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Increased Vancomycin MICs for *Staphylococcus aureus* Clinical Isolates from a University Hospital during a 5-Year Period[▽]

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***Staphylococcus aureus* is one of the most commonly isolated organisms in nosocomial infections. While the prevalence of methicillin-resistant *S. aureus* (MRSA) continues to increase worldwide, there is concern about an increase in vancomycin MICs among *S. aureus* strains. The prevalence of MRSA and vancomycin MIC trends in *S. aureus* from patients in a university hospital were analyzed. Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) reference broth microdilution MIC testing was performed on all clinically relevant *S. aureus* isolates from January 2000 through December 2004. A total of 6,003 *S. aureus* isolates were analyzed. No vancomycin-resistant *S. aureus* isolates were detected. One MRSA isolate had a vancomycin MIC of 8 μg/ml and was confirmed as vancomycin-intermediate *S. aureus*. Among the 6,002 remaining isolates, a shift in vancomycin MICs from ≤0.5 to 1.0 μg/ml was observed during the 5-year period. The percentage of *S. aureus* isolates with a vancomycin MIC of 1 μg/ml in 2004 was significantly higher than the percentage of isolates in 2000 (70.4% versus 19.9%; $P < 0.01$). This vancomycin MIC shift was more notable in methicillin-susceptible *S. aureus*. Our 5 years of routine testing of clinical isolates using the CLSI reference broth microdilution MIC method demonstrated a tendency toward decreasing susceptibility to vancomycin in *S. aureus*.**

Staphylococcus aureus is commonly associated with hospital- and community-acquired infections. Currently, measures to control *S. aureus* infections are challenged by a large and continuing increase in the prevalence of methicillin-resistant *S. aureus* (MRSA) worldwide (1, 20, 31), the spread of highly virulent community-associated MRSA (6, 25), and the emergence of *S. aureus* with reduced susceptibility to vancomycin and other glycopeptides (11, 13, 26, 28, 30). In the United States, approximately 60% of *S. aureus* isolates from intensive-care unit patients with nosocomial infections are MRSA, representing an 11% increase in resistance over the previous 5 years (1998 to 2002) (20). In pediatric intensive-care units, *S. aureus* infections account for approximately 10%, 20%, and 17% of patients with bloodstream infections, surgical-site infections, and pneumonia, respectively (23).

In 1997, the first *S. aureus* isolate with reduced vancomycin susceptibility (MIC, 8 μg/ml) was reported in Japan (13). Since then, six confirmed vancomycin-resistant *S. aureus* (VRSA) (MIC ≥ 32 μg/ml) (3–5, 30, 32; L. M. Weigel, personal communication) and at least 21 vancomycin-intermediate *S. aureus* (VISA) (17, 33) strains have been documented in the United States. A considerable prevalence of heterogeneous VISA (hVISA), in which only a subset of the bacterial population expresses the resistant phenotype, has been reported among *S. aureus* isolates from various geographic and patient populations of the world (9, 12, 27, 32, 33).

Although VRSA and VISA strains that demonstrate high-level resistance to vancomycin are rare among *S. aureus* clinical isolates, there is great concern about the emergence of *S. aureus* with reduced susceptibility to vancomycin due to the high incidence of the organism in causing both health care- and community-associated infections and its well-known virulence and resistance to many other antimicrobial agents (10). Furthermore, analysis of clinical and microbiological data from patients for whom vancomycin therapy failed suggests that the increasing vancomycin MICs for *S. aureus*, even those well within the current susceptible range (MIC ≤ 2 μg/ml) (8), might be a significant risk for vancomycin treatment failure (19, 24). Nevertheless, only limited studies are currently available on *S. aureus* clinical isolates with reduced susceptibility to vancomycin based on routine susceptibility testing in clinical laboratories (15, 28, 29). In this study, vancomycin MICs for *S. aureus* were determined using the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) reference broth microdilution MIC method, and results from 5 years of routine antimicrobial susceptibility testing (2000 through 2004) were analyzed to identify any trends.

MATERIALS AND METHODS

Definition of antimicrobial susceptibility. As stated in the CLSI standards, *S. aureus* isolates with oxacillin MICs of ≤2 μg/ml and ≥4 μg/ml are defined as methicillin-susceptible *S. aureus* (MSSA) and MRSA, respectively (21, 22). During the course of this study, *S. aureus* isolates with vancomycin MICs of ≤4 μg/ml were considered susceptible. VISA was defined by MICs of 8 to 16 μg/ml, and VRSA by MICs of ≥32 μg/ml (22).

***S. aureus* clinical isolates.** The *S. aureus* isolates included in this study were those for which susceptibility tests were routinely performed among isolates obtained from specimens submitted to the UCLA Clinical Microbiology Laboratory from January 2000 through December 2004. The percent susceptibility to vancomycin was calculated by examining the first isolate per patient per year, as recommended in CLSI M39-A2 (7). *S. aureus* isolates were identified by con-

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TABLE 1. Number of *S. aureus* isolates tested for susceptibility from 2000 to 2004 and vancomycin MICs

Yr	No. of strains	No. (%) of strains with vancomycin MIC (μg/ml) of:		
		≤0.5	1	≥2
2000	945	755 (79.9)	188 (19.9)	2 (0.2)
2001	1,026	830 (80.9)	194 (18.9)	2 (0.2)
2002	1,317	851 (64.6)	462 (35.1)	4 (0.3)
2003	1,297	779 (60.1)	515 (39.7)	3 (0.2)
2004	1,418	408 (28.8)	998 (70.4)[a]	12 (0.8)
Total	6,003	3,623 (60.4)	2,357 (39.3)	23 (0.4)

[a] $P < 0.01$ compared to the percentage of *S. aureus* strains with a MIC of 1 μg/ml in 2000.

ventional laboratory approaches, including Gram stain, colony morphology, and slide and/or tube coagulase tests.

MIC determination. MICs for vancomycin and several other antimicrobial agents were determined by broth microdilution, as recommended by CLSI, using in-house-prepared panels (21). Vancomycin was tested at concentrations of 0.5 to 32 μg/ml, and MICs were read manually after 24 h of incubation (21). Quality control was performed by using CLSI-recommended reference strains (21, 22). In addition, eight in-house quality control strains with variable MICs for vancomycin were tested on each new lot of the in-house-prepared panels.

Data analysis. Test results and patient demographic information were downloaded from the Medtech Laboratory Information Systems and subsequently analyzed with the WHONET software (version 5.3). The MIC interpretive criteria listed in NCCLS M100-S14 were used to define susceptibility of *S. aureus* to vancomycin and other antimicrobial agents (22). A two-sided chi-square test was employed to determine the statistical significance between *S. aureus* groups with different MICs.

RESULTS

Vancomycin susceptibility of *S. aureus*. A total of 6,003 *S. aureus* isolates from patients seen at the UCLA Medical Center from 2000 through 2004 were included in this analysis. The overall prevalence of MRSA increased from 24.9% in 2000 to 45.1% in 2004. No VRSA isolates were detected. One *S. aureus* isolate recovered from the blood of a patient in 2001 had an MIC of 8 μg/ml and was confirmed by the CDC as VISA (18). For the remaining 6,002 isolates, a shift in vancomycin MICs from ≤0.5 to 1.0 μg/ml was observed during the 5-year period (Table 1). The percentage of *S. aureus* isolates with a vancomycin MIC of 1 μg/ml in 2004 was significantly higher than that for isolates in 2000 (70.4% versus 19.9%; $P < 0.01$), indicating decreasing susceptibility to vancomycin in *S. aureus*.

TABLE 2. Percentages of *S. aureus* isolates susceptible to nonglycopeptide antimicrobial agents at different vancomycin MICs

Antimicrobial agent	% of MSSA isolates with vancomycin MIC (μg/ml) of:			% of MRSA isolates with vancomycin MIC (μg/ml) of:		
	≤0.5 (n = 2,702)	1 (n = 1,219)	≥2 (n = 11)	≤0.5 (n = 921)	1 (n = 1,138)	≥2 (n = 12)
Ciprofloxacin	95.1	93.0[a]	72.7	6.6	5.4	8.3
Clindamycin	96.0	89.7[a]	72.7	48.3	38.7[a]	25.0
Doxycycline	99.9	99.9	90.9	99.6	98.3[a]	91.7
Erythromycin	76.5	73.4	45.5	5.7	3.3[a]	8.3
Gentamicin	99.1	98.8	81.8	85.5	82.2	75.0
Rifampin	99.7	99.2	100.0	98.1	95.0[a]	100.0
TMP/SMX[b]	98.0	96.3[a]	90.9	95.6	93.8	91.7

[a] Statistical difference compared to *S. aureus* with vancomycin MIC of ≤0.5 μg/ml ($P < 0.05$).
[b] TMP/SMX, trimethoprim-sulfamethoxazole.

Twenty-three *S. aureus* isolates, including 12 MRSA and 11 MSSA isolates, had vancomycin MICs of ≥2 μg/ml during the 5-year period (Fig. 1). Of these, 22 isolates had vancomycin MICs of 2 μg/ml, and the vancomycin MIC for one isolate was 8 μg/ml. Twelve of the 23 *S. aureus* isolates with vancomycin MICs of ≥2 μg/ml were recovered in 2004.

Vancomycin MICs in MRSA and MSSA isolates. The overall percentage of MRSA isolates with a vancomycin MIC of 1 μg/ml was much higher than that for MSSA during the 5-year period (54.9% [1138/2071] versus 31.0% [1219/3932]; $P < 0.001$) (Table 2).

The change in the percentage of isolates with a vancomycin MIC of 1 μg/ml was more noticeable for MSSA, with a statistically significant increase from 12.4% in 2000 to 66.0% in 2004 (Fig. 2) ($P < 0.001$).

Vancomycin MICs in *S. aureus* from different specimen types and patient populations. To determine if there were any differences in vancomycin MICs and trends for *S. aureus* from different specimen sources, vancomycin MICs in *S. aureus* from three types of specimens (blood, wound, and respiratory) were compared. A total of 4,164 (69.4%) *S. aureus* isolates were

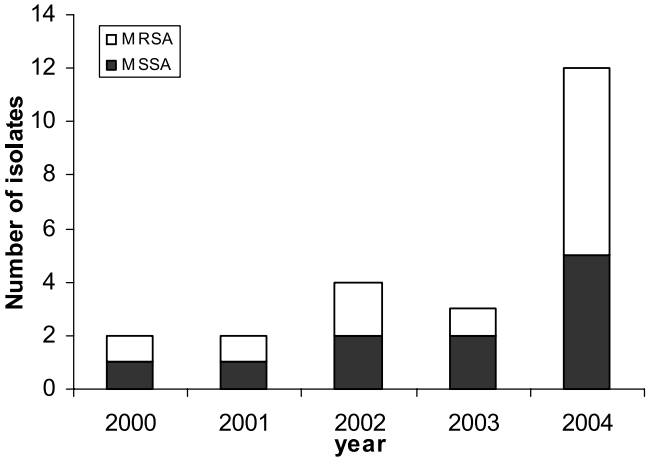

FIG. 1. Numbers of MRSA and MSSA isolates with vancomycin MICs of ≥2 μg/ml from 2000 to 2004.

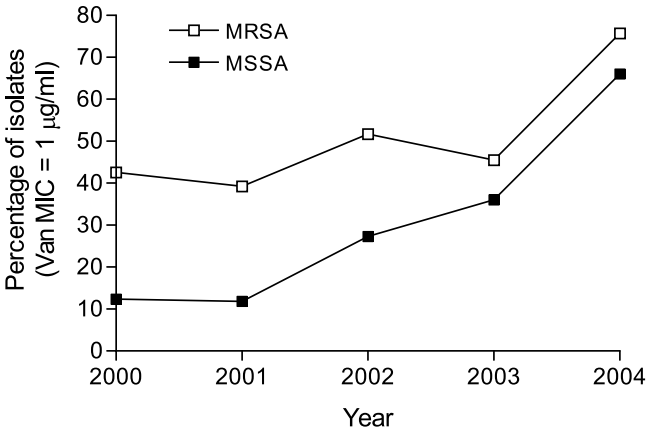

FIG. 2. Percentages of MRSA and MSSA isolates with a vancomycin (Van) MIC of 1 μg/ml from 2000 to 2004.

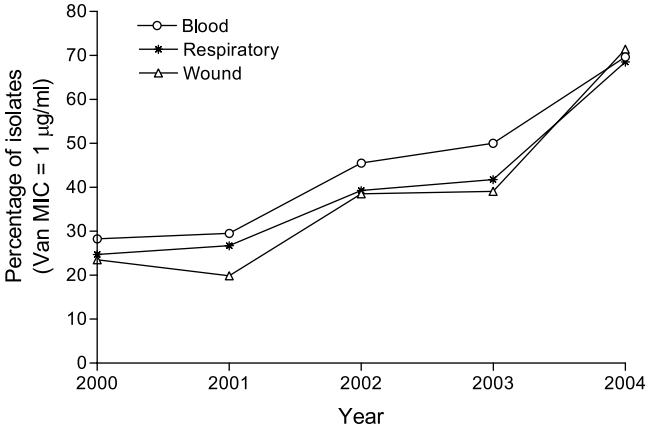

FIG. 3. Percentages of *S. aureus* isolates with vancomycin (Van) MICs of 1 μg/ml from blood, wound, and respiratory specimens from 2000 to 2004.

recovered from these specimens during the 5-year period (blood, $n = 707$; wounds, $n = 1,531$; respiratory, $n = 1,926$). A similar pattern was observed among *S. aureus* isolates recovered from all three types of specimens, showing a gradual increase in the percentage of isolates with vancomycin MICs of 1 μg/ml from 2000 to 2004 (Fig. 3).

There was no significant difference in the occurrences of vancomycin MICs in *S. aureus* isolates from distinct patient populations (e.g., different sex and age groups and outpatients versus inpatients) (data not shown).

Susceptibility to other antimicrobial agents. To determine the relationship between vancomycin MICs and susceptibility to other antimicrobial agents, the MICs for several additional antimicrobial agents were analyzed. Both MRSA and MSSA isolates with vancomycin MICs of ≥1 μg/ml tended to be less susceptible to clindamycin than those isolates with vancomycin MICs of ≤0.5 μg/ml. As shown in Table 2, the susceptibility of an *S. aureus* isolate to various classes of antimicrobial agents was more likely to correlate with oxacillin susceptibility.

DISCUSSION

S. aureus clinical isolates with reduced susceptibility to vancomycin have been reported from selected patient populations and study sites using various testing methods (e.g., agar dilution, broth microdilution, Etest, agar screening, and population studies) (2, 9, 15, 16, 32). However, only a few cases of VRSA or VISA infections have been documented to date. Given increasing reports of failure of treatment of *S. aureus* infections with vancomycin and concerns about varying vancomycin MICs obtained with different test methods (29), it is necessary to examine other parameters to follow this development.

Several studies have examined the prevalence of heterogeneous VISA among *S. aureus* clinical isolates (33). Nevertheless, the role of routine testing for hVISA in the clinical laboratory remains controversial, as the determination of hVISA among *S. aureus* clinical isolates is mainly based on labor-intensive bacterial-population profile analysis. The clinical relevance of the hVISA phenotype to clinical outcomes in patients is still under investigation. Evaluation of the evolutionary and temporal trends of the susceptibility to vancomycin among unselected *S. aureus* clinical isolates tested routinely using the CLSI reference broth microdilution MIC method is an alternative approach. In this study, the vancomycin MICs among a large number of *S. aureus* isolates from a university hospital over a period of 5 years were analyzed. Increased vancomycin MICs in *S. aureus* have been demonstrated by a shift in vancomycin MICs from ≤0.5 to 1.0 μg/ml based on analysis of routine antimicrobial susceptibility testing data obtained from 2000 through 2004 using the CLSI reference broth microdilution MIC method. It is likely that the observed shift reflects the true temporal trend in vancomycin MICs among *S. aureus* clinical isolates, since (i) all the susceptibility tests were performed by the same experienced personnel using a consistent procedure recommended by CLSI; (ii) the MIC for vancomycin on the testing panel was usually definitive and could be easily read; (iii) analysis of quality control data on *S. aureus* reference isolates, which were evaluated simultaneously throughout the study period, showed no significant shift as seen in clinical isolates; and (iv) analysis of coagulase-negative *Staphylococcus* clinical isolates ($n = 3,527$), whose antimicrobial susceptibilities were determined using the same in-house-prepared MIC panels and testing method as for *S. aureus*, showed comparable vancomycin MICs among isolates from different years of the study period: 65.1% of coagulase-negative *Staphylococcus* isolates in 2000 compared to 65.9% of isolates in 2004 had a vancomycin MIC of 1 μg/ml ($P > 0.05$).

The increasing vancomycin MICs in *S. aureus* clinical isolates are also evident from the increasing number of isolates with vancomycin MICs of ≥2 μg/ml during the study period. As shown in Table 2, *S. aureus* isolates with vancomycin MICs of ≥1 μg/ml tend to be less susceptible to other antimicrobial agents. Howden et al., Moise-Broder et al., and Sakoulas et al. reported a high rate of treatment failure with vancomycin in patients with *S. aureus* infections (14, 19, 24). The efficacy of vancomycin in the treatment of *S. aureus* infections decreased for isolates with vancomycin MICs of ≥1 μg/ml (19, 24). The number of clinical failures for patients treated with vancomycin may rise as vancomycin MICs increase to ≥2 μg/ml (19, 24). In January 2006, CLSI revised the vancomycin MIC interpretive criteria for *S. aureus*. *S. aureus* with vancomycin MICs of ≤2 μg/ml and ≥16 μg/ml are currently defined as vancomycin susceptible and vancomycin resistant, respectively, and isolates for which vancomycin MICs are 4 to 8 μg/ml are classified as vancomycin intermediate (8). It is not known whether the increasing number of *S. aureus* isolates with vancomycin MICs of ≥2 μg/ml and the observed shift in vancomycin MICs from ≤0.5 μg/ml to ≥1 μg/ml over the 5 years of our study represent a real clinical problem in the treatment of *S. aureus* infections with vancomycin.

Most previous studies focused on the clinical and microbiological characteristics of MRSA-associated infections. It is noteworthy that there was a significant increase in vancomycin MICs among MSSA clinical isolates in our study. Only 12.4% of MSSA isolates (versus 42.6% of MRSA isolates; $P < 0.01$) had vancomycin MICs of 1 μg/ml in 2000, which increased to 66.0% by 2004 (versus 76% in MRSA; $P > 0.05$). The reason for this change and its clinical significance are unknown. Since higher vancomycin MICs seem to correlate with resistance to several other classes of antimicrobial agents, this finding may

raise concerns for antimicrobial therapy in patients with MSSA infections.

In conclusion, an extremely low prevalence of VRSA or VISA was confirmed among *S. aureus* clinical isolates routinely tested for susceptibility using the CLSI reference broth microdilution MIC method over a 5-year period. Analysis of the evolutionary trend among *S. aureus* clinical isolates demonstrated a tendency toward increasing vancomycin MICs. This may raise more concerns about the potential failure of treatment of *S. aureus* infections with vancomycin and necessitate further investigation of the temporal trend of vancomycin susceptibility in *S. aureus* clinical isolates.

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