

# Multicenter Evaluation of MRSASelect II Chromogenic Agar for Identification of Methicillin-Resistant *Staphylococcus aureus* from Wound and Nasal Specimens

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Hospitals strive to reduce methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence via active surveillance of inpatient populations. Rapid and inexpensive screening methods are utilized when molecular methods are not operationally feasible. In this multisite clinical trial, the utility of Bio-Rad's MRSASelect II was evaluated for MRSA identification from remnant nares and wound swabs. The prevalence of MRSA was 11.1% ( $n = 1,384$ ) from nares samples and 18.1% ( $n = 842$ ) from wound samples. MRSASelect II had an overall concordance of 95.4% (confidence interval [CI] = 94.5% to 96.2%) compared to a broth-enriched reference standard. Comparisons between results, stratified by examination times, exhibited a nonsignificant trend toward increased positivity at prolonged incubation times. Cefoxitin screening of colonies directly from MRSASelect II was 96.7% (95.8% to 97.3%) concordant compared to testing of colonies following broth enrichment. A comparison of MRSASelect and MRSASelect II revealed no statistical differences; however, the latter exhibited earlier positivity, greater selectivity, and more intense indicator staining, which resulted in facilitated differentiation of positive results. MRSASelect II agar is a simple, rapid, and robust method to routinely screen patients for MRSA colonization without the need for additional testing.

Since their initial identification in 1959 (1), methicillin-resistant *Staphylococcus aureus* (MRSA) infections have been known to cause hospital-associated and community-acquired (CA-MRSA) infections that impose a significant economic burden on the U.S. health care system (2). Prompt and cost-effective identification of asymptomatic MRSA carriers and patients with active disease is vital to support infection control practices and bed management operations within health care facilities.

MRSA surveillance programs for patients and health care workers are longstanding practice in some European health care systems and have drastically reduced the incidence of hospital-acquired infections (HAIs) (3, 4). Subsequently, U.S. centers instituted similar approaches. In 2008, the U.S. Center for Medicare and Medicaid Services reduced the amount of financial reimbursement for preventable outcomes during hospitalizations, including HAIs (5, 6). These new regulations forced many U.S. hospitals to implement active surveillance of incoming patients for MRSA. MRSA screening programs that utilize molecular methods are relatively common for laboratories screening at-risk populations or in high-volume situations (7–9). For smaller laboratories or for those screening low-risk populations, the financial investment associated with molecular testing is not practical. Therefore, many rely on culture-based methods to screen their patient populations for MRSA (10, 11).

Traditionally, culture-based methods have centered on a two-step algorithm for MRSA identification whereby *Staphylococcus aureus* is first identified and is subsequently screened for cefoxitin or oxacillin resistance to confirm MRSA identification. Chromogenic agars, while generally not as rapid or sensitive as molecular methods, can provide a cost-effective alternative for identifying MRSA from patient specimens (12–14).

CHROMagar MRSA (CHROMagar, France), Brilliance agar I

(Oxoid, United Kingdom), Brilliance agar II (Oxoid), chromID MRSA (bioMérieux, France), and MRSASelect (MSI; Bio-Rad, USA) are widely used FDA-cleared chromogenic agars for MRSA detection in the United States and Europe. Biochemical properties and resistance to cefoxitin or oxacillin in conjunction with proprietary compounds are used to differentiate MRSA strains from other staphylococcal and nonstaphylococcal organisms.

MRSASelect is FDA-cleared for MRSA detection from nasal and wound swabs and has recently been evaluated for use with blood cultures (14–16). Recently, MRSASelect was reformulated by the manufacturer to improve visual differentiation by deepening the hue of pink color that designates MRSA colonies from non-MRSA colonies and to provide enhanced inhibition of normal flora and non-MRSA organisms found in patient specimens (Bio-Rad, Hercules, CA). This new formulation will be commercially available as MRSASelect II (MSII).

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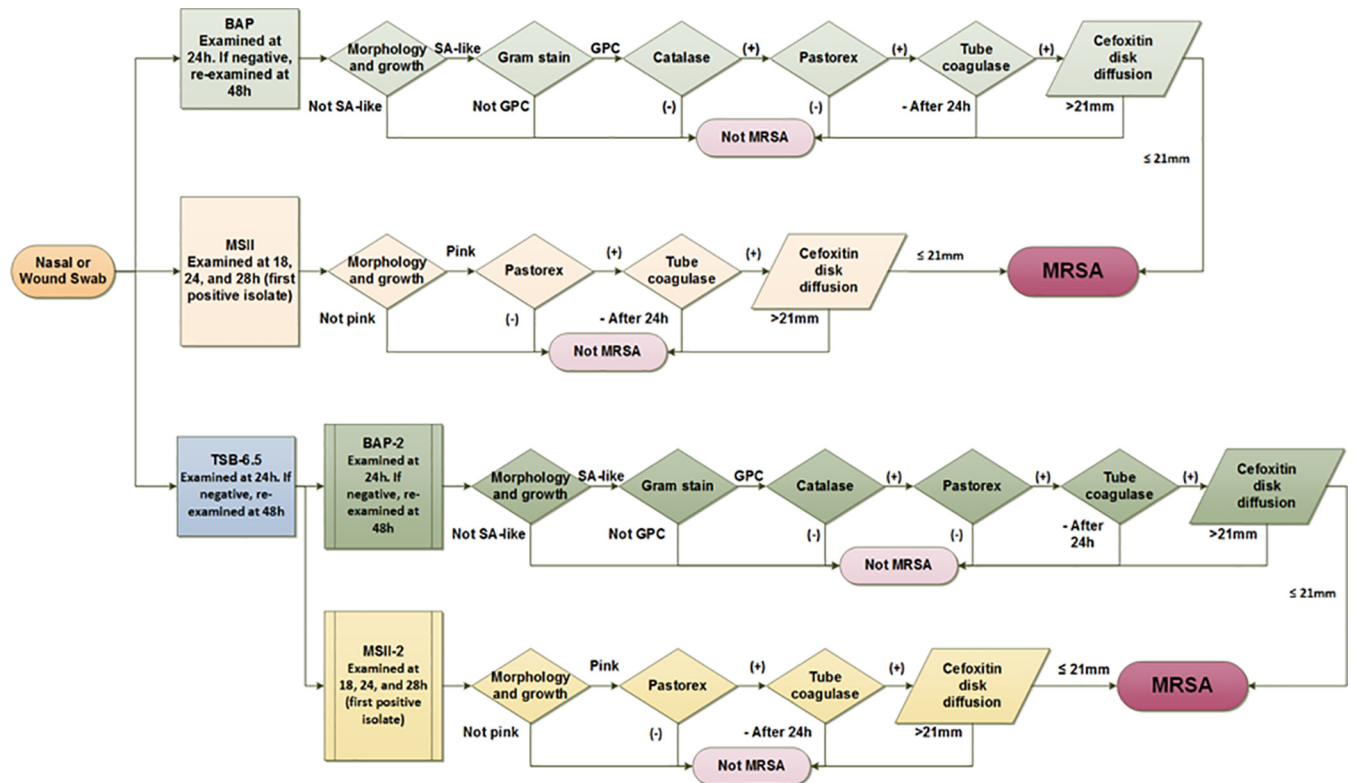


FIG 1 Procedural steps for MRSA identification from nasal or wound swabs. Start and stop of processes are denoted by ovals, procedures are denoted by rectangles, and subprocedures are denoted by lined rectangles. Decision points are symbolized by diamonds, and negative results leading to a stop in testing are marked by blush ovals. Rhomboids represent input/output data leading to a final result.

This study was designed to evaluate the ability of MSII to detect MRSA from nares and wound swabs. The results of bacterial outgrowth on MSII were compared to two reference standards: direct detection of MRSA on a 5% sheep's blood agar plate (BAP) and detection on a BAP following broth enrichment in tryptic soy broth supplemented with 6.5% NaCl (TSB-6.5). These results represent the first multicenter evaluation of the accuracy of MSII for MRSA identification from nares and wound samples processed at three laboratory sites in the midwestern and northeastern United States.

## MATERIALS AND METHODS

**Human subjects.** Procedures were performed in accordance with ethical standards as reviewed by the Institutional Review Boards at Geisinger Health System (GMC; project number 2013-0425), the University of Michigan Health System (project number HUM00079689), and the Medical College of Wisconsin (project number 20768). Samples were collected between September 2013 and June 2014. All patient specimens were deidentified and assigned study numbers prior to use. No exclusion criterion was incorporated into the study design; however, 17 samples were excluded due to contamination of the MSII medium during the course of the study.

**Specimen collection.** Clinical remnant specimens utilized in this study originated from the Geisinger Medical Center (Danville, PA), the University of Michigan Health System (Ann Arbor, MI), and the Medical College of Wisconsin (Milwaukee, WI). Nares swab specimens were collected using ES swabs (Copan Diagnostics, Murrieta, CA). Wound swabs were collected using BBL CultureSwab Liquid Stuart (Becton-Dickinson, Franklin Lakes, NJ) or Copan ES swabs. The experimental design of this study was based on the FDA requirements for clearance of MSII for com-

mercial distribution. The workflow of sample testing is summarized in Fig. 1 and is briefly described below.

**Routine culture on primary BAP.** Specimens included in this study were maintained at 4°C and were inoculated onto BAP or MSII medium less than 24 h after collection. Cultures were incubated for 18 to 28 h at 37°C in ambient humidity and were examined for the presence of bacterial growth. MSII plates that did not exhibit characteristic pink colonies were considered negative after 28 h and were discarded. BAPs with no presumptive *S. aureus* growth were subsequently incubated for an additional 24 h at 37°C and were then reevaluated. If no presumptive *S. aureus* was observed following this additional incubation, the plates were recorded as negative and were discarded.

**Routine culture with NaCl-supplemented broth enrichment.** Following direct inoculation onto BAP or MSII medium, specimen swabs were enriched in 6.5% NaCl-TSB (Remel, Lenexa, KS) at 37°C with ambient humidity for 24 h and were examined for turbidity indicative of bacterial outgrowth. Subsequently, 10  $\mu$ l of turbid broth was subcultured to BAP (TSB-6.5 BAP) and MSII and was incubated for 24 h at 37°C and examined for growth. Nonturbid specimens were reincubated for an additional 24 h and were then reexamined for turbidity. Specimens remaining nonturbid after the second incubation were reported as negative and were discarded.

**Phenotypic confirmation of *Staphylococcus aureus* from positive cultures.** Cultures containing isolates exhibiting typical *S. aureus* colony morphology and reactivity on BAP and TSB-6.5 BAP were visually quantified and tested to confirm the presumptive identification of *S. aureus* based on established morphological and biochemical criteria. Gram-positive cocci exhibiting clustering arrangements when viewed by light microscopy were presumptively identified as *Staphylococcus* spp. and were subjected to additional phenotypic testing for species-level confirmation

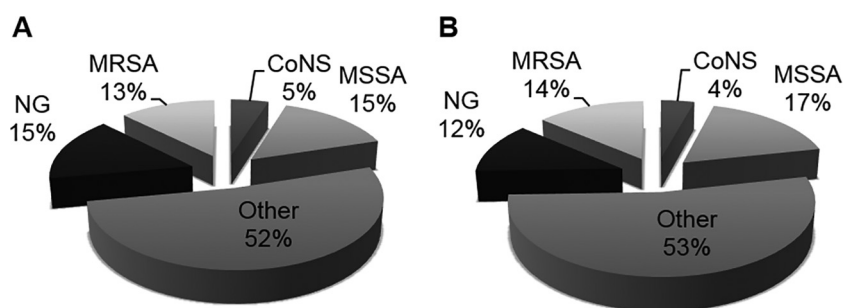


FIG 2 Classification of *Staphylococcus* species from wound and nasal swabs in the population studied. (A) Organisms present on primary culture without enrichment for *Staphylococcus* spp. (B) Prevalence of different staphylococci after TSB-6.5 enrichment. NG, no growth.

by catalase, latex agglutination (Pastorex Staph Plus; Bio-Rad, Hercules, CA), and tube coagulase following the manufacturer's instructions.

**Cefoxitin susceptibility testing.** Confirmed *S. aureus* isolates were tested for cefoxitin resistance according to CLSI guidelines for disk diffusion (17). A 0.5 McFarland standard suspension in phosphate-buffered saline was inoculated onto Mueller-Hinton medium (Remel, Lenexa, KS) with a 30- $\mu$ g cefoxitin disk (Becton-Dickinson, Franklin Lakes, NJ) and was incubated for 18 to 24 h at 37°C. Zones of inhibition were measured with resistant *S. aureus* isolates defined as those producing zone diameters of  $\leq 21$  mm.

**Discrepant testing.** Isolates that grew MRSA from BAP or TSB-6.5 BAP culture and that either failed to grow or failed to develop pink colonies on MSII plates were considered false negatives. Samples that grew pink colonies on MSII but did not have proven MRSA colonies on BAP or TSB-6.5 BAP were considered false positives. False positives and false-negative colonies were tested for the *mecA* gene product PBP2a using the PBP2a culture colony test per the manufacturer's instructions (Alere, San Diego, CA).

**Comparison of MRSAselect II versus MRSAselect.** Positive nasal swab samples ( $n = 32$ ), positive wound culture specimens ( $n = 35$ ), and negative samples ( $n = 51$  nasal, 58 wound) were randomly selected and were simultaneously inoculated onto paired MSI and MSII plates. Plates were incubated at 37°C with ambient humidity and were examined for growth at 18-, 24-, and 28-h time points. Pink colonies indicative of *S. aureus* and white colonies indicative of non-*S. aureus* bacteria were recorded on a scale of 1+ to 3+ based on quantity.

**Statistical analyses.** The reference standard overall was the TSB-6.5 enrichment method. However, for statistical analysis, data were substratified to represent the various options that can be used in clinical laboratories. McNemar's test for paired categorical data was used in 2 by 2 tables for performance comparisons. Wilcoxon's signed-rank test was used for nonparametric data. JMP statistical software version 12 and Excel 2010 were used for all statistical analyses and figure generation.

## RESULTS

**Prevalence of *Staphylococcus* strains in nasal and wound specimens among the study population.** During the course of the study, the prevalence of coagulase-negative *Staphylococcus* (CoNS), methicillin-susceptible *Staphylococcus aureus* (MSSA), and MRSA within the study population was determined. Primary culture on BAP resulted in the following distribution: 5% CoNS, 15% MSSA, 13% MRSA, 52% other organisms (including fungi), and 15% no growth (NG) (Fig. 2A). TSB-6.5 enrichment for staphylococcal species resulted in higher proportions of MRSA (14%) and MSSA (17%) isolation (Fig. 2B). CoNS were also detected in a higher proportion (4%) and other organisms were detected in 53% of the samples (Fig. 2B). Substratification of samples into categories by specimen type resulted in a similar distribution. *Staphylococcus* spp. were isolated in 28% of nares samples (4% CoNS, 11% MRSA, and 13% MSSA), whereas 63% of the samples contained nonstaphylococcal organisms. In comparison, 46% of wound samples contained *Staphylococcus* spp. (4% CoNS, 18% MRSA, and 24% MSSA), and 37% of the samples contained other microorganisms.

### Performance of MSII compared to the reference standard.

Comparing MRSA isolation from TSB-6.5-enriched cultures to direct MSII-plated swabs exhibited an overall concordance of 95.4% (confidence interval [CI] = 94.5% to 96.2%) across the three study sites ( $n = 2,226$ ) (Table 1). Positive and negative agreements were 90.5% (CI = 86.7% to 93.3%) and 96.2% (CI = 95.2% to 97.0%), respectively.

Comparing MRSA detection rates from direct blood agar plating to those of the reference standard demonstrated a high level of

TABLE 1 MRSAselect II performance results compared to those of BAP growth after TSB enrichment<sup>a</sup>

Site	Sample type(s)	No. TN	No. TP	No. FN	No. FP	% PA (95% CI)	% NA (95% CI)	% prevalence (95% CI)	% concordance (95% CI)
1	Nasal	294	42	11	16	79.2 (66.5–88.0)	94.8 (91.8–96.8)	14.6 (11.3–18.6)	92.6 (89.4–94.8)
	Wound	243	51	3	18	94.4 (84.9–98.1)	93.1 (89.4–95.6)	17.1 (13.4–21.7)	93.3 (90.0–95.6)
2	Nasal	187	45	6	13	88.2 (77.6–95.3)	93.5 (89.2–96.2)	20.3 (15.8–25.7)	92.4 (88.5–95.1)
	Wound	203	50	1	11	98.0 (89.7–99.7)	94.9 (91.0–97.1)	19.2 (15.0–24.4)	95.5 (92.3–97.4)
3	Nasal	709	42	7	12	85.7 (73.3–92.9)	98.3 (97.1–99.0)	6.4 (4.8–8.3)	97.5 (96.2–98.4)
	Wound	212	46	1	3	97.9 (88.9–99.6)	98.6 (96.0–99.5)	17.9 (13.8–23.0)	98.5 (96.1–99.4)
Total	Nasal and wound	1,848	276	29	73	90.5 (86.7–93.3)	96.2 (95.2–97.0)	13.7 (12.3–15.2)	95.4 (94.5–96.2)

<sup>a</sup> TN, true negative; TP, true positive; FN, false negative; FP, false positive; PA, positive agreement; NA, negative agreement.

agreement (96.6%; CI = 95.7% to 97.3%). Positive and negative agreements were also high at 91.0% and 97.4%, respectively. Although the performance of direct plating appears elevated, when MSII results were compared to direct blood agar plating without TSB-6.5 enrichment, the overall agreement was only 76% (CI = 74.1% to 77.7%). Positive and negative agreements were markedly reduced to 16.5% and 84.4%, respectively.

Isolates cultivated from discrepant samples between TSB-6.5-enriched and MSII plating were tested for PBP2a production ( $n = 102$ ). Of the 73 false-positive results, PBP2a testing confirmed the presence of MRSA in 43 specimens while 24 samples remained discrepant. The remaining six samples were not available for testing. Of the 29 false-negative results, MRSA was not detected in 11 samples while 18 samples remained discrepant. After the resolution of discrepant samples, the overall agreement between MSII and the reference standard increased to 97.8%. The positive and negative agreements of MSII also improved to 94.7% and 98.4%.

**Performance of MRSASelect II agar on MRSA detection from nasal swabs.** MRSA prevalence from nasal swabs across all study sites was 11.1% (CI = 9.5% to 12.8%;  $n = 1,384$ ) (Table 1). MSII performed with a positive agreement of 84.3% (CI = 77.7% to 89.2%) and a negative agreement of 96.7% (CI = 95.5% to 97.5%). Agreement with TSB-6.5 enrichment was 95.3% (CI = 94.1% to 96.3%). Coupling the use of MSII after TSB-6.5 enrichment yielded a similar prevalence of positive nasal swabs and an agreement of 94.0% (CI = 92.6% to 95.1%). Positive and negative agreements increased to 96.1% and 93.7%, respectively. Comparing direct plating on MSII to direct plating on BAP for MRSA isolation resulted in a positive agreement of only 10.4% (CI = 6.6% to 16.0%) and a negative agreement of 87.4% (CI = 85.5% to 89.2%). The agreement between these two methods was only 78.3%.

**Performance of MSII agar on MRSA detection from wound specimens.** Based on the enriched culture reference method, wound specimen MRSA prevalence was determined to be 18.1% (CI = 15.6% to 20.8%;  $n = 842$ ). MSII performed with an agreement of 95.6% (CI = 94.0% to 96.8%) compared to that of the reference standard. Positive and negative agreements of MSII were 96.7% and 95.4%, respectively. Plating on MSII after TSB-6.5 enrichment had a negative effect on MSII performance; the percent agreement was reduced to 88.0% with no change in prevalence. Positive agreement increased to 98.0% (CI = 94.4% to 99.3%), whereas negative agreement decreased to 85.8% (CI = 83.0% to 88.2%). The agreement between the two methods was 72.1% (CI = 69.0% to 75.0%). Positive and negative agreements decreased to 25.4% and 79.4%, respectively.

**Evaluation of antibiotic susceptibility testing directly from MSII versus that of subculture on BAP.** Confirmatory testing following growth on chromogenic agars is not routinely performed. However, to determine the performance of determinative testing from MSII, a cefoxitin disk diffusion test was performed on all isolates that produced pink colonies on MSII. These results data were compared to the cefoxitin test data from isolates obtained after TSB-6.5 enrichment and BAP subculture. It was determined that the cefoxitin testing directly from MSII agreed 96.6% (CI = 95.8% to 97.3%) with that of the BAP subculture test. Compared to testing after enrichment and isolation, direct cefoxitin testing exhibited positive and negative agreement of 92.2% and 97.3%, respectively.

**Performance comparison between MSII and MSI for MRSA detection.** A subset of frozen samples from one study site (GMC) was randomly selected using Research Randomizer from pools of MSII-positive nasal swabs, MSII-positive wound swabs, and overall MSII-negative samples (18). A total of 176 samples consisting of 83 nasal swabs (32 positives and 51 negatives) and 93 wound swabs (35 positives and 58 negatives) were inoculated on MSI and MSII. MSI and MSII plates were inoculated at the same time and were reviewed for growth at 18-, 24-, and 28-h time points. Statistical comparisons (using MSI as the reference) showed a 96.0% (CI = 92.0% to 98.1%) agreement between MSI and MSII at 24 h. Providing 28 h of incubation improved the agreement to 97.7% (CI = 94.3% to 99.1%). Comparison of MSI and MSII results to the initial MSII result recorded previously yielded 93.8% (CI = 89.2% to 96.5%) and 92.6% (CI = 87.8% to 95.6%) agreement, respectively. These results may be partially explained by changes introduced during freeze-thaw cycling and the long-term storage of the samples at  $-80^{\circ}\text{C}$  (7 to 10 months), potentially resulting in reduced quantities of viable bacteria (19).

Comparison of the intensity of color development between chromogenic agars is difficult to quantify objectively. In this study, two identically trained technicians recorded growth and color development observations to determine relative differences in perceived color when the same sample was cultured on MSI and MSII. Colors were reported as white (no value, indicates non-MRSA organism), light/pale pink (1 value), pink (2 value), and bright pink (3 value). Growth was semiquantitated as 1+, 2+, and 3+.

Comparisons of the differences in growth did not demonstrate statistically significant differences between each medium with a  $P$  of 0.25 at 18 h, a  $P$  of 0.40 at 24 h, and a  $P$  of 0.499 at 28 h (Wilcoxon signed-rank test) between MSI and MSII. Conversely, comparing color development between MSI and MSII demonstrated that plating on MSII resulted in the development of darker shades of pink color development with a  $P$  of  $<0.001$  at 18 h, a  $P$  of  $<0.001$  at 24 h, and a  $P$  of  $<0.05$  at 28 h. Comparison of white colony growth, which is indicative of non-MRSA bacteria, also exhibited an improvement in MSII versus MSI, with significantly less white colonies observed on MSII ( $P < 0.05$ ) at every time point measured (Fig. 3), which is indicative of enhanced medium selectivity.

## DISCUSSION

Surveillance of MRSA colonization among inpatient populations and health care workers has dramatically reduced the number of MRSA-associated HAIs, impacting the associated financial burdens to the patient and the health care system. Between 2005 and 2011, MRSA HAIs decreased by  $>50\%$  while CA-MRSA infections decreased by 5% within the same time period (20). Routine surveillance of MRSA-colonized patients remains an important aspect for case management and infection control due to the prolonged duration of colonization. Studies using standard, non-chromogenic medium for MRSA screening demonstrated that almost 50% of colonized patients remain MRSA-positive after 1 year and that over 20% are MRSA-positive for an additional 4 years post hospitalization (21). It is estimated that 30% of the population carries *S. aureus* asymptomatically with 6% of those isolates being MRSA (22). Recent literature reports MRSA prevalence ranges between 9% to 25% depending on geographic location (9, 23, 24).

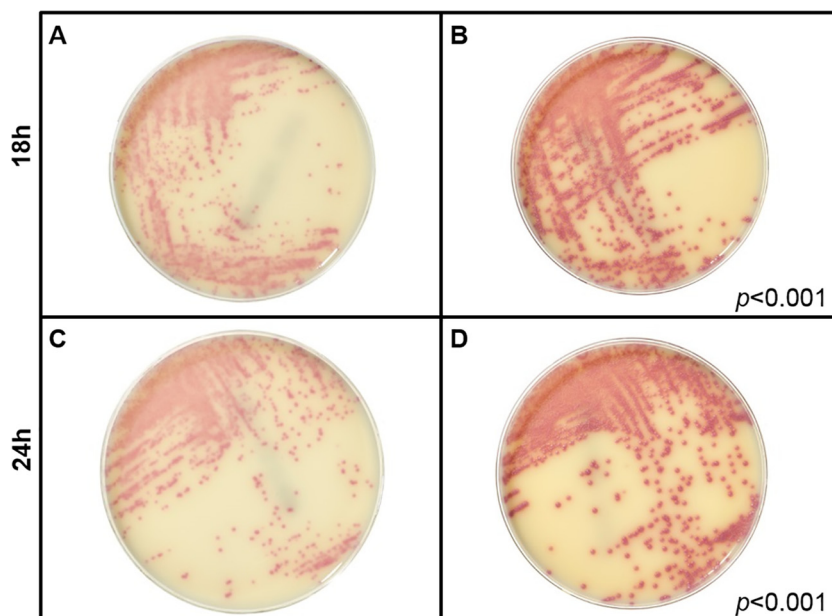


FIG 3 Representative growth of MRSA strain ATCC 43300 on (A and C) MRSASelect and (B and D) MRSASelect II. Photos courtesy of Bio-Rad.

In this study, we report a nasal *S. aureus* prevalence of 28% among three study sites located in the midwestern and northeastern United States. MSSA and MRSA prevalence was identified in 13% and 11% of the total population, respectively. Wound colonization by staphylococci accounted for 55.4% of isolates identified in the culture-positive population studied, with 43.3% of those isolates confirmed as MSSA and 32.4% confirmed as MRSA. Our findings correlate with the latest SENTRY antimicrobial surveillance program report on skin and soft tissue infections (SSTIs), which found that 44.6% of SSTIs in North America were caused by *S. aureus*, with MRSA being the causative agent of 35.9% of those infections (25).

The reported analytical sensitivity for chromogenic agars ranges from 67% to 91.9% for CHROMagar (11, 26) to 100% for chromID MRSA and MSI (11, 12, 27). The fact that each study employed different testing algorithms for sample processing, including the utilization of different reference standards, may contribute to these discrepancies. Relatively few studies have compared chromogenic agars to direct plating on blood agar plates (typically BAP or tryptic soy agar), while others use enrichment in salt-containing broths as the reference standard (13, 26). The CLSI guidelines for *S. aureus* isolation recommend the addition of NaCl to a culture medium like TSB-6.5 in order to enrich the growth of salt-tolerant staphylococci; however, there is no recommendation for a defined concentration of additive salt (17). The literature suggests that NaCl concentrations in enrichment broths range between 0.05% and 10%, with 6.5% being the most common concentration used (11, 14, 28, 29). MSII has a 2.0% NaCl concentration, which allows most salt-sensitive strains to grow while still limiting growth on non-MRSA strains due to its other selective properties. When salt-enriched broths are used as reference standards, the concentrated salt can inhibit the outgrowth of some salt-intolerant strains of MRSA, which hampers comparisons (26).

In this study, we compared the performances of MSII for

MRSA identification with and without TSB-6.5% NaCl enrichment. The overall agreements between MSII and the two reference methods with and without enrichment were 95.4% and 95.0%, respectively. These values were not statistically different when compared by McNemar's test ( $CI = 95\%$ ). Even though the agreement between the two reference methods was high, the agreement using broth enrichment as the reference standard was lower. Identification methods for MRSA based on BAP or TSB-6.5 culture miss about 5% to 10% of positive samples due to low levels of colonization or even due to colonies having morphological characteristics indistinguishable from those of MSSA strains. This known issue, as well as the salt concentration in the TSB-6.5, may have contributed to the lower agreement between MSII and the reference standard compared to direct BAP plating (9). These results highlight the importance of selecting the appropriate culture and enrichment method as a comparative reference.

The 84% agreement between MSII plating and TSB-6.5 enrichment for nasal samples is superior to that reported by Wolk et al. for MSI at the same incubation time (14). In wound specimens, the agreements of MSI and MSII to TSB-6.5 enrichment are similar at 96.0% and 95.6%, respectively (15). Subculture to MSII after TSB-6.5 enrichment had either no effect (nasal swabs) or a negative impact on performance values (wound swabs). It is to be noted that we used the PBP2a assay to test discrepant isolates. This assay can only detect MRSA strains that owe their methicillin-resistance to the *mecA* gene. With the increased isolation of MRSA strains that possess *mecC* and not *mecA*, we can only speculate that we may have missed some true MRSA strains and classified them as false positives (30).

Using frozen samples, the comparison of MSII agar to MSI demonstrated a 96% concordance between the two media at 24 h of incubation and a 97.7% concordance at 28 h of incubation. No significant difference was noted when comparing the growth of positive samples for each agar. Importantly, highly significant differences were found with respect to the color development denot-

ing positive colonies. MSII was reformulated to enhance the contrast between true positive colonies (dark pink) and false-positive or true negative colonies (white to light pink). MSII showed an increased selection against non-MRSA bacteria compared to MSI ( $P < 0.05$ ) at every time point examined, which facilitated evaluation of chromogenic plates. Color intensity was also examined at every incubation time point (18, 24, and 28 h) with MSII exhibiting significantly darker coloration ( $P < 0.001$ ,  $P < 0.001$ , and  $P < 0.05$  for each time point, respectively). To our knowledge, this is the first report of direct susceptibility testing from a chromogenic agar. The high concordance of MRSA-positive colonies tested directly from MSII (96.7%) compared to those isolated after TSB-6.5 enrichment confirms the effectiveness of using direct swab plating on MSII for surveillance purposes. The enhanced chromogenic capacities of MSII can result in faster examination times, translating to a reduced analysis time for the technologist and to a potential decrease in the net cost of MRSA screening. This multi-site study highlights the chromogenic and selective improvements of MRSASelect II as a rapid and robust method for the identification of MRSA from nasal and wound specimens.

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