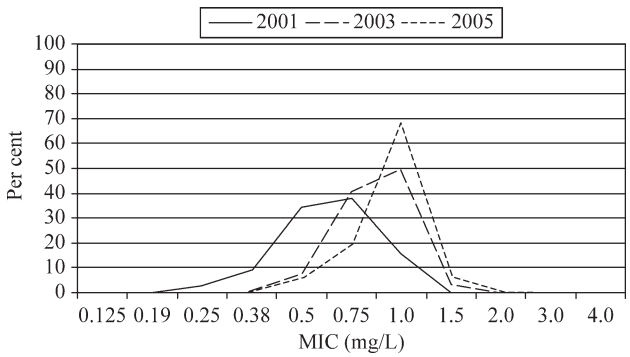

Figure 1. Vancomycin MIC population distribution 2001–05.

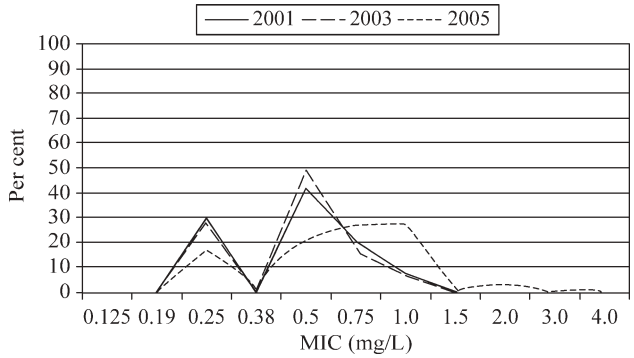

Figure 2. Linezolid MIC population distribution 2001–05.

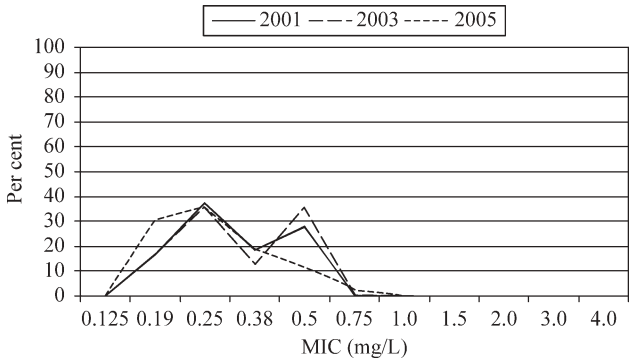

Figure 3. Daptomycin MIC population distribution 2001–05.

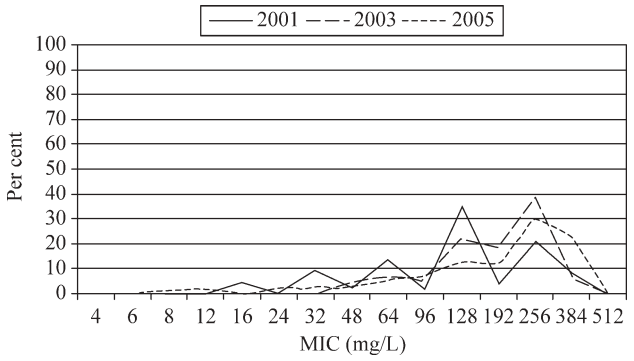

Figure 4. Oxacillin MIC population distribution 2001–05.

comparison with the 2001 median MIC are shown in Table 2. The percentage of MICs less than or equal to the 2001 median MIC significantly declined for vancomycin, linezolid and oxacillin ($P < 0.0001$), indicating a shift to higher MICs over the study period. In contrast, no trend was noted for daptomycin ($P = 0.1361$).

Correlation analysis between MICs of oxacillin and those of vancomycin, linezolid and daptomycin revealed a statistically significant association for all three agents, although the degree of the association was not very strong. The strongest association was noted for linezolid ($r = 0.364$, $P < 0.0001$), followed by vancomycin ($r = 0.353$, $P < 0.0001$) and daptomycin ($r = 0.106$, $P = 0.0063$). Analysis excluding isolates with oxacillin MICs > 256 mg/L did not change the strength or the statistical significance of the associations for any drug.

Discussion

MRSA continues to be a major pathogen in hospital-acquired infections. Over the last 40 years, its incidence has continually increased with recently published estimates of ~60% in ICUs.[9] Recognition of vancomycin-resistant *S. aureus*, vancomycin-intermediate *S. aureus* (VISA) and hetero-resistant *S. aureus* (h-VISA) has caused a great deal of concern as they have been associated with clinical failures.[4,10–12] As decreases in vancomycin susceptibility appear to occur along a continuum, close scrutiny of vancomycin susceptibility trends is warranted.[13]

Studies reporting vancomycin MIC creep with MRSA have produced conflicting results most likely due to the MIC statistic used. Reports from large multicentre surveillance studies have not reported changes in vancomycin susceptibilities over time.[14–17] However, these types of studies are not designed to detect more subtle changes in MIC populations. They typically combine data from multiple institutions, test abbreviated MIC ranges and utilize less-sensitive traditional susceptibility markers (e.g. %S, MIC_{50}, MIC_{90}) in their analyses. Combining data from multiple centres (nationally and/or internationally) can obscure trends that may exist within a given institution(s) or country as a result of differences in patient populations and drug usage patterns. The use of abbreviated MIC ranges will limit the ability to detect shifts and their magnitude in MIC populations, especially if the shifts occur outside the tested range or near the upper end of the range tested. Furthermore, the use of traditional 2-fold dilution MIC testing instead of more precise measurement (intermediate dilutions) may also obscure changes.[6] Finally, traditional susceptibility markers such as the MIC_{50} and the MIC_{90} are not sensitive to changes that can occur well below these arbitrary cutoffs. Moreover, the percentage susceptibility does not change unless the MIC population shifts occur around the susceptibility breakpoint. Interestingly, a recent report from the BSAC Bacteraemia Resistance Surveillance Programme representing multiple centres in the UK and Ireland did report a significant increase in vancomycin MICs for *S. aureus* isolates. However, their analysis, like ours, was based on the changes in the geometric mean MIC and not on traditional susceptibility markers.[18]

Most surveillance studies reporting vancomycin susceptibility changes over time have been reported by single centres and span a similar time period as our study (2000–05).[19–22] Golan *et al.*[19] reported a statistically significant increase in vancomycin MICs over a 4 year period (2002–05) at their institution. The 1.6-fold increase in geometric mean MIC was similar to that reported in our study. The major increase in vancomycin MICs occurred with isolates increasing from ≤ 0.5 to > 0.5 mg/L; the biggest increase being seen with a 4-fold increase in MICs of 2 mg/L. Interestingly, of those organisms with an MIC of 2 mg/L by broth microdilution, <60% were identified by the automated systems studied (Vitek and Vitek 2). This observation may explain in part why vancomycin MIC creep has not been more widely detected. Wang *et al.*[22] recently published their experience from UCLA over a 5 year period for 6603 clinical

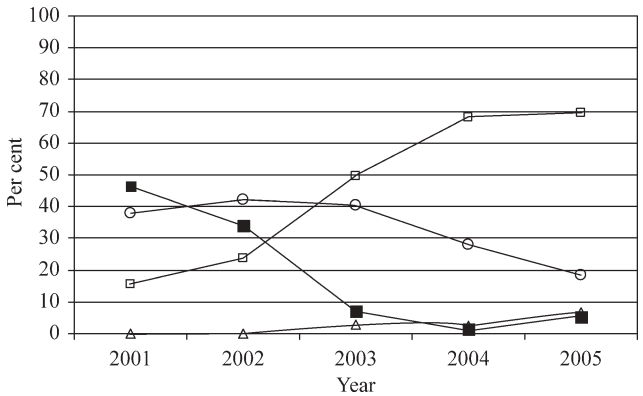

Figure 5. Vancomycin MIC trends 2001–05. Filled in squares, MIC <=0.5; open circles, MIC = 0.75; open squares, MIC = 1.0 and open triangles, MIC > 1.0 mg/L.

Table 2. Percentage of isolates with MICs less than or equal to 2001 median MIC

Drug	2001 Median MIC	2001	2002	2003	2004	2005	*P* value
Vancomycin	0.75 mg/L	84	76	48	29	24	<0.0001
Linezolid	0.50 mg/L	72	82	77	60	41	<0.0001
Daptomycin	0.25 mg/L	54	55	53	51	66	0.1361
Oxacillin	128 mg/L	67	48	38	28	36	<0.0001

S. aureus isolates. They documented a statistically significant 3.5-fold increase in the percentage of *S. aureus* isolates (methicillin susceptible and methicillin resistant) with an MIC=1.0 mg/L, with a similar decrease in isolates with an MIC $\leq$0.5 mg/L. There did not appear to be a change in the percentage of isolates with MICs $\geq$ 2 mg/L. Similar MIC patterns occurred for both MRSA and MSSA isolates, although the increase in MICs = 1.0 mg/L was greater for MSSA (6-fold), compared with the 2-fold increase with MRSA. Similar MIC patterns were noted when isolates were analysed by specimen source and patient population. Finally, Kapedia *et al*.,[20] in a study evaluating data in 2004 compared with 1985, reported significantly higher MIC_{50}, MIC_{90} and percentage of *S. aureus* isolates with MICs $\geq$ 1.0 mg/L in their institution in 2004. Similar to the report from Wang *et al*., these increases were noted for both MRSA and MSSA and were greater for MSSA. Additionally, these investigators reported significantly decreased bactericidal activity over time as evidenced by increased MBC:MIC ratios. In contrast to these previous reports, Rhee *et al*.[21] did not demonstrate any trends in *S. aureus* vancomycin MICs from 1994 to 1999. However, similar to data from Kapedia *et al*., they did demonstrate a significant decrease in the bactericidal activity as described by increasing MBCs.

Although reports of linezolid resistance among *S. aureus* are becoming more frequent,[23–25] reports of MIC creep are infrequent. Golan *et al*.[26] reported a significant trend in increased MRSA linezolid MICs from 2002 to 2005. The geometric mean increased from 1.6 to 3.3 mg/L and the percentage of isolates with MICs of 4 and 8 mg/L increased from 26% to 60% and from 0% to 9%, respectively. They also documented that the automated susceptibility testing system at their institution (Vitek 2) detected <30% of the MRSA isolates with a broth dilution MIC $\geq$ 4 mg/L to MRSA. We also demonstrated an increased linezolid geometric mean; however, the magnitude of the increase was not as great (1.4-fold versus 2.1-fold) and the pattern of the increase was different. Golan *et al.* reported a large increase in the geometric mean for the second year, which subsequently reached a plateau. In contrast, linezolid MICs increased steadily over the study period in our study. The reason for these different patterns is unknown, but may be due to differences in linezolid usage patterns between the two institutions, the baseline MICs (ours were initially lower or more susceptible), different MIC testing methods (Etest versus broth dilution) or other factors. Our data in concert with those from Golan *et al*. suggest that linezolid MIC creep is occurring.

As reported by other investigators, we demonstrated a statistically significant increase in MRSA vancomycin MICs over time. The magnitude of this increase was similar among studies, with the largest increase seen with MICs increasing from $\leq$0.5 to 1 mg/L and smaller increases seen with MICs > 1 mg/L. These increases were consistent across analyses as evidenced by the population distributions as well as the trend analyses and occurred in that range where clinical efficacy may be compromised.[1,3,4,27] We also documented increasing MICs with linezolid and oxacillin, which is consistent with previous reports. The oxacillin data are interesting in that we documented that the degree of resistance may be changing as evidenced by the increased oxacillin geometric mean MICs. The extent of these increases may be underestimated because of the number of isolates with Etest MICs >256 mg/L that were rounded up to the next intermediate Etest dilution. As most susceptibility testing typically stops at oxacillin MICs of 4 mg/L, this shift in MICs would not normally be detected. The significance of this is not known, but associations between oxacillin MICs and MICs of all other agents, although weak, were demonstrated. Thus, selection of *S. aureus* strains with high MICs of one agent may also select for higher MICs of other agents. Interestingly, the association was strongest for vancomycin and linezolid, the only two agents that also demonstrated increasing MICs. Although these associations were determined with oxacillin MICs that were rounded up to the next intermediate Etest dilution, when analysed without these data (rounded MIC values) the strength or statistical significance of these relationships was not affected. Finally, these MIC changes occurred within the susceptible category for vancomycin and linezolid or the resistant category for oxacillin, illustrating a major flaw in the use of categorical susceptibility data for surveillance. Although significant changes in the MIC populations were occurring, the percentage susceptibility remained at 100% for both vancomycin and linezolid. Analysis of other traditional susceptibility markers (e.g. MIC_{50} and MIC_{90}) only demonstrated minor changes which occurred later than those which would have been detected by use of the geometric mean.

The clinical significance of the MIC increases we noted is not clear. However, even subtle changes in MIC populations can substantially alter the pharmacodynamic profile and thus the clinical utility of an agent. For both vancomycin and linezolid, the primary pharmacodynamic parameter predicting efficacy is the AUC/MIC.[28–34] Even one intermediate dilution MIC change can affect the AUC/MIC achieved in an individual patient; similarly, a shift in an MIC population can drastically affect the anticipated target attainment rate associated with a specific AUC/MIC target. Furthermore, the increase in MICs over time may accelerate the development of resistance because of suboptimal drug exposure resulting from usual dosing regimens of vancomycin.[35]

In summary, we documented an increase in vancomycin, linezolid and oxacillin MICs over time in clinical, non-VISA

MRSA blood isolates. For vancomycin and linezolid, these MIC increases were not reliably detected by the percentage susceptible, as they occurred below the susceptibility breakpoint. These data illustrate that significant changes in MICs can occur within susceptible isolate populations. These changes may portend the future development of resistance and may help to explain the clinical failures, especially with vancomycin. Closer scrutiny of susceptibility trends should include MIC population analyses rather than sole reliance on traditional susceptibility markers.

Acknowledgements

The authors wish to acknowledge Sara Yankalev and Conni Otradovec of Cubist Pharmaceuticals for their assistance in the statistical analyses of these data.

Transparency declarations

This work was supported by a grant to G. S. from Cubist Pharmaceuticals. R. W. has also received research funding from Cubist Pharmaceuticals. L. F. is an employee of Cubist Pharmaceuticals.

References

1. Macclayton DO, Suda KJ, Coval KA *et al*. Case–control study of the relationship between MRSA bacteremia with a vancomycin MIC of 2 μg/mL and risk factors, costs, and outcomes in inpatients undergoing hemodialysis. *Clin Ther* 2006; **28**: 1208–16.

2. Moise-Broder PA, Forrest A, Schentag JJ. Relationship between time to eradication *in vivo* and bactericidal activity *in vitro* of vancomycin for MRSA infections. In: *Abstracts of the Forty-third Annual Meeting of the Infectious Diseases Society of America, Chicago, IL*, 2005. Abstract 539, p. 138. Infectious Diseases Society of America, Alexandria, VA, USA.

3. Moise-Broder PA, Sakoulas G, Eliopoulos GM *et al*. Accessory gene regulator group II polymorphism in methicillin-resistant *Staphylococcus aureus* is predictive of failure of vancomycin therapy. *Clin Infect Dis* 2004; **38**: 1700–5.

4. Sakoulas G, Moise-Broder PA, Schentag JJ *et al*. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* 2004; **42**: 2398–402.

5. White RL, Kays MB, Friedrich LV *et al*. Impact of different statistical methodologies on the evaluation of the in-vitro MICs for *Bacteroides fragilis* of selected cephalosporins and cephamycins. *J Antimicrob Chemother* 1993; **31**: 57–64.

6. White RL, Friedrich L, Steinkraus G. MIC creep: early detection with geometric mean and Etest MICs. In: *Abstracts of the Eleventh International Congress on Infectious Diseases, Cancun, Mexico, 2004*. Abstract 62.009, p. 116. International Society for Infectious Diseases, Boston, MA, USA.

7. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: 15th Informational Supplement M100-S15*. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2005.

8. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: 16th Informational Supplement M100-S16*. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2006.

9. Rehm S, Bartlett JG. Challenges in the management of serious infections. *Clin Infect Dis* 2006; **42** Suppl 2: S63–4.

10. Howden BP, Ward PB, Charles PBP *et al*. Treatment outcomes for serious infections caused by methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Clin Infect Dis* 2004; **38**: 521–8.

11. Wong SS, Ho PL, Woo CY *et al*. Bacteremia caused by staphylococci with inducible vancomycin heteroresistance. *Clin Infect Dis* 1999; **29**: 760–7.

12. Plipat N, Livni G, Bertram H *et al*. Unstable vancomycin heteroresistance is common among clinical isolates of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2005; **43**: 2494–6.

13. Hussein FM, Boyle-Vavra S, Shete PB *et al*. Evidence for a continuum of decreased vancomycin susceptibility in unselected *Staphylococcus aureus* clinical isolates. *J Infect Dis* 2002; **186**: 661–7.

14. Cuevas O, Cercenado E, Vindel A *et al*. Evolution of the antimicrobial resistance of staphylococcus spp. in Spain: five nationwide prevalence studies, 1986 to 2002. *Antimicrob Agents Chemother* 2004; **48**: 4240–5.

15. Ena J, Houston A, Wenzel RP *et al*. Trends in gram-positive bloodstream organism resistance: a seven year audit of five glycopeptides and other drugs at a large university hospital. *J Chemother* 1993; **5**: 17–21.

16. Jones RN. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. *Clin Infect Dis* 2006; **42**: S13–24.

17. White RL, Friedrich LV. Assessment of vancomycin MIC creep against staphylococci (1980–2005). In: *Abstracts of the Twenty-sixth Annual Meeting of the American College of Clinical Pharmacy, San Francisco, CA, 2005*. Abstract 156, p. 132. The American College of Clinical Pharmacy, Kansas City, MO, USA.

18. Reynolds R, Livermore DM, BSAC Extended Working Party on Bacteremia Surveillance. Trends in resistance of *Staphylococcus aureus* from blood in the UK and Ireland 2001–2005. In: *The Abstracts of the Forty-sixth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2006*. Abstract C2-1143, p. 124. American Society for Microbiology, Washington, DC, USA.

19. Golan Y, Baez-Giangreco C, O'Sullivan C *et al*. Trends in vancomycin susceptibility among consecutive MRSA isolates. In: *Abstracts of the Forty-fourth Annual Meeting of the Infectious Diseases Society of America, Toronto, Ontario, Canada, 2006*. Abstract LB-11, p. 238. Infectious Diseases Society of America, Alexandria, VA, USA.

20. Kapedia M, Coyle E, Prince R *et al*. *In vitro* activity of vancomycin against *Staphylococcus aureus* isolates from cancer patients. In: *Abstracts of the Forty-fifth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 2005*. Abstract E-807, p. 168. American Society for Microbiology, Washington, DC, USA.

21. Rhee KY, Gardiner DF, Charles M. Decreasing *in vitro* susceptibility of clinical *Staphylococcus aureus* isolates to vancomycin at the New York Hospital: quantitative testing redux. *Clin Infect Dis* 2005; **40**: 1705–6.

22. Wang G, Hindler JF, Ward KW *et al*. Increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital during a 5-year period. *J Clin Microbiol* 2006; **44**: 3883–6.

23. Gales AC, Sader HS, Andrade SS *et al*. Emergence of linezolid-resistant *Staphylococcus aureus* during treatment of pulmonary infection in a patient with cystic fibrosis. *Int J Antimicrob Agents* 2006; **27**: 300–2.

24. Roberts SR, Freeman AF, Harrington SM *et al*. Linezolid-resistant *Staphylococcus aureus* in two pediatric patients receiving low-dose linezolid therapy. *Pediatr Infect Dis J* 2006; **25**: 562–4.

25. Tsiodras S, Gold HS, Sakoulas G *et al*. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *Lancet* **358**: 207–8.

26. Golan Y, McDermott L, Perry L *et al*. Changes in linezolid susceptibility among consecutive MRSA bacteremia isolates. In: *Abstracts of the Forty-sixth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA*, *2006*. Abstract C2-1157, p. 127. American Society for Microbiology, Washington, DC, USA.

27. Hidayat LK, Hsu DI, Quist R *et al*. High-dose vancomycin therapy for methicillin-resistant *Staphylococcus aureus* infections: efficacy and toxicity. *Arch Intern Med* 2006; **166**: 2138–44.

28. Circincione B, Grasela T, Sardella S *et al*. Population pharmacodynamic assessment of linezolid efficacy in community-acquired pneumonia (CAP), skin and soft tissue (SST) infections and bacteremia (BAC). In: *Abstracts of the Fortieth Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, Canada*, *2000*. Abstract 1389, p. 29. American Society for Microbiology, Washington, DC, USA.

29. Craig WA, Andes DR. *In vivo* pharmacodynamics of vancomycin against VISA, heteroresistant VISA (hVISA) and VSSA in the neutropenic murine thigh-infection model. In: *Abstracts of the Forty-sixth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA*, *2006*. Abstract A-644, p. 16. American Society for Microbiology, Washington, DC, USA.

30. Dudley M, Griffith D, Corcoran E *et al*. Pharmacokinetic–pharmacodynamic indices for vancomycin treatment of susceptible and intermediate *S. aureus* in the neutropenic mouse thigh model. In: *Abstracts of the Thirty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA*, *1999*. Abstract 2031, p. 49. American Society for Microbiology, Washington, DC, USA.

31. Louie A, Liu W, Deziel MR *et al*. Pharmacodynamics of linezolid (Lin) in a neutropenic mouse thigh model of staphylococcus aureus (SA) infection. In: *Abstracts of the Forty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC*, *2003*. Abstract A-1865, p. 37. American Society for Microbiology, Washington, DC, USA.

32. MacGowan AP. Pharmacokinetic and pharmacodynamic profile of linezolid in healthy volunteers and patients with gram-positive infections. *J Antimicrob Chemother* 2003; **51** Suppl S2: ii17–25.

33. Moise-Broder PA, Forrest A, Birmingham MC *et al*. Pharmacodynamics of vancomycin and other antimicrobials in patients with *Staphylococcus aureus* lower respiratory tract infections. *Clin Pharmacokinet* 2004; **43**: 925–42.

34. Rayner CR, Forrest A, Meagher AK *et al*. Population (Pop) pharmacodynamics (PD) of linezolid (L) in seriously-ill adult patients (pts) from a compassionate-use protocol. In: *Abstracts of the Fortieth Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, Canada*, *2000*. Abstract 1390, p. 29. American Society for Microbiology, Washington, DC, USA.

35. Austin DJ, Bacall O, Anderson RM. Predicting the emergence of vancomycin-insensitive *Staphylococcus aureus* (VISA) using pharmacokinetic/dynamic models. In: *Abstracts of the Fortieth Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, Canada*, *2000*. Abstract 1394, p. 30. American Society for Microbiology, Washington, DC, USA.