$P < .0001$). Within the time frame of a typical patient care encounter (approximately 30 minutes), there was a <0.02 $\log_{10}$ reduction in virus at 40% RH, while there was a <0.1 $\log_{10}$ reduction at 60% RH. Achieving a 4 log reduction of infectious virus on a mask surface would take 87 hours at 40% RH and 20 hours at 60% RH.

Enveloped bacteriophage Φ6 can survive on the surface of an N95 respirator longer than a single patient care encounter. High levels of virus remaining on a respirator may pose a risk of virus transfer to the wearer during handling and reuse.[4] The use of a bacteriophage provides a simple, low-cost method for evaluating survival and transfer risks; bacteriophages are already used as surrogates in studies of respirator decontamination.[5] Bacteriophage Φ6 was inactivated somewhat more rapidly than H1N1 influenza on N95 surfaces at 60% RH (possibly as a result of the matrix used), and a similar trend of greater inactivation was observed at higher humidity levels.[6] The results are similar to those found for transmissible gastroenteritis virus, a member of the coronavirus family, on respirator surfaces.[7] This suggests that bacteriophage Φ6 is a potential surrogate for studies of human respiratory viruses on personal protective equipment.

The inactivation observed demonstrates that residual virus on a respirator surface is an important factor when reuse is considered. If a respirator is used over an 8- or 12-hour shift, even 90% inactivation during that time raises the possibility that that reuse over multiple patient encounters may add additional viral load to an already contaminated respirator. Therefore, decontamination of respirators is an important consideration in any reuse scenario.[8] Studies of infectious virus reduction[9] suggest that decontamination may be a viable option if pandemic situations or shortages make respirator reuse an alternative that needs to be considered. The design of effective respirator decontamination protocols should include the intervals at which a respirator needs to be decontaminated between uses, as well as how long a respirator should be used before discarding. Virus survival data is needed to model inactivation, decontamination, and recontamination to determine safe and effective reuse protocols. Long-term survival of respiratory viruses on the surface of N95 respirators needs to be taken into account when evaluating decontamination protocols and weighing the risks and benefits of respirator reuse for outbreak and pandemic preparedness.

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Evaluation of Universal Methicillin-Resistant *Staphylococcus aureus* Screening Using Nasal Polymerase Chain Reaction Compared with Nasal, Axilla, and Groin and Throat and Perianal Cultures in a Hospital Setting

To the Editor—Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage by polymerase chain reaction (PCR) methods and early patient isolation could reduce the chances of nosocomial transmission between patients.[1] However, the cost of PCR and MRSA prevalence could influence choice of testing method in a hospital screen-

ing program.[2] We report the potential utility of nasal PCR compared with 3 swab cultures in a tertiary hospital where the overall prevalence of MRSA carriage was 11.8%.[3] The Communicable Disease Centre (CDC) at Tan Tock Seng Hospital in Singapore is the national referral center for human immunodeficiency virus (HIV) infection and other infectious diseases and also houses dermatology inpatients. Routine MRSA screening at the CDC includes the culture of specimens from 5 sites, using 3 swabs upon admission and at discharge: combined swab from nares, axillae, and groin; throat; and perianal, using chromogenic agar plates (MRSASelect, BioRad). From November to December 2011, an additional 200 nasal swabs were obtained and tested for MRSA colonization, using the Xpert MRSA kit (Cepheid).[4] Residual broths before addition of lysis buffer from PCR-positive samples were subcultured onto chromogenic agar.

The median age of the patients screened was 52 (range, 17–100). Seventy-two percent were male, and 28% were female. The composition of patients was dermatology (42%), HIV (36%), and other infectious diseases (22%). When comparing nasal PCR to culture methods from 5 sites, the specificity of nasal PCR was 94.6%, with a sensitivity at 57.1% (Table 1). Of the 6 culture-positive cases missed by nasal PCR, 3 were positive from samples taken from the throat and perianal sites only. Two of these cases were HIV patients. Nasal PCR was able to identify 10 possible MRSA-positive cases from the dermatology cohort, which were culture negative from 5 sites. Four of these cases that were positive via nasal PCR only demonstrated positivity upon subculture onto chromogenic agar.

We have shown that nasal PCR has good specificity even when compared with culture methods from 5 sites. For obvious reasons, one would expect that additional anatomical sites by the culture method would increase the MRSA detection rate.[5] Our previous experience was that the addition of throat and/or perianal swabs to the nares, axillae, and groin culture increased the sensitivity of MRSA detection by 10% in all patient groups.[3] In this study, this was reflected in HIV patients (2 cases), although we cannot rule out the possibility that PCR could have identified those if the swabs from the other sites had also been tested using PCR. In contrast, nasal PCR assay was able to detect 10 additional MRSA-positive cases in dermatology patients not picked up by the conventional culture method. The absence of a positive broth culture for these nasal PCR positive cases does not exclude the possibility of carriage and risk of transmission.

In conclusion, we have demonstrated the utility of nasal PCR as a screening tool for MRSA in view of its high specificity and negative predictive value in our setting. For dermatology patients, our data suggest an advantage of nasal PCR over culture methods with multiple sampling sites, but further larger studies would be needed to confirm this. However, the low sensitivity, especially in HIV cohorts, is a concern. We propose that there is still a need to augment nasal PCR with culture methods, particularly for HIV patients, with sampling and culturing from the throat and perianal sites to minimize false negatives for the time being.

TABLE 1. Comparison of Nasal Polymerase Chain Reaction (PCR) to Culture from 5 Sites

	NAG + throat + perianal swabs		
Nasal PCR	Negative	Positive	Total
Negative	176 (94.6)	6 (42.9)	182[a]
Positive	10 (5.4)	8 (57.1)	18[b]
Total	186[c]	14[d]	200 (100)

NOTE. Data are no. (%). NAG, nares, axillae, and groin.
[a] Negative predictive value, 96.7%.
[b] Positive predictive value, 44%.
[c] Specificity, 94.6%.
[d] Sensitivity, 57.1%.

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Multiple-Locus Variable Number Tandem Repeat Analysis Typing of Vancomycin-Resistant *Enterococcus faecium* in Serbia

To the Editor—Multidrug-resistant *Enterococcus faecium* has become one of the most important nosocomial pathogens causing increasing numbers of nosocomial infections worldwide. In Serbia, after the first report of vancomycin-resistant *Enterococci* (VRE) in 2002,[1] reports on the epidemiology of this bacterium have been scarce.[2] Although pulsed-field gel electrophoresis (PFGE) was long considered the gold standard for typing methods, it has now been replaced by multilocus sequence typing (MLST). Molecular epidemiological studies using MLST identified host-specific genogroups, including 3 hospital-associated *E. faecium* lineages designated lineages 17, 18, and 78.[3] In order to obtain more insight into the molecular epidemiology of VRE in Serbia, we used the relatively fast and cheap multiple-locus variable number tandem repeat analysis (MLVA) typing method[4] to determine the genetic relatedness of a total of 32 VRE isolates isolated between 2007 and 2010 in the following hospitals: Clinical Center of Serbia (23), University Children's Clinic (2), and a gynecology clinic (1), all located in Belgrade, and 3 general hospitals located in Valjevo (8), Zaječar (2), and Ćuprija (1; Table 1). In addition, we included 5 VRE isolates, each representative of the 5 major clones (based on PFGE, comprising 97 strains) of the 194 VRE isolated from hospitalized patients at 4 health institutions in Belgrade between 2002 and 2006 (data not shown).

The identification and antimicrobial susceptibility was performed by a Vitek system using IDGP and AST 586 card. All VRE were resistant to ampicillin, ciprofloxacin, streptomycin high doses, and imipenem and sensitive to linezolid. In total, 86.5% (32/37) of the strains were resistant to teicoplanin. Overall resistance to tetracycline was 70.3% (26/37), and overall resistance to gentamicin high doses was 75.7% (28/37).

MLVA typing of 37 VRE revealed 11 different MLVA types (MTs), including 4 not previously detected MTs (MT-340 to MT-343; Table 1). The most predominant MTs included MT-159 (8/37 [21.6%]), MT-1 and MT-231 (5/37 [13.5%] for both), MT-268 and MT-340 (4/37 [10.8%] for both), and MT-296 and MT-342 (3/37 [8.1%] for both). Unique new types were confined to patients residing at the Clinical Center of Serbia in Belgrade, while the other new types originated from inpatients in Belgrade and Valjevo. Except for MT-334, all MTs found outside Belgrade (MT-1 [3], MT-159 [2], MT-231 [2], MT-296 [1], MT-340 [1], MT-342 [1]) were found in Belgrade as well, indicating widespread distribution of several types.

The most prevalent MT in this study, MT-159, is one of the most common types causing hospital outbreaks and invasive infections in Europe today.[5-8] However, MT-159 was not identified among the 5 representative isolates of major clones of the 194 VRE, suggesting that MT-159 has spread recently and quickly, covering the territory of at least 2 metropolitan areas in Serbia. Only 2/8 members of that type were sensitive to high dozes of gentamicin, while resistance to all other antibiotics was uniform, suggesting possible clonal spread of that MT. Similar findings could be observed with MT-231, with 4/5 members sensitive to tetracycline and resistant to all other antibiotics tested except linezolid.

In contrast, the second-most predominant type, MT-1, identified among 2/5 representative isolates of major clones from Belgrade (representing 45/194 isolates) and in 2 other metropolitan areas of Serbia (3 strains), was the most widespread type in terms of time and space. Furthermore, that type was previously also identified among 2 VRE strains isolated at another hospital in Belgrade in 2005.[2] Some other studies that followed population changes of enterococci over long periods identified MT-1 from the beginning of the investigation and documented its span over 10 and 11 years, respectively.[7,9]

MLST analysis was performed on 12 isolates, including 1 representative of each MT that comprised more than 2 isolates,[10] those with unique new MT, and the 5 representatives of major clones from Belgrade and revealed in total 9 different sequence types (STs).

MT-1 is composed of multiple STs, thus making a polyclonal population.[9] Indeed, 2 members of the MT-1 type from our study have been ascribed different sequence types, ST-17 and ST-554. Werner speculated that if isolates of ST-17 represent a rather ancient clonal type prone to recombination and thus divergent PFGE patterns for a long time, then ST-203 and ST-18 could constitute rather recent hospital-adapted clones. ST-203 from our study, ascribed to newly appearing MT-159, and newly introduced MLVA type MT-341 belonging to ST-18 support this hypothesis. Werner's speculation was based on German experience with molecular epidemiology of enterococci starting in the 1990s. It seems that evolution of VRE in Serbia could have a similar pattern. Hospital origin of at least 5 STs from our study (ST-17, ST-18, ST-252, ST-426, and ST-427), belonging to the Bayesian analysis of population structure subgroup 3-3, could be confirmed, according to Willems' findings.[3]

MLST proved its superiority over MLVA when 2 separate sequence types (ST-17 and ST-554), marked as 2 different pulsotypes by PFGE in 2 strains that could be distinguished,