Practical Strategies for Detecting and Confirming Vancomycin-Intermediate \textit{Staphylococcus aureus}: a Tertiary-Care Hospital Laboratory’s Experience

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The clinical microbiology laboratory plays a critical role in the detection of \textit{Staphylococcus aureus} with decreased susceptibility to vancomycin. Staff education and rapid laboratory response are of utmost importance. We report on our laboratory’s experience and provide recommendations for the identification and confirmation of vancomycin-intermediate \textit{S. aureus}.

Despite the low incidence of vancomycin-intermediate \textit{Staphylococcus aureus} (VISA) worldwide, VISA remains a significant concern. The VISA strains isolated at the Medical Center of the University of California at Los Angeles (UCLA) mark the fifth of eight sets of strains confirmed by the Centers for Disease Control and Prevention (CDC) (Fred Tenover, personal communication) in the United States (1, 2, 2a, 3, 6). Internationally, cases have been reported in Japan, Korea, and Europe (3, 4, 5). Expedient confirmation of VISA isolates from clinical specimens is critical to patient care and infection control. Clinical microbiologists must be knowledgeable about procedures that can accurately detect VISA. In addition, when a VISA strain is found, results must be effectively communicated to health care providers and public health officials. Here we report on our laboratory’s experience with the isolation, detection, and confirmation of two VISA isolates from a single specimen. Our goal is to highlight specific issues that must be considered when VISA is suspected in a clinical specimen.

On 8 June 2000, a biliary drainage specimen was submitted to the Clinical Microbiology Laboratory of the UCLA Medical Center for bacterial culture. The specimen was obtained from a transhepatic biliary drainage catheter from a home health care patient with multiple hepatic abscesses who had received long-term vancomycin therapy for methicillin-resistant \textit{S. aureus} (MRSA) infection.

Following overnight incubation, examination of the primary plates revealed large colonies of \textit{S. aureus} (isolate 1) and pinpoint colonies of lactose-negative, gram-negative rods which were subsequently identified as \textit{Stenotrophomonas maltophilia}. Broth microdilution susceptibility testing of \textit{S. aureus} isolate 1 demonstrated oxacillin and vancomycin MICs of >16 µg/ml and a vancomycin MIC of 4 µg/ml. These results remained unchanged after 24 h of incubation. In our laboratory it is policy to inoculate a purity plate (blood agar plate) at the time that broth microdilution MIC test plates are inoculated. The purity plate for \textit{S. aureus} (isolate 2) demonstrated a positive slide coagulase test confirmed the identification of these colonies as \textit{S. aureus} (isolate 2), and susceptibility testing was performed. The results of the broth microdilution test read the next morning (18 h) revealed an oxacillin MIC of >16 µg/ml and a vancomycin MIC of 4 µg/ml. These results remained unchanged after 24 h of incubation. In our laboratory it is policy to inoculate a purity plate (blood agar plate) at the time that broth microdilution MIC test plates are inoculated. The purity plate for \textit{S. aureus} (isolate 2) demonstrated

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multiple colony types resembling staphylococcus and was considered to possibly contain a mixture of isolates. A Gram stain of the well that contained 2\( \mu \)g of vancomycin per ml was performed and showed only gram-positive cocci in clusters. The contents of this well and the positive control well were subcultured onto blood agar plates to check for purity. The susceptibilities of the two distinct colonial phenotypes, one comprising small yellow-white isolates (\( S. \) \textit{aureus} isolate 2a) and the other comprising pinpoint grey-white isolates (\( S. \) \textit{aureus} isolate 2b), were determined (Fig. 1). At 18 h, the vancomycin MIC was 4\( \mu \)g/ml for isolates of both phenotypes. At 24 h, the vancomycin MIC had increased to 8\( \mu \)g/ml for isolates of both phenotypes. Interestingly, the oxacillin MICs for isolates 2a and 2b were 16 and 0.5\( \mu \)g/ml, respectively. Vancomycin MICs were determined by the E-test (AB BIODISK, Solna, Sweden) and were 6\( \mu \)g/ml for both isolates. In addition, both isolates grew on vancomycin at 6\( \mu \)g/ml on brain heart infusion screen agar (Hardy, Santa Barbara, Calif.). Laboratory personnel notified hospital infection control, the Los Angeles County Public Health Department (LACPHD), and CDC. Isolates were subcultured onto Trypticase soy agar slants (BBL Microbiology Systems, Sparks, Md.) for shipment to LACPHD and CDC. The initial isolates sent to CDC were not confirmed to be VISA isolates. After further investigation at our laboratory, it was discovered that in order to expedite the confirmation process, the slants had been incubated for only 4 h prior to shipping. An important feature of VISA is its slow growth (8). It was suspected that this 4-h incubation

FIG. 2. Algorithm for detection and confirmation of VISA. This algorithm is a general guideline for workup of VISA isolates in the clinical laboratory. Algorithms should be designed for the specific hospital and patient population. Abbreviations: BHI, brain heart infusion; Vanco, vancomycin.
that patients with VISA infection are likely to respond poorly to vancomycin therapy. Thus, receipt of information that a patient is responding poorly to vancomycin should warrant appropriate tests for VISA. (v) Determine if the susceptibility method in your laboratory is likely to detect VISA. If not, determine if a backup method is warranted. (vi) Define a mechanism that can be used to communicate the results indicating probable isolation of a VISA isolate to appropriate health care workers in a timely manner.

Algorithms for VISA detection can be designed to best fit the institution and the patient population (Fig. 2). Information on how to detect and report VISA is available through the CDC website (www.cdc.gov/ncidod/hip/lab/factsheet/gisa.htm and www.CDC.gov/ncidod/hip/ARESIST/search.htm).

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