Letters to the Editor

Failure of a PCR Screening Method to Detect MRSA

To the Editor:

Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) have become an increasing problem worldwide since the initial report of resistance some 30 years ago.[1] The increased morbidity, mortality, and cost associated with MRSA infections are a major incentive to control the spread of this organism within acute-care facilities.[2] Since 1995, healthcare facilities in Ontario, Canada, have experienced spread of an epidemic strain of MRSA throughout the province.[3]

In an effort to control this spread, most hospitals have instituted screening of all admissions with a past history of hospitalization or nursing home admission within the previous 6 months. Because of this increased level of surveillance and the associated cost, and to provide more rapid turnaround times, our laboratory adopted a multiplex polymerase chain reaction (PCR) screening method that detects the *mec*A and nuclease (*nuc*) genes. We report a *nuc*-negative strain of *S aureus* that was missed using this test, which led to us modify our screening method.

MRSA can be detected by routine susceptibility testing, oxacillin/salt screening plates, PCR, and more recently by using the MRSA-screen latex agglutination test.[4-7] In our laboratory, nasal and rectal swabs from suspected MRSA carriers are inoculated onto mannitol salt plates with 6 mg/L oxacillin. Twenty-four hours later, once growth is visible, the *Staphylococcus* is harvested from the plate and suspended in sterile distilled water to a turbidity of 0.5 McFarland.

Two microliters of this suspension are then used for the detection of MRSA by multiplex PCR. This multiplex PCR amplifies the *nuc* and *mec*A genes, and the resulting amplicons are detected by electrophoresis on a 0.1% agarose gel containing ethidium bromide.[5,6] The presence of both genes is indicative of MRSA. Methicillin-resistant coagulase-negative staphylococci may occasionally yield yellow colonies on the screen plate, but are easily distinguished from MRSA by the absence of the *nuc* gene on PCR.

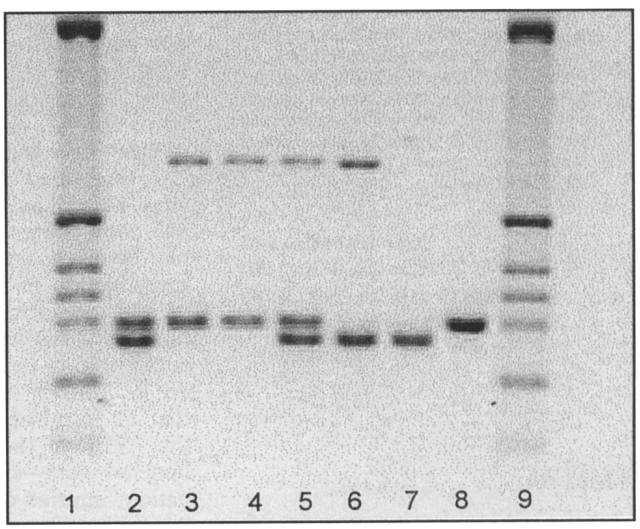

FIGURE. Multiplex polymerase chain reaction for *nuc*, *mec*, and *fem* genes: lanes 1 and 9 Lambda ladder; lane 2 *nuc*-positive, *fem*-negative methicillin-resistant *Staphylococcus aureus* (MRSA); lanes 3 and 4 *nuc*-negative, *fem*-positive MRSA; lane 5 *nuc*-positive, *fem*-positive MRSA; lane 6 *nuc*-positive, *fem*-positive methicillin-sensitive *S aureus* (MSSA); lane 7 *nuc*-positive, *fem*-negative MSSA; lane 8 *mec*A-positive, coagulase-negative *Staphylococcus*.

Recently, a patient colonized with MRSA was missed by our screening test. The isolate, which was *mec*A positive and *nuc* negative by PCR, was identified as a methicillin-resistant coagulase-negative *Staphylococcus*. An astute technologist noted that a wound isolate from the same patient looked like *S aureus*, despite its being DNAse negative. Biochemical reactions and gas liquid chromatographic profiles of cell-wall fatty acids confirmed the identity of the isolate as *S aureus*. This led us to add detection of the *fem* gene to our multiplex PCR to identify *nuc*-negative strains of MRSA; following this, a similar strain was identified from a second patient. Both strains were identical by pulsed-field gel electrophoresis, and further investigation showed that the index patient had briefly shared a room with this patient.

We would recommend that any laboratory using PCR to detect the *nuc* and *mec*A genes be aware that *nuc*-negative MRSA strains exist and consider instead using the *fem* and *mec* genes to avoid this problem if such strains are present in their institution. At present, we screen for all three genes in our laboratory. Using our primers, the *fem* gene usually is not amplified in the presence of the *nuc* gene in MRSA or methicillin-susceptible *S aureus;* however, *nuc*-negative strains of MRSA are consistently positive for the *fem* and *mec*A genes (Figure).

We do not know whether in the past we have missed MRSA strains using our screening method, but we have not identified any further *nuc*-negative strains, nor found any in our culture collection of MRSA, so they do not appear to be common.

REFERENCES

1. Barrett FF, McGhee RF Jr, Finnland M. Methicillin-resistant *Staphylococcus aureus* at Boston City Hospital: bacteriologic and epidemiologic observations. *N Engl J Med* 1968;79:441-448.
2. Boyce JM. Methicillin-resistant *Staphylococcus aureus* in hospital and long-term care facilities: microbiology, epidemiology, and preventive measures. *Infect Control Hosp Epidemiol* 1992;13:725-737.
3. McGeer A, Low D, Conly J, Campbell I, Devlin R, Simor A, et al. Methicillin-resistant

Staphylococcus aureus in Ontario. *Can Commun Dis Rep* 1997;23:45-46.
4. Chambers HF. Methicillin-resistant staphylococci. *Clin Microbiol Rev* 1988;1:173-186.
5. Vannuffel P, Gigi J, Ezzedine H, Vandercam B, Delmee M, Wauters G, et al. Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. *J Clin Microbiol* 1995;33:2864-2867.
6. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of *nuc* gene. *J Clin Microbiol* 1992;30:1654-1660.
7. Cavassini M, Wenger A, Jaton K, Blanc DS, Bille J. Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. *J Clin Microbiol* 1999;37:1591-1594.

Zafar Hussain, MD
Michael A. John, MD
London Health Sciences Centre
London, Ontario, Canada

Déjà Vu . . . All Over Again? The Importance of Instrument Drying

To the Editor:

Although the risk of patient infection following flexible endoscopy is reported to be very low, investigations linking nosocomial infection (and pseudo-infection) to endoscopes contaminated with waterborne microorganisms have been published.[1,2] In 1991, a report documenting patient infection and pseudo-infection caused by two models of automated flexible endoscope reprocessors (AFERs), whose internal components were colonized with *Pseudomonas aeruginosa* and *Mycobacterium chelonae,* was published in *Morbidity and Mortality Weekly Report (MMWR)*.[3] Discussed in this report (and several others) is the importance of drying the endoscope to prevent bacterial colonization during storage.[1-3] Drying is typically achieved by rinsing each of the endoscope's channels with 70% alcohol (to facilitate drying), followed by forced air.[1-3]

More recently, an outbreak linked to endoscopes contaminated with *P aeruginosa* was reported in *MMWR*.[4] Investigators of this outbreak, referred to as cluster 3, concluded that inadequately trained healthcare staff improperly connected bronchoscopes to an AFER, resulting in multiple patient infections and one fatality (for unclear reasons, the fatality was not reported in this *MMWR)*.

Although several noteworthy recommendations were provided in the *MMWR,*[4] absent was a needed discussion of the importance of drying the endoscope to prevent patient infection. Also not discussed in this *MMWR*[4] was whether the AFER's filtered rinse water and the hospital's water supply, water faucets, and sink drains were sampled microbiologically.

Obtaining cultures of these sites, as well as of the AFER's internal components and water filters, has been recommended during an investigation to identify the source of, and risk factors for, patient infection.[1,2] Indeed, these sampling data are crucial to the conclusion reported by *MMWR* that human error—that is, inappropriate connection of the AFER to the bronchoscope by hospital staff—was the cause of the patient injuries described in cluster 3.[4] If the filtered rinse water had been sampled and found to be contaminated with *P aeruginosa* (the outbreak microorganism in cluster 3), then the *MMWR*'s conclusion, which suggests patient-to-patient transmission, would likely be incomplete, and the reported patient injuries might have occurred even if hospital staff had properly connected the bronchoscopes to the AFER.

Although effective and routinely employed by healthcare facilities to reprocess bronchoscopes and gastrointestinal endoscopes, AFERs have their limitations. For example, unlike other processes that use heat, gas, or a plasma to disinfect or sterilize reused instruments, current AFERs immerse the endoscope in a liquid chemical sterilant, requiring that the endoscope be rinsed with a large volume of water to remove potentially toxic chemical residues. This step is arguably the Achilles' heel of AFERs (and other liquid-chemical sterilant-based processes), as the success of these devices is therefore vulnerable to, and depends significantly on, the quality of the rinse water, which is difficult to monitor and control. Rinse water that contains microorganisms can recontaminate the endoscope and result in patient infection, even if the cleaning and chemical immersion steps were effective.[1-3]

Only the use of sterile water would virtually eliminate the risk of recontaminating instruments with waterborne microorganisms during rinsing. But using bona fide sterile water for rinsing can be problematic in the clinical setting, and producing it in the healthcare setting is likely to be expensive and impractical and presumably would require periodic microbiological monitoring to ensure the process's effectiveness. In lieu of sterile water, AFERs typically rinse the endoscope with tap water that has been passed through a bacterial filter rated at 0.1 or 0.2 μm. These filters are designed to produce bacteria-free, but not sterile, water in the healthcare setting. Moreover, these water filters are not fail-safe and, with repeated use, have been reported to fail, allowing microorganisms to pass.[1]

Whereas the contribution of cleaning and disinfecting the endoscope to the prevention of patient infection is well recognized, the importance of drying and properly storing the endoscope is sometimes overlooked.[4,5] (Whether the hospital involved in cluster 3 dried the endoscope before storage is unclear.[5]) Why might a healthcare facility fail to dry its endoscopes before storage? Plausible explanations include misunderstanding the inherent limitations of water filters and confusion over the definitions of, and microbiological differences between, tap water, bottled sterile water, and filtered water claimed to be bacteria-free or sterile.[5] Using a 70% alcohol rinse followed by forced air to dry the endoscope before storage is well documented and has been shown to prevent nosocomial infection caused by rinse water contaminated with *P aeruginosa,* mycobacteria, and other opportunistic pathogens.[1,2,5] This practice is recommended whether using tap water or water labeled as bacteria-free or sterile.[5]

In conclusion, to prevent patient infection caused by inadequately dried endoscopes, I encourage federal regulatory agencies and professional endoscopy and infection control organizations to reemphasize the importance of thoroughly drying and properly storing the endoscope. Also encouraged, to prevent confusion and patient infection, are discussions aimed at clarifying and detailing the definitions and microbiological dif-