Molecular Laboratory Tests for the Diagnosis of Respiratory Tract Infection Due to *Staphylococcus aureus*

Lance R. Peterson

Department of Pathology and Laboratory Medicine, NorthShore University HealthSystem (NorthShore) and University of Chicago, Evanston, Illinois

When *Staphylococcus aureus* is the cause of ventilator-associated pneumonia or a bacterial infection following influenza, the infections are devastating if not treated promptly. Disease due to methicillin-resistant *S. aureus* (MRSA) continues to be of concern throughout most of the United States. Currently, the U.S. Food and Drug Administration (FDA) has cleared polymerase chain reaction tests for detection of MRSA in nasal swab specimens; however, there are no FDA-cleared tests for identifying *S. aureus* in purulent respiratory secretions. The real-time polymerase chain reaction tests for *S. aureus* (primarily MRSA) in nares provide results in \<2 h and have sensitivities ranging from 95% to 100%, with specificities of 96%–99%; these results are comparable to that of standard cultures, which can take up to 3–4 days for final results. The FDA is encouraged to work closely with industry providers to expedite the evaluation and clearance process for molecular diagnostic devices detecting *S. aureus* (including MRSA) in the diagnosis of respiratory tract infection.

The global plague of antibiotic-resistant infections is recognized as a serious threat to health care worldwide [1]. This threat is accompanied by a steady decrease in the research and development of new antimicrobial agents. One of the key pathogens is methicillin-resistant *Staphylococcus aureus* (MRSA) [1], which can cause a destructive necrotizing pneumonia with high mortality [2]. We need accurate and fast laboratory detection of infection. Unfortunately, until very recently, there was virtually no federal funding for research into the diagnosis and treatment of bacterial infectious diseases [3].

The purpose of this review is to (1) discuss the role of *S. aureus* (including MRSA) in pneumonia; (2) review the promise and reality of molecular diagnostic testing for respiratory tract infection (RTI); (3) summarize the currently available molecular technology based on the published literature; and (4) describe broad interventions that can reduce the likelihood of MRSA as a potential pathogen in RTI.

**STAPHYLOCOCCUS AUREUS AS A PATHOGEN IN PNEUMONIA**

Many cases of hospital-acquired pneumonia are due to *Pseudomonas aeruginosa, Acinetobacter* species, and *S. aureus* [2]; *S. aureus* is the number one cause of ventilator-associated pneumonia, accounting for 24.4% of cases [4]. In U.S. hospitals, ~50% of *S. aureus* infections involve MRSA. The estimated number of MRSA-related hospitalizations more than doubled between 1999 and 2005 [5]. Of note, there has been some apparent success in the reduction of invasive infection across the United States since 2005, but most of the improvement was related to bloodstream infection [6], which may be tied to improvement in the care of central venous catheters. The lack of a reduction in the rate of MRSA disease associated with documented improvement in hand hygiene performance suggests any reduction in national MRSA invasive infection is more
complex than a general improvement in basic infection control practice [7]. Additionally, Delorme et al [8] recently reported a 183% increase in MRSA infections among long-term care facility residents during the period 2006–2007, with an overall increase in disease among inpatients and outpatients of 77%—a disease rate >100-fold higher than the U.S. national rate of tuberculosis [9]. In fact, by 2005, MRSA was causing greater mortality in the United States than were tuberculosis, *Salmonella* infection, influenza, and human immunodeficiency virus–AIDS combined [10]. Clearly, now is the time for research into better understanding the effective detection and control strategies to deal with MRSA, perhaps particularly for RTI.

Importantly, MRSA-associated ventilator-associated pneumonia likely has a higher mortality rate than does that due to methicillin-susceptible *S. aureus* (MSSA). The difference is largely due to inadequate initial therapy [11]. In developed countries, the incidence of community-acquired pneumonia (CAP) in adults is estimated to range from 5 to 11 cases per 1000 persons per year, with ~20% of patients requiring hospital admission [12]. *S. aureus* is not a common etiology of CAP, with a reported incidence of 1%–5% of CAP cases [12]. The incidence is likely substantively higher during outbreaks of influenza.

Community-associated MRSA pneumonia may be an emerging infection, with 15 and 10 cases reported to (CDC) in the 2003–2004 and 2006–2007 influenza seasons, respectively [12]. The recent 2009 pandemic influenza A (H1N1) season highlighted the potential problem of *S. aureus* as a cause of pneumonia [13]. As seen in Figure 1, influenza can be a significant illness during a substantial part of the year, and the 2009–2010 season was marked by pandemic novel influenza virus [14]. During the period from May through August 2009, there were 77 postmortem lung specimens examined from the United States. Bacteria were detected using a broad-range polymerase chain reaction (PCR) assay targeting 16S ribosomal DNA gene in extracted tissue samples. Concurrent bacterial infection was found in 29% of the 77 cases. Of the bacterial infections detected, 10 were caused by *Streptococcus pneumoniae*, 7 by *S. aureus* (one should assume at least some were MRSA), 2 by *Streptococcus mitis*, and 1 by *Haemophilus influenza*; 4 cases involved multiple pathogens [13]. Thus, although *S. aureus* (including MRSA) is not highly prevalent as a cause of outpatient pneumonia, it can become an important cause of morbidity and mortality in both the inpatient and community settings.

**POTENTIAL FOR THE RAPID DIAGNOSIS OF PNEUMONIA**

One of the historic and potentially reliable tests for rapid diagnosis is the Gram stain. Sputum Gram stains were prospectively evaluated in 1390 patients with CAP who were hospitalized during the period of January 2002 through June 2008. Of the 1390 patients, 12.8% fulfilled the criteria for study inclusion (ie, a high-quality sputum sample was available and the same pathogen was recovered from both blood and sputum cultures). The Gram stain results suggested the presence of
pneumococci in 82% of patients, staphylococci in 76%, and gram-negative bacteria in 79%. Specificity was very good, at 93% for pneumococci and 96% for both staphylococci and gram-negative bacteria [15]. The main limitation with this Gram stain results is the absence of antimicrobial susceptibility test information that can guide therapy. Further development of molecular diagnostics will eventually make Gram stains obsolete, but for now they can be helpful.

From the molecular viewpoint, a promising development is the recognition of a soluble triggering receptor (sTREM-1) on myeloid cells that can be measured in respiratory secretions [16]. sTREM-1 is expressed by neutrophils, macrophages, and mature monocytes. An initial report suggested that sTREM-1 levels can separate bacterial pneumonia from noninfectious causes of pulmonary inflammation. Measurement of sTREM-1 in 148 patients detected the presence of bacterial or fungal infection with a sensitivity of 98% and a specificity of 90% [16]. It is disappointing that a subsequent investigation did not confirm the original observations [17]. The second study prospectively evaluated 105 adult patients undergoing mechanical ventilation who underwent bronchoalveolar lavage (BAL). The diagnostic sensitivity of sTREM-1 was 42.1% with a specificity of 75.6% [17]. The study included 19 patients that were classified as having definitive ventilator-associated pneumonia, which was defined as a positive quantitative BAL culture result. There were also 21 patients (20.0%) who clearly did not have infection [17]. The detection of host response protein(s) holds much promise for the future specific diagnosis of infectious diseases, but the field is still in its infancy, and additional research is needed to understand the precise human biological response to each unique infectious pathogen.

REAL-TIME PCR FOR THE DIAGNOSIS OF PNEUMONIA

Real-time PCR (rtPCR) has revolutionized the detection of pathogens by combining the PCR chemistry with probe detection of amplified products into a single technology that provides amplification and target detection in <2 h [18–20]. Amazingly, the 10-year anniversary of this technology was in 2008. Early application of rtPCR focused on detection of MRSA and, later, MSSA from nasal surveillance swabs. At least 3 instruments and their associated assays have been cleared by the U.S. Food and Drug Administration (FDA) for diagnostic use [21–23]. These elegant assays specifically detect S. aureus and distinguish those strains that are MRSA; with these tests, there is no concern with any interference from coagulase-negative staphylococci that may be present (typically as contaminants) in the respiratory samples. There is one full report of the use of this type of test, in a modified version, for the detection of S. aureus (MSSA and MRSA combined) and of MRSA in wound cultures [24]. However, no one has yet published data on the use of such tests for the specific diagnosis of pneumonia due to S. aureus or MRSA.

One potential use of these new rtPCR tests is to determine the S. aureus colonization status of patients admitted with CAP and to then determine whether the colonization does, or does not, predict the potential for MRSA as the agent of the lower RTI. In a study of 5779 nasal rtPCR tests for MRSA, done within 24 h of a clinical culture for any type of infection, a positive nasal MRSA test result predicted a 12.9-fold increased likelihood of MRSA as a cause of the infectious disease [25]. Specifically for pneumonia, 5.6% of 402 respiratory cultures grew MRSA, with a relative risk equal to an 18.3-fold higher likelihood of MRSA causing the RTI when the patient was nasally colonized with MRSA [25]. However, on a cautionary note, 33% of MRSA infections occurred in patients who had a negative nasal MRSA test result. The overall sensitivity, specificity, and negative predictive value for using nasal colonization status to predict RTI with (or without) MRSA were 75%, 90%, and 98%, respectively [25]. Thus, although the knowledge of MRSA nasal colonization status is helpful in deciding whether someone may have a MRSA-associated pneumonia, the accuracy of prediction is far from sufficient.

A multicenter, preclinical study was done to assess the utility of rtPCR using the Xpert MRSA/SA SSTI (Cepheid) test on 114 clinical wound swab specimens, with culture serving as the reference method. The results of that study are shown in Table 1 [24]. As can be seen from the results, although the overall ability to detect of S. aureus was excellent, there was some erroneous determination as to whether the S. aureus was MRSA or MSSA, which could lead to some major treatment errors (eg, treatment of MRSA with a β-lactam) unless all samples are cultured with susceptibility tests performed. Also, all the available rtPCR tests only detect S. aureus and MRSA, so if any other potential pathogens are suspected, full culture and sensitivity testing are necessary.

Two other studies performed by a single research group assessed both wound cultures and respiratory specimens. They investigated the preclinical BD GeneOhm combined StaphSR assay, which is an rtPCR test for detection of S. aureus (MSSA and MRSA) and compared it with an in-house assay for MSSA (NorthShore-developed MSSA) and the FDA-cleared BD GeneOhm MRSA Assay for Table 1. Results of a Multicenter Xpert MRSA/SA SSTI (Cepheid) Trial on Clinical Wound Swabs Testing for Staphylococcus aureus and MRSA

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity, % (n/N)</th>
<th>Specificity, % (n/N)</th>
<th>NPV, % (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA and MRSA</td>
<td>100 (65/65)</td>
<td>96.6 (67/59)</td>
<td>100 (57/57)</td>
</tr>
<tr>
<td>MRSA</td>
<td>97.1 (34/35)</td>
<td>96.2 (76/79)</td>
<td>98.7 (76/77)</td>
</tr>
</tbody>
</table>

NOTE. Data are from [24]. MSSA, methicillin-susceptible S. aureus; NPV, negative predictive value.
nal colonization on 200 clinical samples. The results are shown in Table 2. MSSA was found in 49 specimens by culture, and MRSA was found in 40 samples [26–27]. With regard to the 3 false-positive MSSA rtPCR results, all subjects were receiving anti-staphylococcal therapy at the time of testing, and for 2 patients, cultures of samples from the same body site subsequently grew MSSA. There were 4 false-negative MSSA rtPCR results, 3 of which involved mixed bacterial growth, with rare to few S. aureus isolates in the culture. Of the 2 false positive MRSA rtPCR results, no explanation could be found for the false positive result. There were also 3 false-negative MRSA rtPCR results, 2 could not be explained. Of the 34 respiratory samples in this study, 2 grew MRSA and 4 grew S. aureus (MSSA); all were correctly identified by the molecular diagnostic tests when compared with the diagnostic laboratory culture. Interestingly, one of the false-negative MRSA rtPCR test results was from a sputum sample that had been initially reported as mixed flora by the clinical laboratory, and MRSA was only detected as part of the research study [26–27], suggesting the sensitivity of the new rtPCR assays is very close to that of routine culture.

Finally, one other report used the FD- cleared BD GeneOhm MRSA Assay for nasal colonization to analyze 196 sputum specimens obtained from 156 patients for MRSA [28]. These authors used a less robust extraction method and found that the rtPCR test had a sensitivity and specificity of 97% and 92%, respectively, for 32 culture-positive samples [28]. Of the 12 false-positive rtPCR test results in this study, 10 involved patients who had an MRSA-positive clinical or surveillance culture within 2 weeks of the rtPCR assay. Nine samples (5%) remained unresolved after repeated testing and therefore could not provide a result; this was most likely because a relatively simple extraction method was used for processing a complex sample, such as sputum [28]. All of the clinical samples in these studies were purulent material for which the physician suspected infection. The rtPCR tests performed adequately but not as well as culture for determining the presence of S. aureus and distinguishing which strains were MRSA.

Although these tests may not be quite ready for clinical application in the detection of MSSA and MRSA as a cause of pneumonia, there is reason for optimism that, with very little effort, both the Cepheid and BD-GeneOhm assays could be clinically useful for the detection of S. aureus in RTI specimens and could provide immediate determination of methicillin susceptibility. Such information would be very useful in helping to avoid the unnecessary use of antimicrobial agents targeted against MSSA or MRSA in the initial empirical therapy for pneumonia.

**ELIMINATING MRSA AS A POTENTIAL PATHOGEN**

Reports continue that MRSA is a pathogen of increasing importance. As noted earlier, Delorme et al [8] assessed the problem of staphylococcal disease in Northeastern Ohio during 2006–2007. They found that 59% of the 1612 S. aureus isolates were MRSA and that the burden of disease was 589 infections per 100,000 inhabitants; there was an overall increase in staphylococcal infection of 77% between the 2 years [8]. Furthermore, they observed an increase in the rate of S. aureus disease for all age groups, with a 58% increase among outpatients and a 43% increase among inpatients. In this population, 66% of infected persons had no risk factors [8]. The burden of disease in 2005 was >100-fold that of tuberculosis in the United States [9]. Thus, effective diagnosis of MRSA in any disease syndrome with potentially serious consequences, such as pneumonia, is critical.

Another approach for reducing the risk of MRSA pneumonia is to eliminate the possibility of MRSA as a cause of any disease by universal screening, contact isolation, and decolonization. One 3-hospital health care organization deploying this method found that the MRSA disease rate decreased by >60% in 1 year [29], and they concomitantly found it cost-effective [30]. The Veterans Administration (VA) health care system of 153 hospitals reported on their first 21 months of universal screening with contact isolation of MRSA-positive patients, encompassing 1,213,646 admissions covering 5,296,757 patient-days of care. The VA recorded a 24% reduction in the rate of MRSA disease throughout the system for the patients who were not treated in the intensive care unit (ICU; *P* = .04) and a 77% reduction for ICU patients (*P* < .001) after 21 months [31]. Relevant to this review on using molecular testing for staphylococcal

### Table 2. Results of a Single-Center Study of the BD GeneOhm Real-Time Polymerase Chain Reaction Test for Methicillin-Susceptible Staphylococcus aureus (MSSA) and Methicillin-Resistant S. aureus (MRSA), Compared with a US Food and Drug Administration–Cleared Test for Nasal MRSA Surveillance (BD GeneOhm) and an In-House Test for MSSA (NorthShore) on 200 Clinical Samples

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA BD GeneOhm</td>
<td>96 (89–99)</td>
<td>97 (92–99)</td>
<td>97 (90–99)</td>
<td>96 (91–99)</td>
</tr>
<tr>
<td>NorthShore Developed MSSA</td>
<td>94 (87–98)</td>
<td>99 (95–100)</td>
<td>99 (94–100)</td>
<td>96 (90–99)</td>
</tr>
<tr>
<td>MRSA BD GeneOhm</td>
<td>95 (83–99)</td>
<td>99 (96–100)</td>
<td>95 (83–99)</td>
<td>99 (96–100)</td>
</tr>
<tr>
<td>MRSA Nasal</td>
<td>98 (87–100)</td>
<td>98 (94–99)</td>
<td>91 (78–97)</td>
<td>99 (97–100)</td>
</tr>
</tbody>
</table>

**NOTE.** Clinical samples included 123 wound samples, 34 respiratory specimens, 20 tissue specimens, 16 body fluid specimens, 2 catheter samples, and 5 other samples [26–27]. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
pneumonia, the rate of ventilator-associated pneumonia due to MRSA decreased 53% while the ventilator use rate decreased 19.7% (P < .001); non-ventilator-associated pneumonia due to MRSA associated with hospitalization also was reduced by 67% in the VA program [31]. However, the “search and destroy” intervention for MRSA (widespread surveillance and contact isolation with or without decolonization) remains controversial [32]. If the goal is to reduce MRSA-associated respiratory tract disease, it may be necessary to adopt the search and destroy as a national policy, as is in effect in the Netherlands [33].

**SUMMARY AND CONCLUSIONS**

Molecular diagnostics are redefining how we manage infectious diseases, and this trend will accelerate. Same-day (ie, in <4 h) diagnostics are here for many viral and bacterial tests. Unfortunately, at the present time, the only rapid molecular diagnostic tests for use in pneumonia are those for tuberculosis and viral respiratory infection; many lack sufficient sensitivity, and the viral diagnostic tests do not discriminate between infection and colonization [34–35]. Point-of-care molecular tests, planned for the near future, will further speed diagnostic information available to physicians who are treating an infectious disease [36]. This will place the experts in test interpretation and therapy at the heart of clinical management. Developing a reliable test for detection of *S. aureus* and MRSA as a cause of pneumonia remains a challenge [37], but it is not insurmountable. The current technical challenges for RTIs are (1) developing simple and robust specimen-processing procedure(s) before PCR testing; (2) modestly enhancing the sensitivity and specificity of the *S. aureus* and MRSA tests already available for clinical infection diagnosis; and (3) multiplexing the tests for the relatively few major pathogens (bacterial and viral) specifically associated with unique disease syndromes, such as CAP, hospital-acquired pneumonia, and ventilator-associated pneumonia.

Rapid tests for the specific detection of *S. aureus* and MRSA in respiratory secretions are very close to being clinically useful. The US FDA should be encouraged to work closely with the appropriate industry providers so as to bring these tests into clinical use as soon as can be reasonably done. The tests can be of great help to the practicing physician in the accurate detection of staphylococcal RTI. The deployment of these molecular diagnostic tests can lower patient morbidity and mortality, as well as limit the use of antimicrobials directed against (susceptible) *S. aureus* or MRSA to those patients who are really need treatment for *S. aureus* infection.

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**References**