NHANES 2001–2002 Data Release
July 2004
Documentation for Laboratory Results

Laboratory 35 (LAB35) – Methicillin-Resistant *Staphylococcus aureus* (MRSA)

(1) Documentation File Date –September 2006

(2) Documentation File Name – Laboratory 35 – Methicillin-Resistant *Staphylococcus aureus*

(3) Survey Years Included in this File Release – 2001-2002

(4) Component Description

Staphylococcus aureus is one of the most common causes of skin and soft tissue infection in both the health care and community settings. Antimicrobial resistance in *S. aureus* has increased dramatically, particularly in the hospital, where the rapid emergence of methicillin-resistant *S. aureus* (MRSA) and the appearance of *S. aureus* isolates with resistance to vancomycin have led to concern that this organism may become untreatable with currently available antimicrobials. Previously limited to hospitals, MRSA infections have been increasingly reported in the community. However, no prospective, population-based prevalence study has been done to measure the prevalence of MRSA in the community, and no national surveillance exists to provide a reliable national population estimate.

(5) Sample Description

5.1 Eligible Sample

Participants aged 1 year and older were tested.

(6) Description of the Laboratory Methodology

Nasal swabs were first examined for proper labeling and integrity; they were then plated on mannitol salt agar (MSA), a selective media for the isolation of *S. aureus*. MSA plates were incubated at 35°C for 48 hours. Mannitol fermenting colonies (yellow or gold) were selected from the MSA plates and subcultured to trypticase soy agar + 5% sheep blood plates (BAP) and incubated at 35°C overnight. MSA plates with little or no growth were re-incubated at 35°C overnight, and plates with non-mannitol fermenting growth were held at room temperature. These plates were reexamined the next day, and any yellow or gold colonies were subcultured to BAP.

Overnight cultures on BAP are first screened using Staphaurex, a rapid latex kit for the identification of *S. aureus* (Remel, Lenexa, KS). A tube coagulase test using rabbit plasma with (ethylenedinitrilo) tetraacetic acid (EDTA) is then performed on

Staphaurex-negative isolates from BAP with morphology consistent with *S. aureus* and Staphaurex-positive isolates with morphology inconsistent with *S. aureus* (non-hemolytic). Staphaurex-positive isolates and Staphaurex-negative tube coagulase-positive isolates are identified as *S. aureus* and saved for further testing. Staphaurex-positive, tube coagulase-negative isolates are discarded.

S. aureus isolates are screened for methicillin resistance following the National Clinical and Laboratory Standards Institute (NCCLS) disk diffusion method. Overnight cultures from BAP are plated on Mueller-Hinton (MH) agar, and a 1-µg oxacillin (OX) disk is placed on the inoculated plate. Zone diameters are measured and recorded after 24-h incubation at 35°C (susceptible, ≥ 13 mm; intermediate, 11 mm–12 mm; resistant, ≤ 10 mm).

Isolates resistant to OX (MRSA), intermediate to OX, and every 10th isolate susceptible to OX (MSSA) by disk diffusion are saved for additional testing of organism characteristics. These tests include antibiotic susceptibility testing (MIC) by using broth microdilution using NCCLS reference methods; strain typing by pulsed-field gel electrophoresis (PFGE) using *SmaI* enzyme; singleplex polymerase chain reaction (PCR) for detection of enterotoxins, toxic shock syndrome toxin-1, and Panton-Valentine leukocidin toxin; and SCC-mec cassette typing by PCR.

(7) Laboratory Quality Control and Monitoring

The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed quality control and quality assurance instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

(8) Data Processing and Editing

Nasal swab specimens were processed, stored and shipped to Division of Healthcare Quality Promotion, National Center for Infectious Diseases, National Centers for Disease Control and Prevention, Atlanta, GA for analysis. Detailed specimen collection and processing instructions are discussed in the LPM. Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the **Description of the Laboratory Methodology** section.

(9) Data Access

All data are publicly available.

(10) Analytic Notes for Data Users

The analysis of NHANES 2001–2002 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 2001–2002 Household Questionnaire Data Files contain demographic data, health indicators, and other related information collected during household interviews. They also contain all survey design variables and sample weights for these age groups. The phlebotomy file includes auxiliary information such as the conditions precluding venipuncture. The household questionnaire and phlebotomy files may be linked to the laboratory data file using the unique survey participant identifier SEQN.

10.1 *S. aureus* present and organism identified as MRSA

If LBXMS1 = 1 and LBXM1 = 1, further organism characteristic testing was performed.

Isolates resistant to OX (i.e., MRSA), isolates intermediate to OX, and every 10th isolate susceptible to OX (i.e., MSSA) by disk diffusion were saved for additional testing of organism characteristics. These tests include antimicrobial susceptibility testing (minimum inhibitory concentration [MIC]) by broth microdilution using NCCLS reference methods; strain typing by pulsed field gel electrophoresis (PFGE) using *SmaI* enzyme; singleplex polymerase chain reaction (PCR) for detection of enterotoxins, toxic shock syndrome toxin-1, and Panton-Valentine leukocidin toxin; and SCC-mec cassette typing by PCR.

10.2 *S. aureus* present and organism identified as MSSA

Every 10th isolate susceptible to OX (i.e., MSSA) by disk diffusion was saved for additional testing of organism characteristics. These tests include antimicrobial susceptibility testing (MIC) by broth microdilution using NCCLS reference methods; strain typing by PFGE using *SmaI* enzyme.

Other organism characteristic (e.g., enterotoxin) testing is still in progress. These data will be added to this file in a future release.