Published in final edited form as:

Pediatr Infect Dis J. 2014 February ; 33(2): 216–218. doi:10.1097/01.inf.0000436281.18687.0c.

No Evidence of Vancomycin Minimal Inhibitory Concentration Creep or Heteroresistance Identified in Pediatric *Staphylococcus aureus* Blood Isolates

Jennifer L. Goldman, MD^{1,3,*}, Christopher J. Harrison, MD^{1,3}, Angela L. Myers, MD, MPH^{1,3}, Mary Anne Jackson, MD^{1,3}, and Rangaraj Selvarangan, PhD^{2,3}

¹Division of Pediatric Infectious Diseases, Children's Mercy Hospitals & Clinics, Kansas City, Missouri, USA

²Department of Microbiology, Children's Mercy Hospitals & Clinics, Kansas City, Missouri, USA

³University of Missouri - Kansas City School of Medicine, Kansas City, Missouri, USA

Abstract

We evaluated vancomycin minimum inhibitory concentration (MIC) trends by three methods (broth microdilution, Etest, Vitek 2®) in 208 *S. aureus* blood isolates from 2006–2009 and assessed for heteroresistance. Vancomycin MICs did not increase nor was heteroresistance identified. Etest yielded higher MIC results than the other two methods. No MIC was > 2 µg/ml by any testing method.

Keywords

Staphylococcus aureus; vancomycin; resistance; heteroresistance

Introduction

Over the past two decades, *Staphylococcus aureus* has emerged as a common cause of invasive infection in children with an increase in methicillin-resistant *S. aureus* (MRSA) disease [1]. Vancomycin is frequently utilized as empiric therapy for presumed invasive *S. aureus* disease and increasing vancomycin minimum inhibitory concentrations (MIC) for *S. aureus* have been observed in adult tertiary care settings [2]. These increasing MICs have been associated with treatment failures [3] and greater morbidity and mortality in adults with invasive *S. aureus* infections [4,5]. Limited data are available assessing trends in vancomycin resistance in pediatric hospital settings [6,7] and no studies are available evaluating potential *S. aureus* vancomycin heteroresistance (subpopulations of resistant *S. aureus* within a larger population of vancomycin susceptible bacteria) in children.

*Corresponding Author: Jennifer Goldman MD, Division of Pediatric Infectious Diseases, Children's Mercy Hospitals & Clinics, 2401 Gillham Rd., Kansas City, MO 64108, Telephone: 816-234-3061 Fax: 816-346-1328 jlgoldman@cmh.edu.

Conflicts of interest: The authors have no conflicts of interest

We measured *S. aureus* vancomycin MICs to assess for increasing resistance from 2006–2009. Three MIC testing methods were utilized as there have been documented discrepancies between automated, manual dilution and Etest methods. Heteroresistance testing was performed on all MRSA isolates. Potential associations between vancomycin MIC and clinical outcomes were also assessed.

Materials and Methods

Isolates

208 blood cultures where *S. aureus* was the single species isolated from patients cared for at Children's Mercy Hospitals and Clinics (CMHC) from January 1, 2006 through October 15, 2009 were included in the study. If a patient had multiple positive cultures, the earliest dated isolate was used. Four patients included had two episodes of *S. aureus* bacteremia 60 days apart during separate hospitalizations.

Inoculum

All isolates were subcultured on sheep blood agar overnight and diluted to a 0.5 McFarland standard concentration. Further dilution of inoculum was performed to yield 5×10^4 CFU/well for broth microdilution (BMD) per CLSI guidelines [1]. Additionally, the 0.5 McFarland standard concentration was directly applied to Mueller Hinton agar media per Etest manufacturer's instructions. For heteroresistance testing, all MRSA blood isolates were subcultured on sheep blood agar overnight, diluted to 0.5 McFarland standard concentration and directly applied to Mueller Hinton agar supplemented with 5% sheep blood according to Glycopeptide Resistance Detection (GRD) Etest manufacturer's instructions. Colony count was performed to validate inoculum.

Susceptibility testing

Vancomycin MICs for all isolates were determined by an automated testing system (Vitek 2®) at the original time of positive culture. Blood isolates were retrieved from -80°C storage and tested by both BMD according to CLSI guidelines [8] and Etest according to manufacturer's instructions (AB bioMérieux) simultaneously. Microdilution 96 well plates containing dilutions of vancomycin (stock, CMHC pharmacy) were prepared in order to have comparable antibiotic concentrations to the range on Etest strips so that MIC results from both methods could be compared. Quality control was performed daily with positive control wells utilizing *S. aureus* ATCC 29213. All results were interpreted by one investigator to minimize inter-observer variability.

Heteroresistance testing

GRD Etest (AB bioMérieux) was used to perform vancomycin heteroresistance testing on all MRSA blood isolates. A GRD strip consisting of a double-sided gradient with vancomycin and teicoplanin was applied to the inoculated plate. Quality control was performed daily with ATCC 29213 (MSSA), 700698 (MRSA/vancomycin susceptible) and 700699 (MRSA/reduced resistance to vancomycin) strains. All results were interpreted by one investigator at 24 and 48 hours. A result was consistent with hVISA if the Etest GRD

strip was 8 mg/L for either vancomycin or teicoplanin and the standard vancomycin Etest MIC was 4 mg/L.

Clinical Data

Clinical data from each patient in which a *S. aureus* blood isolate was available were obtained from CMHC data submitted into the Pediatric Health Information System (PHIS). PHIS is an administrative database that contains data from 43 not-for-profit, tertiary care pediatric hospitals in the United States. Data quality and reliability are assured through a joint effort between the Children's Hospital Association (formerly Child Health Corporation of America) (Shawnee Mission, KS) and participating hospitals. Utilizing data from PHIS allowed us to efficiently link study laboratory results to the clinical patient data (length of hospital stay; all-cause mortality) and cost information (total hospitalization cost).

Statistics

Wilcoxon-Rank sum test was applied to analyze continuous clinical outcome data and χ^2 or Fisher's exact was utilized to compare dichotomous variables. Cochran-Armitage trend test was used to assess trends in methicillin-susceptible *S. aureus* (MSSA) and MRSA over time and MIC trends. All analyses were two tailed, and a *P* value of <0.05 was considered significant. Statistical analysis was performed using SAS® 9.2 Software. The Institutional Review Board of CMHC approved this study.

Results

All vancomycin MICs determined by Vitek 2® and BMD were less than 2 µg/ml while 5 isolates had an MIC of 2 µg/ml by the standard Etest method. No significant increase in vancomycin MIC was evident during the study time period. No MIC values were greater than 2 µg/ml by any testing method, thus all were susceptible to vancomycin by CLSI criteria [9]. When analyzing MSSA (n=137) and MRSA (n=71) separately, no trend in vancomycin MIC was observed. No MRSA isolate exhibited vancomycin heteroresistance utilizing the Etest GRD.

Differences between BMD and standard Etest MIC results (Table 1) were observed, and 94% of isolates had higher MIC results by Etest when compared to BMD. Over half of those with higher MIC results by Etest (104/195; 53%) resulted in MIC values that were the equivalent to 1-well dilution higher, with the remaining (91/195; 47%) standard Etest MICs equivalent to 2-well dilutions higher when compared to BMD, based on the study dilution design (well concentrations of 0.5, 0.75, 1, 1.5 and 2 µg/ml). When comparing BMD to Vitek 2®, only 44 (21%) had different MIC values with the vast majority (95%) different by only 1-well dilution. Overall, Vitek 2® results were more consistent with BMD while standard Etest appeared to overestimate MICs when compared to the standard method of BMD.

Clinical patient data were available for 187 patients (63 MRSA and 124 MSSA). There were no differences in median length of hospital stay, median hospital cost, or all-cause mortality when comparing patients infected with a staphylococcal isolate with a MIC < 1 µg/ml

compared to those with a MIC ≥ 1 $\mu\text{g/ml}$ or when comparing patients with MSSA versus MRSA bacteremia.

Discussion

In the adult population, the trend during 2000–2005 toward increasing vancomycin MIC values for *S. aureus* [2,10], so-called “MIC creep”, has led to the concern that vancomycin may no longer be appropriate to treat invasive staphylococcal infection in adults where the MIC is ≥ 2 $\mu\text{g/ml}$. Further, the increase in vancomycin MICs has been associated with worse outcomes in adults with invasive *S. aureus* disease [4,5]. We did not observe a similar trend in our pediatric population during the study period. Our data are consistent with other reports in which a significant vancomycin MIC increase in pediatric populations was not demonstrated. [6,7] There is not a clear explanation for the difference in MIC trends between children and adults. However, it may be related to differences in vancomycin usage (both frequency of use and dosing strategies) in children and adults leading to differences in resistance patterns of *S. aureus*. Our study is the first to specifically evaluate all blood isolates during a specified period.

We also evaluated the prevalence of hVISA in our pediatric population with invasive MRSA. Evaluation of the presence of vancomycin heteroresistance is challenging as there is a lack of standardized methodology for identification and the gold standard, population analysis profile to vancomycin exposure curve ratio (PAP-AUC), can be labor-intensive and is typically unavailable in clinical laboratory settings. We utilized the Etest GRD method as a screening test to detect vancomycin heteroresistance for all MRSA isolates in our population. This method has demonstrated relatively high specificity and sensitivity when compared to PAP-AUC, suggesting it may be an adequate screening test.[11] However, confirmation of positive tests by PAP-AUC is warranted. Although no vancomycin heteroresistance was identified among our isolates, observations should be confirmed in other pediatric populations because of the potential associated clinical complications and poor outcomes reported in adult cases.

Storage of *S. aureus* isolates can potentially impact vancomycin susceptibility results and is a recognized limitation of this study. Furthermore, results over a more extensive study period may potentially demonstrate an increasing trend in vancomycin MICs. Finally, a larger sample size may be required to detect heteroresistance.

In our study, Vancomycin MIC results varied across testing methods, with the standard Etest resulting in a higher value 94% of the time when compared to BMD. This could be problematic in clinical care as a falsely elevated vancomycin MIC may deter a clinician from utilizing vancomycin in a scenario in which it would otherwise be the preferred first-line therapy. Therefore it is essential for clinicians to be aware of the testing method employed for susceptibility testing in their institution and consider MIC results in light of the method when selecting vancomycin as the preferred antimicrobial agent.

Acknowledgements

We are thankful for the assistance of Ashley Sherman, CMHC's Associate Research Biostatistician, for providing statistical analysis of the data and for Matt Hall, with the Children's Hospital Association, for assistance with the use of the PHIS database.

We are also thankful to Ralph Kauffman for reviewing the manuscript and providing guidance.

Funding disclosures: Dr. Goldman is supported by grant T32 HD069038 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (Kearns, PI). Funded by the National Institutes of Health. Children's Mercy Hospitals & Clinics receives grant support from Forest Laboratories Inc., from Glaxo SmithKline, and Gilead laboratories for projects on which Dr. Harrison is PI.

References

1. Gerber JS, Coffin SE, Smathers SA, Zaoutis TE. Trends in the incidence of methicillin-resistant *Staphylococcus aureus* infection in children's hospitals in the United States. *Clin Infect Dis*. 2009; 49:65–71. [PubMed: 19463065]
2. Steinkraus G, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001–05. *J Antimicrob Chemother*. 2007; 60:788–794. [PubMed: 17623693]
3. Kullar R, Davis SL, Levine DP, Rybak MJ. Impact of Vancomycin Exposure on Outcomes in Patients with Methicillin-Resistant *Staphylococcus aureus* Bacteremia: Support for Consensus Guidelines Suggested Targets. *Clin Infect Dis*. 2011; 52:975–981. [PubMed: 21460309]
4. Soriano A, Marco F, Martinez JA, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis*. 2008; 46:193–200. [PubMed: 18171250]
5. Lodise TPGJ, Evans A, Graffunder E, Helmecke M, Lomaestro BM, Stellrecht K. Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. *Antimicrob Agents Chemother*. 2008; 52:3315–3333. [PubMed: 18591266]
6. Mason EOLL, Hammerman WA, Hulten KG, Versalovic J, Kaplan SL. Vancomycin MICs for *Staphylococcus aureus* vary by detection method and have subtly increased in a pediatric population since 2005. *J Clin Microbiol*. 2009; 47:1628–1630. [PubMed: 19403769]
7. Zheng X, Qi C, Arrieta M, O'Leary A, Wang D, Shulman ST. Lack of increase in vancomycin resistance of pediatric methicillin-resistant *Staphylococcus aureus* Isolates from 2000 to 2007. *Pediatr Infect Dis J*. 2010; 29:882–884. [PubMed: 20453708]
8. M07-A8 As. , editor; Wayne, PA., editor. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Clinical and Laboratory Standards Institute; 2009.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 19th informational supplement M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
10. Wang G, Hindler JF, Ward KW, Bruckner DA. Increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital during a 5-year period. *J Clin Microbiol*. 2006; 44:3883–3886. [PubMed: 16957043]
11. Leonard SN, Rossi KL, Newton KL, Rybak MJ. Evaluation of the Etest GRD for the detection of *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *J Antimicrob Chemother*. 2009; 63:489–492. [PubMed: 19136530]

TABLE 1Yearly number and percentage of *S. aureus* vancomycin minimum inhibitory

concentration (µg/ml) by testing method				
MIC (µg/ml)	2006 N (%)	2007 N (%)	2008 N (%)	2009 N (%)
BMD				
0.5	1 (2)	3 (5)	2 (4)	2 (5)
0.75	48 (76)	41 (70)	33 (70)	26 (67)
1	12 (19)	12 (20)	11 (24)	10 (25)
1.5	2 (3)	3 (5)	1 (2)	1 (3)
Etest				
0.5	0	0	1 (2)	0
0.75	2 (3)	1 (2)	1 (2)	0
1	23 (36)	26 (44)	16 (34)	9 (23)
1.5	35 (56)	31 (52)	28 (60)	30 (77)
2	3 (5)	1 (2)	1 (2)	0
Vitek 2®*				
<=0.5	NA	NA	3 (6)	16 (41)
<=1	63 (100)	59 (100)	39 (83)	NA
1	0	0	4 (9)	23 (59)
2	0	0	1 (2)	0

* Vitek 2® antibiotic susceptibility cards utilized since mid-2008 included <=0.5 µg/ml.

NA = not applicable.