

Vancomycin MICs for *Staphylococcus aureus* Vary by Detection Method and Have Subtly Increased in a Pediatric Population Since 2005[∇]

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Vancomycin MICs for *Staphylococcus aureus* isolates in a pediatric hospital with a high rate of staphylococcal infections were examined for any increase over a 7-year period. A broth microdilution scheme allowed direct comparison of the MICs generated by this method to MICs generated by Etest. MICs generated by both methods were determined with the same inoculum suspension. One hundred sixty-five *S. aureus* isolates were selected on the basis of the patients having been bacteremic or having received vancomycin as the definitive therapy for their infections. Of the 165 isolates, 117 were methicillin-resistant *S. aureus* and 48 were methicillin-susceptible *S. aureus*. Forty-seven were acquired in the hospital (nosocomial), 56 were community acquired, and 62 were community onset-health care associated. All but one isolate tested by broth microdilution had MICs of <1.0 µg/ml, while 96% of these same isolates tested by Etest had MICs of ≥1 µg/ml. A significant increase in MICs that occurred after study year 4 (2004 to 2005) was demonstrated by the Etest ($P < 0.00007$) but not by broth microdilution. MICs were not different for isolates of community or health care origin, regardless of methodology. The proportion of isolates with Etest MICs of <1 and ≥1 µg/ml between children with bacteremia for ≤5 days and >5 days ($P = 0.3$) was not different. We conclude that MICs for pediatric isolates have increased slightly since 2005 and therapeutic decisions based on vancomycin MICs need to be made by considering the methodology used.

Recent studies have reported a steady increase in vancomycin MICs for *Staphylococcus aureus* that may be, in part, due to the increase in the use of vancomycin in response to community-acquired (CA) methicillin-resistant *S. aureus* (MRSA) (18). Also, some studies report that vancomycin MICs between 1.5 and 2.0 µg/ml are predictors of a poor therapeutic response in adults (15). The decrease in vancomycin susceptibility is difficult to assess by percentage reporting because the MIC increases are subtle, would all be classified as susceptible by using 2009 Clinical and Laboratory Standards Institute (CLSI) interpretive breakpoints, and are only detected by using a more closely spaced (arithmetic) dilution scheme versus the standard geometric dilution scheme (16). We report the first study of vancomycin MIC trends for *S. aureus* isolates from children comparing Etest and modified broth microdilution (BMD) schemes.

MATERIALS AND METHODS

Isolates. *S. aureus* isolates were identified by the Microbiology Laboratory at Texas Children's Hospital and transferred to the infectious disease laboratory on a daily basis as part of an ongoing surveillance study (10). Isolates were cataloged and stored at -80°C after one passage. Isolates were retrieved and tested in batches over a 3-week period by the two methods on the same day with the same inoculum. Demographic and clinical information from the medical record was entered into a database. The Institutional Review Board of Baylor College of Medicine approved this study. Over the 7 years of surveillance, we identified 1,108 systemic infections; 497 were with MRSA, and 611 were with methicillin-susceptible

S. aureus (MSSA). Isolates for this study (study years 1 through 7, August 2001 through July 2008) were selected on the basis of the patient's being bacteremic for 1 or more days ($n = 117$) or if the patient was nonbacteremic, vancomycin being the definitive antibiotic used to treat the infection ($n = 48$). The source of the infection (CA, community onset-health care associated [CO-HCA], or nosocomial [N]) was assigned on the basis of published definitions (10).

Inoculum. The inoculum was prepared from overnight growth on sheep blood agar and diluted to a 0.5 McFarland standard concentration. Etest plates were inoculated directly from this dilution in accordance with the manufacturer's instructions. For BMD, the inoculum was further diluted to obtain a final concentration of 5×10^4 CFU/well (3). The CLSI broth microdilution method was used to obtain MICs and minimum bactericidal concentrations (MBCs) for four isolates in a separate experiment (3). The inoculum was verified by colony counting. Both tests were performed at the same time with the same inoculum preparation.

MIC testing. BMD was performed with Mueller-Hinton broth (BBL BD Microbiology Systems, Cockeysville, MD) according to the methods of the CLSI (3). Plates were prepared and used on the same day as testing. The broth dilution tests were modified from the standard twofold dilution scheme to an arithmetic dilution so that results could be compared directly to the values generated by the Etest rather than rounding the values. This was accomplished by combining two separate twofold dilutions on the same plate. The Etest was performed according to the manufacturer's instructions with Mueller-Hinton agar (BBL BD Microbiology Systems, Cockeysville, MD). All tests were read at 24 h after incubation at 35°C. Quality control was performed with *S. aureus* ATCC 29213, and the resulting values had a median of 1.0 µg/ml by both methods.

Statistics. Statistical analysis was performed with True Epistat (Epistat Services, Richardson, TX). Fisher's exact test or χ^2 analysis was used for dichotomous variables, and χ^2 analysis for trend was used when appropriate. All analyses were two tailed, and a P value of <0.05 was considered significant.

RESULTS

One hundred sixty-five isolates of *S. aureus* were selected for study. One hundred seventeen (71%) were MRSA, and 48 (29%) were MSSA. Fifty-six were CA, 62 were CO-HCA, and 47 were N. All isolates were susceptible to vancomycin (MIC, ≤ 2 µg/ml) by either test method.

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TABLE 1. *S. aureus* vancomycin MICs over 7 years measured by Etest and BMD^a

Test and study yr	No. (%) of isolates with MIC ($\mu\text{g/ml}$) of:					
	0.375	0.5	0.75	1.0	1.5	2.0
Etest						
1	0	0	0	4 (44)	4 (44)	1 (11)
2	0	0	1 (8)	6 (50)	4 (33)	1 (8)
3	0	0	3 (14)	11 (43)	16 (29)	5 (14)
4	0	0	1 (3)	11 (23)	19 (63)	5 (11)
5	0	0	2 (8)	5 (35)	16 (42)	4 (15)
6	0	0	0	1 (13)	26 (77)	1 (10)
7	0	0	0	1 (6)	16 (88)	1 (6)
BMD						
1	4 (44)	5 (56)	0	0	0	0
2	9 (75)	3 (25)	0	0	0	0
3	17 (49)	15 (43)	2 (6)	1 (3)	0	0
4	16 (44)	17 (47)	3 (8)	0	0	0
5	16 (59)	10 (37)	1 (4)	0	0	0
6	14 (50)	13 (46)	1 (4)	0	0	0
7	6 (33)	10 (56)	2 (11)	0	0	0

^a The results shown are for selected isolates obtained at Texas Children's Hospital from 2001 to 2008.

Since the BMD method uses a geometric dilution and the Etest dilution progression is arithmetic, comparison of the two results is problematic; this is usually resolved by rounding the Etest result up. In this study, we sought to overcome this difficulty by using the same dilution progression as the Etest so that the two results could be compared without numerical manipulation. This was accomplished by combining two geometric vancomycin dilutions into one that becomes the arithmetic gradient used by the Etest. There were striking but consistent differences between the MICs generated by the BMD test and the Etest (Table 1). By BMD, all but one isolate had MICs of less than 1 $\mu\text{g/ml}$. In contrast, with the same staphylococci tested by Etest, 158 isolates (96%) had MICs of 1, 1.5, or 2 $\mu\text{g/ml}$. All isolates were categorized as susceptible by CLSI breakpoint when tested by either method (4). The inoculum suspension for both tests was prepared at the same time and confirmed by colony counting to be at the proper final concentration for the BMD and Etest procedures. The Etest result was always higher than the BMD MIC, usually with differences of 2 or more antibiotic concentrations (wells) between methods. In only one instance (0.5 $\mu\text{g/ml}$ by BMD, 0.75 $\mu\text{g/ml}$ by Etest) were the MICs within 1 dilution of each other. Twenty isolates had MICs within 2 dilution, 86 isolates within 3 dilutions, and 58 within 4 dilutions.

The Etest results showed that there had been an increase in vancomycin MICs over the study period (Table 1). Measured by the Etest, this vancomycin MIC increase was manifested as a shift in the proportion of isolates with an MIC of 1 to 1.5 $\mu\text{g/ml}$ that started in study year 4 (August 2004 to July 2005) (χ^2 analysis for trend, $P = 0.000007$) and a similar increase in the proportion of isolates from 1.5 to 2 $\mu\text{g/ml}$ (χ^2 analysis for trend, $P = 0.04$). This MIC increase was not demonstrated by the BMD method as a shift from 0.375 to 0.5 or 0.75 $\mu\text{g/ml}$ (χ^2 analysis for trend, $P = 0.39$).

There were no differences in the vancomycin MICs for 50 or 90% of the strains tested by either method, regardless of the isolate's susceptibility or resistance to methicillin. MICs for CA, CO-HCA, and N isolates did not differ significantly by

either technique. One hundred seventeen patients were bacteremic for 1 to 17 days (median = 3 days). In bacteremic patients, there was no correlation between the MIC and the duration of positive blood cultures. In addition, the proportion of isolates with an MIC of <1 or ≥ 1 $\mu\text{g/ml}$ was not significantly different in patients with bacteremia for ≤ 5 days versus those with bacteremia for >5 days ($P = 0.297$).

A sample of four isolates with Etest MICs of 0.75, 1.5, and 2 $\mu\text{g/ml}$ was selected for comparison testing by Etest, BMD, and broth macrodilution (to include subculture for MBC generation). The Etest, BMD, and macrodilution MICs were markedly different, but the MBC generated by macrodilution was within 1 dilution of the Etest MIC.

DISCUSSION

There is evidence in the literature that vancomycin MICs are increasing incrementally (creep) and that values generated by the Etest vary from those produced by other methods, including CLSI standard BMD (6, 14, 17). Direct comparisons of the values generated by traditional twofold dilutions to the continuous dilution results produced by the Etest (which are usually rounded up for comparison) are difficult. Etest MICs are a gradient derived from the combination of two doubling dilutions resulting in half dilutions that appear to be more precise and are said to correlate with treatment outcome (8).

Using standard BMD, Wang et al. found an increase in vancomycin MICs for *S. aureus* isolates from ≤ 0.5 to 1.0 $\mu\text{g/ml}$ that was more notable for MSSA isolates over a 5-year period (2000 to 2004) (18). Steinkraus et al., using Etest, found an increase in the percentage of MRSA isolates from blood cultures between 2001 and 2005 (16). This is in contrast to reports of stable vancomycin MICs over similar periods of time as revealed by BMD (1, 7, 9). These studies indicate that vancomycin susceptibility results are highly dependent on methodology and perhaps isolate source. Prakash et al. compared the Etest and CLSI broth and agar dilution results for 101 isolates and found them to be very similar to those we report here,

except that by CLSI BMD, 76.2% had an MIC of 1.0 $\mu\text{g/ml}$, compared to 3% of the isolates in the present study by the same method (12). Using agar dilution, Prakash et al. found that 11.9% of their isolates had an MIC of 2 $\mu\text{g/ml}$ versus only 2.97% of the isolates by broth dilution. In contrast, in these same 101 isolates from bacteremic patients, with the same inoculum suspension used for broth and agar testing, 88% had MICs of 1.5 or 2 $\mu\text{g/ml}$ by the Etest. It is not stated whether these isolates were from adults or children.

We believe our study is unique in that all of the isolates used were from children who were either bacteremic or treated solely with vancomycin. Also, by modifying the BMD to the same concentrations as the Etest, we were able to directly compare Etest and CLSI BMD results, an arithmetic dilution scheme similar to that used by Boyle-Vavra et al. and Holmes and Jorgensen (2, 7). We also evaluated vancomycin MICs for MSSA, as well as MRSA, isolates. Initial empirical antibiotic therapy for a severe infection in which *S. aureus* is among the possible pathogens typically includes vancomycin, and 48 h or more may be required before antibiotic susceptibility test results are available. Furthermore, vancomycin may be continued although the isolate is MSSA when a copathogen, especially a coagulase-negative staphylococcus, is also recovered or the patient has a severe hypersensitivity to β -lactam antibiotics.

Emerging studies are finding that vancomycin therapeutic failures might be linked to isolates with higher vancomycin MICs that are still categorized as susceptible by CLSI breakpoint ($\leq 2 \mu\text{g/ml}$) (5, 8, 11). Most of these studies are of adults infected with MRSA. However, we found no difference in vancomycin MICs whether the isolate was MRSA or MSSA. Guidelines developed or under consideration for vancomycin treatment of staphylococcal infections are directed mainly at MRSA infections and focus on elevated MICs still in the susceptible range, i.e., MICs of $\geq 1.5 \mu\text{g/ml}$ (13). Our study points out the dilemma this presents in that 72% of our isolates had vancomycin MICs of $\geq 1.5 \mu\text{g/ml}$ when measured by Etest yet only one isolate had an MIC of 1 $\mu\text{g/ml}$ by conventional BMD. Since many clinical microbiology laboratories perform susceptibility testing by methods other than the Etest, basing antibiotic selection on MICs must be done cautiously and with the knowledge of the methodology employed.

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