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# Should Higher Vancomycin Trough Levels Be Targeted for Invasive Community-Acquired Methicillin-Resistant *Staphylococcus aureus Infections* in Children?

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## Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) isolates with vancomycin minimal inhibitory concentrations (MIC's) ≥ 1.5 μg/mL have been associated with poorer clinical outcomes and treatment failures in adults. We evaluated vancomycin MIC's in 71 invasive pediatric community-acquired MRSA (CA-MRSA) isolates from 2004-2008 using the E-test micro-method and the E-test macro-method. The modal MIC by micro-method was 1.5 μg/mL, and median vancomycin MIC's did not increase over time.

### Keywords

MRSA; vancomycin; methicillin resistance; *Staphylococcus aureus*; pediatric; invasive

## Introduction

Since the emergence of methicillin-resistant *S. aureus* (MRSA), vancomycin has been a key antimicrobial agent for the treatment of MRSA infections [(1, 2)]. Concerns surrounding the long-term use of vancomycin as a primary therapy were confirmed when the first vancomycin–intermediate *S. aureus* (VISA) isolate was diagnosed in Japan in 1996 [3]. Recently, it was shown in adult patients that strains with minimal inhibitory concentrations (MIC's) ≥ 1.5 μg/mL, though not above the susceptibility breakpoint of 2 μg/mL, were associated with clinical failure [(4, 5)]. This increase in vancomycin MICs over time is defined as MIC creep [(6, 7, 8)].

Because of MIC creep, tissue penetration of vancomycin, and other factors, the vancomycin MIC breakpoints were lowered in 2006. According to these breakpoints, an isolate with an MIC of ≤ 2 μg/mL is considered susceptible to vancomycin, an isolate with intermediate resistance has an MIC of 4 - 8 μg/mL, and an isolate with an MIC ≥16 μg/mL is resistant. Some bacterial colonies within the staphylococcal population, on exposure to vancomycin, develop an intermediately resistant phenotype known as hVISA (heterogeneous-vancomycin intermediate *S. aureus*), a phenotype that may be responsible for treatment failures despite overall vancomycin susceptibility [(2, 6)].

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Most data regarding MIC creep has been collected from adult isolates. It is unclear whether this phenomenon occurs in MRSA isolates from pediatric patients, specifically those classified as community-associated MRSA (CA-MRSA). CA-MRSA isolates would be expected to have lower vancomycin MIC's when compared with hospital-associated MRSA isolates due to the lack of selective vancomycin pressure in the community. We hypothesized that vancomycin MIC's have not changed significantly over time in the pediatric population, since risk factors such as frequent vancomycin exposure and foreign bodies such as catheters or prosthetic joints are not likely to be present in children with CA-MRSA disease. To evaluate this, we studied the vancomycin MICs for pediatric CA-MRSA isolates from 2004-2008 based on site of infection.

## Materials and Methods

### *S. aureus* clinical isolates

Since 2004, all pediatric CA-MRSA isolates at Vanderbilt Children's Hospital (VCH) have been archived. Each isolate represents a unique pediatric patient. Isolates are considered to be CA-MRSA based on application of CDC-criteria [(9)]. For this study, we analyzed vancomycin MIC's for 71 previously characterized invasive pediatric CA-MRSA isolates collected from 2004-2008 that were viable in culture and in which site of infection and date were known. These 71 invasive CA-MRSA isolates were randomly selected from a de-identified pediatric clinical isolate database of 1,376 unique isolates using a random number generator. In 706 isolates, unambiguous notation of site of infection was available; from these, 80 were from patients with invasive MRSA disease and 71 were viable in culture and had molecular features characteristic of CA-MRSA.

Isolates were initially classified as MRSA by the clinical laboratory of VCH and subsequently confirmed by our laboratory based on growth on mannitol salt agar plates containing oxacillin and a positive latex agglutination test for clumping factor (Staphaurex, Remel). DNA was extracted and purified and was used as template for PCR detection of *nuc* and *mecA* genes and for SCC*mec* typing, as described elsewhere [(10)]. Genotyping of isolates was performed by pulse-field gel electrophoresis and/or repetitive element sequence based PCR [(11)].

### E-test Micro- and Macro-methods

*S. aureus* strain ATCC 29213 was used as the reference strain for both E-test methods (AB-Biodisk, Solna, Sweden), which were performed according to the manufacturer's guidelines. For the micromethod, a 0.5 McFarland standard was prepared in sterile saline, inoculated onto Mueller-Hinton agar, and incubated for 24 hours at 35°C. For the macromethod, a 2 McFarland standard was inoculated onto brain-heart infusion (BHI) agar and incubated for 48 hours at 35°C. Testing of the clinical isolates was done in a single laboratory, and the results were recorded by a single observer. *S. aureus* isolates with vancomycin MICs by micromethod of ≤ 2 μg/mL were considered susceptible (VSSA) based on Clinical and Laboratory Standards Institute (CLSI) guidelines. Intermediate susceptibility to vancomycin (VISA) was defined by MICs of 4 to 8 μg/mL, and vancomycin resistance (VRSA) by MICs of ≥16 μg/mL.

Analysis of MIC's over time and by site of infection was performed using the Kruskal-Wallis H method. A P value of 0.05 was considered statistically significant. All analysis was performed with SPSS Version 16.0.

## Results

To confirm that each of the 71 isolates clinically determined to be community-associated were also genotypically consistent with CA-MRSA, we performed SCC*mec* typing and genotyping by PFGE or rep-PCR. All of the isolates had SCC*mec* IV cassette and belonged to USA300, the current epidemic clone in the US. Of the 71 invasive isolates, 47 (66.2%) were from joint infections (Table 1).

Overall, the modal MIC by micro-method was 1.5 μg/mL. Fifty-six isolates had an MIC ≥ 1.5 μg/mL. Three isolates had an MIC of 2 μg/mL, but are considered susceptible by CLSI breakpoints. Only 15 isolates had an MIC < 1.5 μg/mL. Median and mean vancomycin MICs did not increase over time (P = 0.245). Similarly, MIC values were not significantly different across different infection sites (P = 0.952, IQR = 1.5-1.5 μg/mL).

By the macro-method, 2 isolates (one from 2005, one from 2004) had vancomycin concentrations of 8 and 12 μg/ml, respectively. These isolates were susceptible by the micro-method, a characteristic consistent with hVISA. By the macromethod, changes in vancomycin concentrations over time in both the mean and the median were not different (P = 0.052, IQR = 3 to 4 μg/mL), nor were the differences by infection sites (P = 0.085, IQR = 3 to 4 μg/mL).

## Discussion

The modal vancomycin MIC of 1.5 μg/mL in our isolates is higher than the previously reported modal MIC of 1.0 μg/mL for *S. aureus* [(2)]. This has clinical importance if treatment with vancomycin is considered, since MRSA isolates with vancomycin MIC's ≥ 1.5 μg/mL have been associated with poorer clinical outcomes and vancomycin treatment failures in adults, despite the fact that they are lower than the vancomycin susceptibility breakpoint [(4,5)].

Though vancomycin is the mainstay of treatment for most children with invasive CA-MRSA infections, the pharmacologic properties of the drug, such as poor penetration into lung and bone [(12)] and potential for nephrotoxicity [(12)], are challenging. For this reason, many choose clindamycin for first-line therapy of uncomplicated osteoarticular disease, given the likelihood of susceptibility and excellent bioavailability. However, for bacteremia or complicated osteoarticular disease, vancomycin is still recommended by most, and clinicians typically use plasma trough values to evaluate safety and therapeutic window. At our institution, target trough values are set between 5 – 12 μg/mL; however, in recent years, many clinicians have pushed the troughs to 10-15 μg/mL, particularly for osteoarticular disease or pulmonary disease where vancomycin concentrations are only 10-15% of plasma [(12)]. In this study, we demonstrated that 56 of 71 isolates had MIC values ≥ 1.5 μg/mL. For patients with bone or joint infections caused by strains with vancomycin MICs of 1.5 μg/mL, troughs of no less than 10 μg/mL are likely needed for the drug to be fully effective.

We did not see a creep in MIC's of vancomycin during the period studied. These findings agree with some authors who have reported steady vancomycin MIC's over time [(2, 8)], but disagree with others, who have reported the existence of a vancomycin “MIC creep” [(6, 7)]. Most of this work has been done in hospital-associated MRSA (HA-MRSA); therefore, the same selective pressures generating MIC creep are likely not present in this cohort of patients with CA-MRSA. Additionally, by the macromethod, 2 isolates with vancomycin concentrations of 8 and 12 μg/mL, respectively, are considered to be hVISA. Currently, the E-test macromethod is considered the most sensitive screening method for detecting

hVISA [13]. Although they represent a low percentage, they might be clinically significant, especially since there are no previous studies that address hVISA in a pediatric population. The two hVISA isolates were from years 2004 and 2005, implying that this is not a growing phenomenon in this collection of isolates.

One limitation of this study is that the sample size depended on all the available and viable pediatric invasive CA-MRSA isolates at our institution. However, there were no systematic biases towards CA-MRSA isolates with higher vancomycin MIC's because until 2009 very few CA-MRSA had formal MIC testing. Another limitation is that we were unable to assess the association between vancomycin MIC and clinical outcomes, since clinical information was unavailable for the patients – a prospective study would be the most definitive way to determine this relation. Last, since many clinical laboratories utilize the E-test because of its cost-effectiveness, we chose to use this method for MIC determination. While this method can overestimate the MIC when compared to broth microdilution [14], the MIC by E-test may be more reliable in predicting vancomycin treatment outcomes [15, 16].

Clinicians should recognize that MRSA isolates, including those that are epidemiologically and genotypically CA-MRSA, may have higher vancomycin MIC's than expected and that this might complicate response to treatment. We recommend that all pediatric patients treated with vancomycin for invasive CA-MRSA disease, particularly those with osteoarticular disease or pneumonia, have formal MIC testing of their staphylococcal isolate (micro-method) to guide serum vancomycin target trough concentrations.

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**Table 1**

**Median, mean, mode, and range for vancomycin MIC's according to year and site of infection by E-test Micro- and Macro-methods**

| | | Micro-Method (μg/mL) | | | | Macro-Method (μg/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N (%) | Median (IQR)[a] | Mean (95% CI)[b] | Mode | Range | Median (IQR)[a] | Mean (95% CI)[b] | Mode | Range |
| **Year** | | *P = 0.245*[c] | | | | *P = 0.052*[c] | | | |
| 2004 | 12 (16.9) | 1.5 (1.5-1.5) | 1.5 (1.4-1.6) | 1.5 | 1.5-2 | 5.0 (4-6) | 5.3 (3.8-6.9) | 6.0 | 3-12 |
| 2005 | 22 (31.0) | 1.5 (1.5-1.5) | 1.3 (1.2-1.5) | 1.5 | 1-1.5 | 4.0 4-4) | 3.9 (3.3-4.4) | 4.0 | 3-8 |
| 2006 | 12 (16.9) | 1.5 (1-1.5) | 1.4 (1.2-1.5) | 1.5 | 1-1.5 | 3.5 (3-4) | 3.5 (3.2-3.8) | 3.0 | 3-4 |
| 2007 | 15 (21.1) | 1.5 (1.5-1.5) | 1.4 (1.3-1.5) | 1.5 | 1-1.5 | 4.0 (3-4) | 3.9 (3.4-4.5) | 4.0 | 3-6 |
| 2008 | 10 (14.1) | 1.5 (1.5-1.5) | 1.5 (1.3-1.7) | 1.5 | 1-1.5 | 4.0 (3-4) | 3.6 (3.2-4.0) | 4.0 | 3-4 |
| **Site of Infection** | | *P = 0.952*[c] | | | | *P = 0.085*[c] | | | |
| Bone | 7 (9.9) | 1.5 (1-1.5) | 1.4 (1.1-1.6) | 1.5 | 1-1.5 | 3.0 (3-6) | 4.0 (2.7-5.3) | 3.0 | 3-6 |
| Blood | 6 (8.5) | 1.5 (1.5-1.5) | 1.5 (1.5-1.5) | 1.5 | 1.5-1.5 | 4.0 (3.8-6.5) | 4.8 (2.9-6.8) | 4.0 | 3-8 |
| Joint | 47 (66.2) | 1.5 (1.5-1.5) | 1.4 (1.3-1.5) | 1.5 | 1-2 | 4.0 (3-4) | 3.7 (3.5-3.9) | 4.0 | 3-6 |
| CSF | 1 (1.4) | 1.5 (1.5-1.5) | 1.5 (NA) | 1.5 | 1.5-1.5 | 4.0 (4-4) | 4.0 (NA) | 4.0 | 4-4 |
| Pleural Fluid | 6 (8.5) | 1.5 (1-1.6) | 1.4 (1-1.8) | 1.5 | 1-2 | 5.0 (3.8-7.5) | 5.8 (2.4-9.2) | 4.0 | 3-12 |
| Pericardial Fluid | 2 (2.8) | 1.5 (1.5-1.5) | 1.5 (1.5-1.5) | 1.5 | 1.5-1.5 | 3.0 (3-3) | 3.0 (3-3) | 3.0 | 3-3 |
| Visceral abscess | 1 (1.4) | 1.5 (1.5-1.5) | 1.5 (NA) | 1.5 | 1.5-1.5 | 4.0 (4-4) | 4.0 (NA) | 4.0 | 4-4 |
| Deep tissue abscess | 1 (1.4) | 1.5 (1.5-1.5) | 1.5 (NA) | 1.5 | 1.5-1.5 | 6.0 (6-6) | 6.0 (NA) | 6.0 | 6-6 |
| **Total** | **71 (100)** | **1.5 (1.5-1.5)** | **1.4 (1.4-1.5)** | **1.5** | **1-2** | **4.0 (3-4)** | **4.0 (3.7-4.4)** | **4.0** | **3-12** |

[a] IQR, Inter-Quartile Range

[b] 95% Confidence Interval

[c] Kruskal-Wallis H method

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