Recent Advances in MRSA Surveillance Detection

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According to a 2007 study by the Association for Professionals in Infection Control and Epidemiology (APIC), 1.2 million US hospital patients may be infected with methicillin-resistant Staphylococcus aureus (MRSA) each year; and up to 119,000 patients may die from the infection.1

MRSA is a common cause of health-care-associated infections, and its prevalence continues to increase worldwide.2,3 MRSA infections are a major cause of morbidity and mortality, and are associated with excess hospital costs.4-6 The recent emergence of community-acquired MRSA, in patients without established risk factors, has posed additional concern for the health care community.2,7-9 Rapidly identifying patients who are carriers and promptly placing them on isolation precautions can prevent further transmission of this organism.10-12 Currently, specimens for active surveillance cultures (ASCs) most commonly are obtained from the anterior nares.13-15 Other body sites, such as the throat, groin, and gastrointestinal tract, as well as wounds can serve as unsuspected reservoirs for this organism.16-20

As national attention to MRSA increases, many professional organizations, such as the Society for Healthcare Epidemiology of America, the Centers for Disease Control and Prevention, APIC, and the Institute for Healthcare Improvement 5 Million Lives Campaign, have recommended that ASCs be performed as part of a protocol to reduce the spread of MRSA and other multidrug-resistant organisms.4,10,21,22 Some states and federal facilities such as Veterans Affairs hospitals have mandated MRSA ASCs for all hospital admissions.5,23 Studies have shown that, in a significant number of patients, ASCs detect MRSA colonization that is not detected by routine clinical cultures.24,25 Expanding the number of ASCs performed increases the demand for isolation beds. However, ASCs also make it possible to discontinue isolation precautions on previously colonized patients if it is determined that they are no longer carriers of MRSA. In one study, performing ASCs on multiple body sites led to the discontinuation of isolation precautions for 21% of the patients tested.26 For hospitals that preemptively isolate patients with a history of MRSA, implementation of polymerase chain reaction (PCR) methods for readmission screening for MRSA can reduce isolation days by 54% and related costs by 45%.27,28 As the demand for ASCs and pressure to prevent the spread of MRSA increases, more rapid tests with shorter turnaround times can speed the institution of isolation precautions and have been shown to significantly reduce subsequent MRSA transmission.24,29,30 This review describes the advances that have been made recently in the methodologies used to detect MRSA in the United States.
Chromogenic agars are relatively inexpensive, require in their health care setting (Table 1).13,29,30,32,33 Offset by a reduction in subsequent MRSA transmission.14,29 Laboratories must decide if the costs associated with the more rapid detection of MRSA can be offset by a reduction in subsequent MRSA transmission in their health care setting (Table 1).13,29,30,32,33

### Traditional Methods

Traditionally, the recovery and identification of MRSA have been culture-based. Specimen swabs are inoculated onto selective or nonselective media, with or without broth enrichment. Subsequent identification and susceptibility testing of staphylococcal colonies must be performed. With traditional culture-based methods, it often takes 2 to 5 days to detect MRSA.15,31

With the advent of new methodologies, laboratories have a variety of choices for MRSA surveillance testing that greatly decreases the time to detection. Two such methodologies include culture-based chromogenic agar and molecular PCR. Chromogenic agars can detect MRSA in as few as 16 hours,5 but incubation for 24 to 48 hours is required before the results can be reported as negative. PCR methods can detect MRSA within 1 to 4 hours.5,32 Traditional methods are more time-consuming and have longer turnaround times but are relatively inexpensive compared with the newer methodologies.14,29 Laboratories must decide if the costs associated with the more rapid detection of MRSA can be offset by a reduction in subsequent MRSA transmission in their health care setting (Table 1).13,29,30,32,33

### Chromogenic Agar Media

Chromogenic agars are selective and differential media designed to isolate and identify MRSA directly from anterior nares specimens. The media incorporate various selective agents to inhibit the growth of other bacteria and yeast and to select for the growth of MRSA. Coloration of the colonies as a consequence of use of the chromogenic substrate by specific bacterial enzymes identifies the organism without further susceptibility testing.14,34-37 As a result, the workload in the laboratory is greatly reduced, and turnaround times to reporting are much shorter than with traditional methods.5,38 Chromogenic agars are relatively inexpensive, require less technical expertise than molecular testing, and may be advantageous in the case of mixed infections with methicillin-sensitive *S. aureus* (MSSA) and MRSA, which can be missed with the use of nonselective agar.14,32 Three commercially available formulations are described in the next sections, and their performance characteristics are summarized in Table 2.

**BBL™ CHROMagar™ MRSA**

**Principle**

BBL™ CHROMagar™ MRSA (BD Diagnostics) is a chromogenic medium supplemented with inhibitory agents and cefoxitin to select for the growth of MRSA. Hydrolysis of the chromogenic mixture by MRSA produces mauve-colored colonies. (It should be noted that this is not the same formulation as CHROMagar, Paris, France.)

**Procedure**

The plates are incubated aerobically between 35°C and 37°C, while exposure to light is avoided. The plates are approved to be examined between 20 and 28 hours, and if mauve-colored colonies are observed, no confirmatory test is needed for the identification of MRSA. All plates without mauve-colored colonies require an additional 24 hours of incubation. When mauve-colored colonies are detected at 48 hours, a coagulase confirmation test is recommended.14,34 Approximately 85% of MRSA will be detected within 24 hours of incubation.14,34

Of note, BBL™ CHROMagar™ MRSA II (BD Diagnostics) is a new chromogenic medium that is intended to detect MRSA from multiple body sites. This formulation recently has been evaluated and has been submitted to the FDA for review. BBL™ CHROMagar™ MRSA II currently is not for sale in the United States.39,40

**MRSASelect™**

**Principle**

MRSASelect™ (Bio-Rad Laboratories) is a chromogenic medium that is supplemented with an antibiotic-antifungal mixture and an optimized salt concentration to select for the growth of MRSA. An enzymatic cleavage of the chromogenic substrate by MRSA will produce small, pink-colored colonies.

**Procedure**

The plates are incubated aerobically between 35°C and 37°C, while exposure to light is avoided. The plates are approved to be examined between 18 and 28 hours, and if small, pink-colored colonies are observed, no

### Table 1. Comparison of Chromogenic Agars and PCR Methods

<table>
<thead>
<tr>
<th>Product</th>
<th>Method</th>
<th>Cost</th>
<th>Technical Time, min</th>
<th>Turnaround Time, h</th>
<th>Complexity of Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBL™ CHROMagar™ MRSA</td>
<td>Culture-based</td>
<td>$6.90</td>
<td>1-2</td>
<td>20-48</td>
<td>High</td>
</tr>
<tr>
<td>MRSASelect™</td>
<td>Culture-based</td>
<td>$8.40</td>
<td>1-2</td>
<td>18-28</td>
<td>High</td>
</tr>
<tr>
<td>Spectra™ MRSA</td>
<td>Culture-based</td>
<td>$7.89</td>
<td>1-2</td>
<td>24</td>
<td>High</td>
</tr>
<tr>
<td>chromID™ MRSA</td>
<td>Culture-based</td>
<td>$3.00</td>
<td>1-2</td>
<td>24</td>
<td>High</td>
</tr>
<tr>
<td>BD GeneOhm™ MRSA</td>
<td>Real-time PCR</td>
<td>$29.50</td>
<td>5-9</td>
<td>1-1.25</td>
<td>High</td>
</tr>
<tr>
<td>GeneXpert™ MRSA</td>
<td>Real-time PCR</td>
<td>$42.00</td>
<td>1</td>
<td>1.25</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction

* Adapted from references 13, 29, 30, 32, and 33.
confirmatory test is needed to identify MRSA. Plates should not be incubated for more than 28 hours.  

**Spectra™ MRSA**

**Principle**

Spectra™ MRSA (Remel Inc) is a chromogenic medium that is supplemented with a combination of antibacterial compounds to select for the growth of MRSA. A phosphatase enzyme present in all MRSA acts on the chromogenic mix to produce denim blue-colored colonies. The medium also includes compounds that encourage the production of the phosphatase enzyme to improve the sensitivity and specificity of this product.

**Procedure**

Plates are incubated aerobically between 35°C and 37°C, and it is not necessary to avoid exposure to light. The plates are approved to be examined at 24 hours, and if denim blue-colored colonies are observed, no confirmatory test is needed for the identification of MRSA. Plates should not be incubated for more than 24 hours.

**chromID™ MRSA**

**Principle**

chromID MRSA (bioMérieux) is a chromogenic medium comprised of a rich nutritive base of different peptones and a combination of antibiotics including cefoxitin to select for the growth of MRSA. Activity of the α-glucosidase by MRSA produces green-colored colonies.

**Procedure**

Plates are incubated aerobically between 35°C and 37°C, while minimizing exposure to light. The plates are approved to be examined after 24 hours and if any green-colored colonies are observed, no confirmatory test is needed for the identification of MRSA. Some strains of *S. aureus* that have the meca gene but have a low cefoxitin minimum inhibitory concentration (MIC) (<4 mcg/mL) may not develop on mediums that contain antibiotics other than cefoxitin.

Rare strains of *S. aureus* that do not have the meca gene may develop characteristic colonies after 24 hours of incubation on some of these mediums. Choosing among these media may depend on the workflow and staffing patterns in the laboratory. Laboratories that inoculate media during evening or night shifts would need to schedule a technologist to read the plates during those shifts if a full 24 hours of incubation is required.

**Polymerase Chain Reaction**

PCR is an automated technique used to detect a target sequence of DNA that is unique to an organism. To detect MRSA, molecular testing focuses on the meca gene, which confers methicillin resistance and is located in the *Staphylococcus* cassette chromosome mec (SCCmec). Assays simultaneously target sequences within the SCCmec and a sequence specific to *S. aureus* within the orfX gene.

Recent developments of commercially available real-time PCR molecular assays have offered new options for MRSA surveillance testing and have decreased the time to detection from that achieved with chromogenic agar methods. Some studies have shown that PCR is more sensitive than selective media, but at least one study reported PCR to be less sensitive than a selective medium. Two commercially available assays are described here, and their performance characteristics are summarized in Table 2.

**BD GeneOhm™ MRSA**

**Principle**

BD GeneOhm™ MRSA (BD Diagnostics), formerly IDI-MRSA™, is a real-time multiplex PCR assay that can detect the presence of MRSA directly from anterior nares specimens. In this assay, 6 primers amplify target sequences near the insertion site of SCCmec. Molecular beacons are used to detect the amplified targets of the SCCmec and the orfX gene. An internal control not found in MRSA also is amplified unless PCR inhibition occurs. This reaction takes place in the SmartCycler (Cepheid Diagnostics, Inc), which measures the fluorescence of each beacon simultaneously.

**Procedure**

Specimen swabs are broken off into a broth buffer tube, vortexed, and then lysed by high-speed centrifugation. The sample is washed and concentrated, heated at 95°C for 2 minutes, then placed in a cooling block. The lysate, master mix, and control DNA are added to

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**Table 2. Performance Characteristics of MRSA Detection Methods**

<table>
<thead>
<tr>
<th>Product</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBL™ CHROMagar™ MRSA</td>
<td>93.5-95.2</td>
<td>96.7-99.7</td>
<td>84.4</td>
<td>99.9</td>
</tr>
<tr>
<td>MRSASelect™</td>
<td>94-96</td>
<td>98-99</td>
<td>97</td>
<td>73</td>
</tr>
<tr>
<td>Spectra™ MRSA</td>
<td>95.2-95.4</td>
<td>99.1-99.7</td>
<td>93.4-99.8</td>
<td>98.2-99.8</td>
</tr>
<tr>
<td>chromID™ MRSA</td>
<td>94.2</td>
<td>97.2</td>
<td>91.1</td>
<td>98.2</td>
</tr>
<tr>
<td>BD GeneOhm™ MRSA</td>
<td>88-96.1</td>
<td>93.5-99</td>
<td>61.1-94</td>
<td>97-99</td>
</tr>
<tr>
<td>GeneXpert® MRSA</td>
<td>69.2-96.5</td>
<td>90.4-98</td>
<td>78.3-90.4</td>
<td>96.3-99.6</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *Staphylococcus aureus*; NPV, negative predictive value; PPV, positive predictive value

Adapted from references 5, 14, 27, 32-34, and 45.
the specimen reaction tubes. After a quick centrifugation, the tubes are placed into the instrument. The results are displayed on a computer screen as “POS,” “NEG,” “Unresolved,” or “ND” (not determined). For specimens with a result of “Unresolved,” the steps must be repeated after a freeze–thaw procedure described by the manufacturer. For specimens with a result of “ND,” the steps must be repeated with a new swab. The instrument can accommodate 14 specimens with 2 controls, and the test usually is performed in batches. Although the assay is complete in approximately 60 to 75 minutes, the actual turnaround time for a specimen in a laboratory that performs this assay only once per day averages between 13 and 19 hours. However, when assays are run several times per day, the results could be available in just a few hours.

The assay can detect MRSA strains representing SCCmec types and subtypes I through V. False-positives have occurred with MSSA and most likely result from the absence of the mecA gene in strains that have retained a residual SCCmec right-extremity fragment that is amplified by this method. The BD GeneOhm™ MRSA assay is FDA-approved only for nasal samples, but studied pooled samples have been validated for non-nasal body sites.

**GeneXpert® MRSA**

**Principle**

GeneXpert® MRSA (Cepheid Diagnostics, Inc.) is a fully automatic and integrated real-time PCR assay for the detection of MRSA from anterior nares specimens. This platform combines sample purification, amplification, and detection in a single sample cartridge and allows random access to the instrument. This assay target sequence incorporates the insertion site of the SCCmec and a sequence in the **orfX** gene.

**Procedure**

Specimen swabs are broken off into the elution reagent tube and vortexed. The eluate is transferred to a notched opening in the cartridge. The lid is closed, and the cartridge is placed into the instrument. The results are displayed in the view window in no more than 66 minutes and are reported as “MRSA positive,” “MRSA negative,” “INVALID,” “ERROR,” or “NO RESULT.” A specimen with an interpretation of “INVALID,” “ERROR,” or “NO RESULT” needs to be repeated with a new swab.

This assay can detect strains of MRSA representing SCCmec types and subtypes I through V and IVa. It is likely that false-positives may occur, as with other methods detecting targets within the SCCmec. As with all PCR technologies, a positive test result does not necessarily indicate that organism is viable and that false-negatives may occur if mutations or polymorphisms in primers or probe-binding regions and would fail to detect a new or unknown variant of MRSA as described by Laurent et al.

It has been found that gene-based amplification-based methods can best detect highly unstable strains of MRSA that rapidly lose SCCmec upon subculturing. The recent FDA 510(k) summary stated that the performance of the GeneXpert® MRSA assay was comparable with that of the BD GeneOhm™ MRSA assay.

**Legislation**

Most states are currently involved in some level of legislation regarding MRSA control. These range from study initiatives to reduce the spread of MRSA, voluntary or mandatory reporting of hospital-acquired infections, or screening high-risk patients upon admission to a health care facility. To date, 15 states have enacted laws with regard to MRSA: Washington, Nevada, California, Texas, Minnesota, Illinois, Tennessee, South Carolina, Virginia, Pennsylvania, Connecticut, Maine, Nebraska, Missouri, and New Jersey. Of these states, 10 require surveillance screening for MRSA colonization upon hospital admission. The states of New York and Massachusetts have pending legislation and both would require surveillance screening, if passed.

Legislation also has been introduced in Congress regarding screening for MRSA. S. 1305 would initially require hospitals to screen high-risk patients, but would be expanded to include all inpatient admissions by 2014.

**Conclusion**

The prevalence of MRSA is increasing in both the health care setting and the community. Because MRSA is a major cause of morbidity, mortality, and increased hospital costs, the effort to reduce the transmission of this organism has become a national issue. An essential component in the strategy of hospital infection control programs is to perform ASCs to rapidly identify and isolate patients who are found to be carriers of MRSA. The recent advances in the methodologies for performing ASCs offer several options to health care institutions. Significant reductions in the rate of transmission have been reported with the institution of same-day testing. Although molecular methods are expensive relative to culture-based methods, they may still prove to be cost-effective in preventing transmission and infection with MRSA.

**References**

10. Muto CA, Jerinigan JA, Ostowsky BE, et al. SHEA guidelines for preventing nosocomial transmission of multidrug-resistant strains of


