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Prevalence and Characteristics of Heteroresistant Vancomycin-Intermediate *Staphylococcus aureus* Bacteremia in a Tertiary Care Center[▽]

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Infections with *S. aureus* with heterogeneous intermediate resistance to vancomycin (hVISA) are occurring more frequently. The detection of these infections, their prevalence, clinical characteristics, and significance are controversial. During 2003 and 2004, all blood culture isolates of methicillin-resistant *Staphylococcus aureus* (264 patients) at the Sheba Medical Center, Tel Hashomer, Israel, were assessed for hVISA by using the Etest macromethod. A total of 16 patients (6%) were positive for hVISA. Resistance to teicoplanin alone and to vancomycin alone using the Etest macromethod was found in 14 and 10 patients, respectively. Standard MICs to vancomycin were between 1 to 4 mg/ml. Most of these isolates (12 of 16 [75%]) would have been missed without specific testing. The median number of bacteremic days was 4. Seven patients had positive blood cultures for more than 5 days. Twelve patients died, and for eight of these the deaths were directly related to hVISA sepsis. We found that hVISA bacteremia was prevalent in our institution, and we suggest seeking hVISA in patients with persistent *S. aureus* bacteremia.

Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are responsible for 50% of hospital acquired *S. aureus* infections with increasing morbidity and mortality (13). A recent meta-analysis demonstrated increased mortality in bacteremia associated with MRSA compared to methicillin-susceptible *S. aureus* (MSSA) (5). Glycopeptides are considered the drug of choice for treating MRSA bacteremia and sepsis. *S. aureus* strains with reduced vancomycin susceptibility were first reported in 1997 (9). *S. aureus* strains with reduced vancomycin susceptibility include vancomycin-resistant *S. aureus* strains (VRSA; MIC $\geq$ 16 μg/ml) and vancomycin-intermediate *S. aureus* strains (VISA; MIC = 4 to 8 μg/ml). Until 2006, the MIC for VISA was defined as 8 to 16 μg/ml by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards), and these guidelines are still the ones approved by the U.S. Food and Drug Administration for vancomycin; the MIC for heterogeneous VISA (hVISA) strains was defined by the presence of subpopulations of VISA at a rate of 1 organism per 10^5 to 10^6 organisms (14, 15, 20, 23). Up till April 2006 only four cases with infections due to VRSA have been reported, which exhibit the *vanA* gene complex found in vancomycin-resistant enterococci (2, 3, 12). A few dozen VISA strains have been reported in recent years, which have slower growth rates, thickened cell walls, and reduced levels of PBP4. The underlying genetic and biochemical mechanism by which this occurred is not known. The majority of these cases with VISA have occurred in patients who were treated with vancomycin for prolonged periods of time (7, 11, 19). hVISA seems to be the stage that precedes the development of VISA, and it is believed that vancomycin creates a selective pressure for these resistant strains (21).

The best method for detecting hVISA is controversial. Methods are not standardized, and no clear guidelines have been issued. Population analysis profiling (PAP) is considered the gold standard, but this approach is labor-intensive and expensive. Thus, it is not realistic to use such a method for a routine service in a busy hospital setup. Various other methods used to detect hVISA were described and compared by Walsh et al. (22). Of the numerous methods reviewed, the Etest macromethod was superior to other methods and had good sensitivity and specificity compared to PAP (sensitivity, 96%; specificity, 97%). The Etest macromethod assesses the susceptibility of *S. aureus* to vancomycin and teicoplanin by using an inoculum density of 2.0 McFarland as opposed to the 0.5 McFarland density used in the standard Etest. In addition, the breakpoint definitions are different. In order to define hVISA, the MIC under these special conditions needs to be $\geq$8 μg/ml for both vancomycin and teicoplanin or $\geq$12 μg/ml for teicoplanin, whereas the MIC under the standard conditions for vancomycin is $<$8 μg/ml. The prevalence of hVISA has been ill defined in most studies performed thus far (8, 11).

The clinical significance of hVISA bacteremia has been difficult to assess; however, the question of clinical failure with vancomycin has been raised. In the present study we assess the prevalence and clinical characteristics of hVISA bacteremia in a tertiary care hospital over a 2-year period using the Etest macromethod. We chose this method since it can be applied in a busy laboratory when considering both labor and costs.

MATERIALS AND METHODS

From January 2003 to December 2004, all *S. aureus* bloodstream isolates at the Sheba Medical Center, Tel Hashomer, Israel, were identified by using standard methods (16). All isolates were assessed for hVISA by using the macromethod Etest. All tests were performed on fresh samples since reversion of resistance after laboratory manipulation has been reported (1). Strains were grown for 24 h on blood agar plates. Randomly selected single colonies were inoculated into fresh Mueller-Hinton broth and grown overnight. Portions (50 μl) of 2.0 McFarland suspensions were pipetted onto brain heart infusion agar plates. Etest strips (AB Biodisk) for vancomycin and teicoplanin were applied on the

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TABLE 1. MICs of vancomycin and teicoplanin as determined by the Etest macromethod and the standard Etest for 16 isolates determined to be hVISA[a]

Patient no.	MIC (μg/ml) of[b]:		
	Vancomycin (EMM)	Teicoplanin (EMM)	Vancomycin (standard Etest)
1	8	16	4
2	16	12	NA
3	12	12	2
4	12	12	4
5	4	12	1
6	4	12	1
7	4	12	1
8	16	12	2
9	8	8	2
10	8	12	2
11	4	16	2
12	6	12	2
13	8	12	4
14	24	16	2
15	6	12	4
16	12	12	2

[a] The CLSI breakpoints available at the time the study was performed associated VISA with a vancomycin MIC of 8 to 16 μg/ml (16). Recently, the breakpoints have been changed, and current CLSI breakpoints (implemented during 2006) associate VISA with vancomycin MICs of 4 to 8 μg/ml (14, 15).
[b] EMM, Etest macromethod. NA, not available.

same plate. Plates were incubated at 35°C for 48 h and then read. The criteria for defining hVISA when the Etest macromethod is used included vancomycin and teicoplanin MICs of ≥8 μg/ml or a teicoplanin MIC of ≥12 μg/ml. In addition, the MIC for vancomycin was assessed by using the standard Etest method according to manufacturer's instructions. The criteria for defining VISA were an MIC of vancomycin of ≥4 μg/ml according to the 2006 revised CLSI guidelines (14, 15). Laboratory workers were blinded to patients' clinical characteristics.

The medical files of patients with hVISA bacteremia were reviewed for baseline demographic and clinical characteristics including age, sex, primary diagnosis, days of persistent bacteremia, vancomycin therapy, vancomycin levels, and outcome.

The study protocol was approved by the hospital's IRB.

RESULTS

During the study period 264 patients were identified with MRSA bacteremia. Using the Etest macromethod, 16 (6%) of these patients were diagnosed with hVISA bacteremia (Table 1). According to the CLSI breakpoints available at that time, none of the isolates would have been identified as VISA by the standard Etest (MIC 8 to 16 μg/ml). Only four isolates (25%) would have been characterized as VISA by the standard Etest using the new CLSI breakpoints for VISA detection (MIC = 4 to 8 μg/ml) (14–16). The Etest macromethod for vancomycin alone would have identified only 7 of 16 patients (44%), and the macromethod for teicoplanin alone would have identified 14 of 16 patients (88%).

Patients characteristics are presented in Table 2. The median age was 78 years (range, 2 to 88 years). Ten patients (63%) were males. Four patients (25%) had underlying malignancy. In seven patients (44%) a surgical procedure was the primary reason for hospitalization. All were treated with vancomycin, and in all but three patients the treatment was not changed since hVISA continuous bacteremia was not considered an indication for changing therapy. In three patients the treatment was eventually changed. Two patients received quinupristin-dalfopristin, and one received linezolid. In four patients the vancomycin levels were not available. Most vancomycin levels, whether the first measurement or the mean value, were within acceptable norms. No patients had vancomycin levels lower than 5 mg/liter, and in five patients the first vancomycin measurement was less than 10 mg/liter. The median number of days patients had positive hVISA blood cultures was 4 (range, 1 to 57 days). Seven patients (44%) had blood cultures positive for more than 5 days. Five died, and three deaths were attributable to hVISA sepsis. Five patients (31%) had positive hVISA cultures for more than 20 days. Three of them died because of hVISA sepsis. Twelve (75%) patients died during the hospitalization. In eight patients (50%) death was directly related to hVISA sepsis. Of the four

TABLE 2. Characteristics of patients with isolates identified as hVISA

Patient no.	Age (yr)	Gender	Primary diagnosis[a]	Days with positive blood culture	First vancomycin serum level (mg/liter)	Mean vancomycin serum level (mg/liter)	Death	Death attributable to hVISA sepsis
1	84	F	IHD	42	NA[b]	NA	No	
2	88	F	Orthopedic surgery	57	NA	NA	Yes	Yes
3	80	F	GIT malignancy	9	8.2	>15	Yes	No
4	58	M	T-cell lymphoma	1	6.2	10–15	Yes	No
5	77	M	Anoxic encephalopathy	1	11.4	10–15	Yes	No
6	65	F	Vascular surgery	3	13.4	>15	Yes	Yes
7	87	M	Carcinoma of rectum	1	7.2	5–10	Yes	No
8	87	M	Social allocation	4	NA	NA	Yes	Yes
9	24	M	Severe burns	1	6	5–10	No	
10	54	M	Brain lymphoma	1	NA	NA	Yes	Yes
11	80	F	CABG	7	20.8	10–15	Yes	Yes
12	65	M	Orthopedic surgery	34	5.3	>15	Yes	Yes
13	71	M	CABG	4	11.9	>15	Yes	Yes
14	78	M	Vascular surgery	30	15.3	>15	Yes	Yes
15	2	F	Tetrology of Fallot	1	13.3	10–15	No	
16	82	M	Orthopedic surgery	24	13.8	>15	No	

[a] IHD, ischemic heart disease; GIT, gastrointestinal tract; CABG, coronary artery bypass graft surgery.
[b] NA, not available.

patients with VISA isolates, two died and in one of them death was attributed to sepsis.

DISCUSSION

In this study we surveyed all MRSA-positive blood cultures at the Sheba Medical Center, Tel Hashomer, Israel, for the presence of hVISA by using the Etest macromethod during a 2-year period. Sixteen patients (6%) were positive for hVISA. Several research groups have addressed the prevalence of hVISA infections (6, 10, 11, 17), and the frequency of hVISA in these publications is in the range of 0 to 74%. A review that summarized data from several studies (11) reported a prevalence of 1.64%. In most of these publications surveillance was not performed routinely, the sample size was small, the historical isolates were analyzed together with current ones, or the methods for identifying hVISA differed between studies. Thus, the interpretation of these studies is difficult. In a recent prospective study performed in France the prevalence of hVISA was 11% (8). We chose to use the Etest macromethod for detecting hVISA since this method has a high sensitivity and specificity compared to the population analysis method and is not labor-intensive or costly. Indeed, we could apply this method in a tertiary care center as a routine service. An additional advantage of our study is that all hVISA were detected in real time. None of the samples were frozen or thawed prior to testing. This approach enhanced our ability to accurately detect hVISA strains since laboratory manipulation can affect resistant results (1).

Our clinical data suggest that an hVISA bacteremic infection denotes a worse clinical course since several patients in our sample had prolonged bacteremia, and the outcome for these patients was generally dire. Vancomycin is considered to be inferior to penicillin for treating staphylococcal infections susceptible to methicillin, and it has been demonstrated that clinical failures occur more readily when patients with MSSA endocarditis are treated with vancomycin (18). In addition, patients with MRSA infections are usually older and have more comorbidities. Thus, it is not surprising that these patients had a worse outcome compared to patients with MSSA bacteremia (5). Do patients with hVISA infections treated with vancomycin have an even worse prognosis? Charles et al. (4) demonstrated that patients with hVISA bacteremia were more likely to have high bacterial load and that vancomycin failed to cure these patients, although there was no excess mortality compared to MRSA patients. However, the small number of hVISA patients in that study limits the generality of the conclusions. Other studies were retrospective in nature, and the sensitivity of detecting hVISA infections was not clear. Thus, it seems premature to conclude that hVISA infections have no clinical significance. The results in the present study suggest that these infections are not trivial, with 75% mortality rates for all hVISA patients and 50% hVISA-attributable mortality. In a meta-analysis comparing the mortality associated with MRSA and MSSA bacteremia the mortality attributed to MRSA infection was 33% (if calculated from the data in Table 1 in reference 2). This figure might be even lower since we can assume that some of these cases were actually hVISA-related deaths. Further study with a direct comparison of patients with hVISA to patients with MRSA infection is needed to reach a more definitive conclusion. We did not find a direct correlation between the MIC and the outcome of patients. Patients that had VISA infections did not fare worse than other patients for whom the vancomycin MICs were lower. However, the sample size here was small.

It has been suggested that the reason for clinical failure and the development of hVISA relates to treatment with suboptimal vancomycin levels. Our data do not support this conclusion. Most patients had vancomycin trough levels of >5 mg/liter in the first measurement taken, and most patients had high trough vancomycin levels throughout their treatment course.

This study was performed during 2003 and 2004. At that time none of the hVISA isolates would have been considered to have reduced susceptibility to vancomycin. Recently, the breakpoints for resistance have been changed, and thus four patients can be considered to have had VISA bacteremia. This did not necessarily affect their outcomes since two of these patients survived. The revised definitions would have probably affected the antibiotic regimen these patients would have received. It remains to be seen whether a different clinical approach would have changed the outcomes of such patients. However, even with the revised definitions most hVISA patients would have escaped detection in our facility.

In summary, we have demonstrated that hVISA isolates are more common than previously thought. Detecting these isolates necessitates specific laboratory diagnosis even when the new CLSI guidelines for detecting VISA are used. We propose to incorporate the Etest macromethod routinely since this information is clinically important.

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