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Original Article

Poor Specificity of Screening Tests for the Detection of *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin

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Abstract

Between July 2001 and December 2003, *Staphylococcus aureus* with reduced susceptibility to vancomycin was detected by screening tests in 22 isolates at our institution. This report is a cautionary note about the results of screening tests without confirmatory testing for the laboratory identification of vancomycin-intermediate *S. aureus*.

Introduction

Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to glycopeptides (known as SA-RVS, SARGS, GISA or VISA) have emerged in many countries,[1] and represent yet another therapeutic and infection control challenge. Although isolates with homogeneous resistance to vancomycin (minimum inhibitory concentration [MIC] = 8 mg/L) continue to be rare, there are increasing reports of heteroresistant vancomycin-intermediate *S. aureus* (hVISA), often with vancomycin MICs in the 1-4 mg/L range. [1,2] Unfortunately, vancomycin-resistant *S. aureus* may not be detected by automated susceptibility tests.[3]

Some isolates identified as vancomycin-intermediate *Staphylococcus aureus* (VISA) by commonly used screening methods may not be confirmed by population analysis profile studies (PAP). This report describes our hospital network's experience with the detection of VISA among isolates of MRSA and the pitfalls related to the poor specificity of screening tests for the detection of these organisms.

Methods

Microbiology Screening Methods

In our laboratory all isolates of *S. aureus* were identified as MRSA on the basis of a positive latex test result (Staph Latex; Remel, USA), positive Dnase reaction, and susceptibility testing using the Vitek AMS GPS-426 card (bioMèrieux, USA). An oxacillin MIC ≥ 4 mg/L confirmed the isolate as methicillin resistant.

MRSA isolates were then further screened for reduced susceptibility to vancomycin. However, the microbiology screening methods for identifying VISA in our institution have changed over the last three years (Figure 1).[4] Isolates of MRSA from blood cultures were screened directly by a macro-method,[5] and PAP was performed to confirm the vancomycin susceptibility of any screen positive MRSA. For sites other than blood cultures, all MRSA strains were initially screened for reduced vancomycin susceptibility by the method described by Hiramatsu *et al*.[6] However, formal vancomycin MICs were substituted with the macro-method in June 2003, with results ≥ 4 mg/L reported as VISA and PAP testing was delayed.

Confirmatory Testing

The PAP test performed consisted of serially diluting a late log phase broth culture of MRSA and plating onto agar with increasing concentrations of vancomycin. A graph was then drawn of viable count versus vancomycin concentration.[7] Beta-lactam antagonism testing between aztreonam and vancomycin was also examined on isolates that screened positive as VISA.[8] Use of PAP as a "gold standard" confirmatory test[1,4] was not immediately performed during the latter half of 2003. Clinical inconsistencies and doubt surrounding the screening results for VISA led to all VISA isolates being subjected to PAP commencing in 2004, with the results analyzed retrospectively.

Infection Control Measures

Contact isolation precautions, as recommended by the Society for Healthcare Epidemiologists of America (SHEA),[9] were adopted in our hospitals to prevent transmission of multidrug-resistant *S. aureus*.

Results

The first case of VISA in our institution was identified in July 2001. By December 2003, reduced susceptibility to vancomycin was detected in 22 MRSA isolates from 21 patients (Table 1). These isolates were from clinical cultures in 18 patients; blood cultures,[6] endotracheal aspirates,[5] sputum,[2] other.[5] Three isolates were screening swabs of the nose[2] and groin.[1]

Table 1: Results of MRSA isolates which screened positive as vancomycin - intermediate *S. aureus* (VISA)

Date of isolation	Site	Vancomycin MIC for Blood Cultures (mg/L)	Vancomycin MIC for isolates from other sites (mg/L)	Vancomycin macromethod screen Etest (mg/L)	PAP	Final Identification
4th Dec 2001	1. Left tibial tissue	-	6	-	Negative	MRSA
5th Dec 2001	2. CVC tip	-	6	-	Negative	MRSA
10th Dec 2001	3. Blood Culture	3	-	-	Negative	MRSA
20th Jul 2003	4. Sputum	-	-	12	Negative	MRSA
21st Jul 2003	5. ETA	-	-	6	Negative	MRSA
18th Jul 2003	6. ETA	-	-	6	Negative	MRSA
25th Aug 2003	7. Tenckhoff	-	-	8	Negative	MRSA
26th Aug 2003	8. Blood Culture	-	-	6	Negative	MRSA
8th Sep 2003	9. Blood Culture	-	-	6	Negative	MRSA
17th Sep 2003	10. Blood Culture	-	-	6	Negative	MRSA
29th Dec 2003	11. Groin swab	-	-	8	Negative	MRSA
19th Nov 2003	12. Nasal swab	-	-	6	Negative	MRSA
21st Jul 2001	13. Blood Culture	8	-	-	Positive	VISA
21St Jul 2001	14. Blood Culture	4	-	-	Positive	VISA
3rd Sep 2001	15. ETA	-	8	-	Positive	VISA
9th Oct 2001	16. Right knee tissue	-	6	-	Positive	VISA
23rd Oct 2001	17. Nasal swab	-	6	-	Positive	VISA
6th Jan 2003	18. Blood Culture	4	-	-	Positive	VISA
22nd Mar 2003	19. Urine	-	2	-	Positive	VISA
16th May 2003	20. Sputum	-	12	-	Positive	VISA
22nd May 2003	21. ETA	-	-	8	Positive	VISA
25th Sep 2003	22. ETA	-	-	16	Positive	VISA

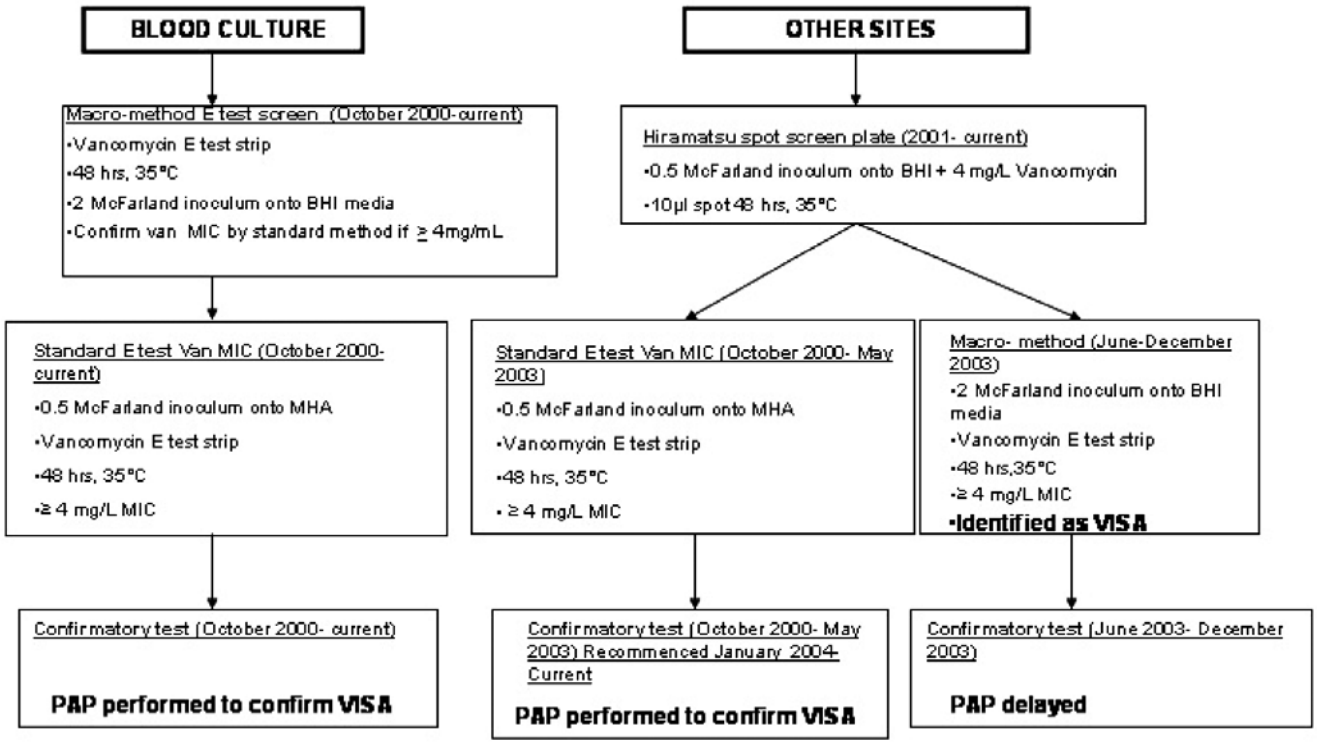
Among the 22 isolates, the MIC to vancomycin ranged between 2mg/L and 12 mg/L and 6-16 mg/L by the macro-method screening. PAP performed on these 22 isolates from 21 patients confirmed that 10 specimens from nine patients fulfilled criteria for VISA. PAP did not confirm that the other 12 isolates were VISA. Among these 12 false-positive isolates, the modal MIC was 6mg/L and, surprisingly, one isolate had a vancomycin MIC of 12 mg/L by the macro-method (Table1).

Discussion

The mechanisms of vancomycin resistance are unknown however VISA isolates exhibit a thickened cell wall and appear to be induced by vancomycin.[6] It is suspected that low vancomycin levels in the early stages of therapy could induce resistance or select out for vancomycin resistant strains. VISA is not just a laboratory phenomenon but clearly associated with serious infections and treatment problems. Many countries have reported serious clinical infections with VISA however treatment efficacy has not been systematically assessed.[6, 10] There have been reports in the literature of glycopeptide treatment failure where the glycopeptides vancomycin and teicoplanin are no longer effective in some cases of MRSA infection.[11, 12]

This report describes the pitfalls of detecting *Staphylococcus aureus* with reduced susceptibility to vancomycin. The most accurate form of vancomycin susceptibility testing for staphylococci is a nonautomated MIC method in which the organisms are incubated for a full 24 hours before reading results.[3] Disk-diffusion tests, including the Stokes method, do not detect VISA strains.[2] Screening methods for detecting vancomycin resistance have been shown to lack sensitivity and reproducibility in some studies.[13]

The macro-method utilizing the Etest is designed to detect heteroresistance since it allows detection of resistant subpopulations.[15] Although the screening Etest is highly sensitive for the detection of VISA, it has poor specificity and is not a confirmatory method. This was illustrated at our institution by the detection of a false-positive strain of VISA with a vancomycin Etest MIC of 12 mg/L by the macromethod. For the above reasons, the most accurate confirmatory test for VISA is the PAP; however this method is labor intensive and not suitable for small laboratories.[1, 14] There is no recognized confirmatory test with a rapid turnaround time, and often, as we found at our institution, there was a delay of several days after the screening results were known before the results of PAP were available.



BHI= Brain heart infusion agar, MHA= Muller Hinton agar, PAP= Population analysis profile, MRSA= Methicillin resistant *S. aureus*, VISA= Vancomycin- intermediate *S. aureus*

Figure 1: Microbiology screening methods for identifying VISA

SHEA has addressed the problem of detection and isolation of multidrug-resistant organisms such as VISA.[9] They recommend active surveillance cultures of hospitalized patients to detect colonization, screening the contacts of any index cases to determine the extent of transmission within the facility,[12] and strict adherence to contact precautions for such patients. Obviously, false-positive results derived from screening tests are therefore responsible for additional and unnecessary laboratory costs, including labor and the costs of media to process screening specimens.

The identification of multidrug-resistant pathogens also has therapeutic consequences.[1] A change of antibiotics may be undertaken based on a positive VISA result from a screening test. In the case of a false-positive result for VISA, the new therapy may not confer any benefit and could be harmful. In addition, other treatments, such as surgery to debulk infected prosthetic material, may be considered if the causative organism is identified as VISA.[12] The results of this are increased financial costs and patient costs measured by patient morbidity and mortality. Clearly, in order for hospitals to adhere to existing recommendations,[9,12] microbiology laboratories need to adopt screening methods to detect reduced vancomycin susceptibility that are sensitive and specific. Confirmatory tests also need to be less time consuming and labor intensive with a shorter turnaround time.

The laboratory identification of vancomycin – intermediate *S. aureus* is problematic, and no consensus standard in methodology currently exists. Our report is a cautionary note to our peers about the results of screening tests in the absence of confirmatory testing. We recommend that confirmatory testing by PAP be performed expeditiously on all MRSA isolates that screen positive as VISA. It is advisable that isolates be sent to a reference laboratory with skill and expertise in the PAP procedure. This will result in faster, less time consuming and labour intensive confirmation of results.

Continued vigilance in enforcing infection-control measures and improved use of antimicrobials are crucial in controlling VISA. A consensus on the optimal laboratory detection methods for VISA is required.

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