JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2011, p. 1119–1121 Vol. 49, No. 3
0095-1137/11/$12.00 doi:10.1128/JCM.02601-10

Methicillin-Resistant *Staphylococcus aureus* Colonization at Different Body Sites: a Prospective, Quantitative Analysis▽

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Received 22 December 2010/Accepted 23 December 2010

We quantified methicillin-resistant *Staphylococcus aureus* (MRSA) carriage. The greater the $\log_{10}$ count in samples from the nares, the greater the likelihood that other body sites had been colonized. Log_{10} counts among body sites were correlated. The greatest sensitivity value (98%) was determined for the combined results from 2 sites: the nares and the groin.

Heavy nasal *Staphylococcus aureus* carriage is a risk factor for invasive infection (20) and an independent risk factor for surgical site infection (8). Numbers of colonized body sites have been found to correlate inversely with successful decolonization in some (19) but not all (16) studies. Methicillin-resistant *S. aureus* (MRSA) in a wound or urine is more often associated with environmental contamination than MRSA at other sites (4). The number of body sites colonized with MRSA and colonization of the groin correlate with the degree of environmental contamination in patient rooms (13). Studies have measured yields of MRSA cultures of different body sites (2, 3, 5, 6, 11, 15, 19). However, few studies have assessed differences in quantities of MRSA at different sites (14). This study was designed to elucidate the quantities of MRSA at different body sites and the interrelationships of colonizations at those sites.

After institutional review board (IRB) approval, patients were enrolled 18 September 2007 to 11 March 2008. Samples were collected by the use of sterile swabs (BBL CultureSwab liquid Stuart medium; Becton Dickinson, Franklin Lakes, NJ) from consenting inpatients who were more than 18 years of age and who had been previously identified as MRSA positive as part of an active surveillance program during the year prior to enrollment on the basis of positive cultures from nares or who otherwise had produced MRSA-positive clinical cultures during the current period or during hospitalization in the year prior to enrollment. Patients were excluded from the study who presented with an obstruction of the nares by a medical device, with nasal jewelry, or with nose, groin, axilla, or perineal trauma or who had applied drugs, ointments, or sprays to their nares, axilla, groin, or perineum within 6 weeks prior to enrollment. Subjects were enrolled once. Swab 1 was inserted 1 cm into each nasal vestibule and rotated two complete revolutions while in contact with the nasal membrane; swab 2 was rotated in a Z pattern for 10 s in each axillary fold from the anterior to posterior lateral axillary line; swab 3 was rotated in a Z pattern for 10 s from the anterior lateral to anterior medial aspect of each groin; and swab 3 was rotated in a Z pattern from the anterior to posterior perineum for 5 s. Swabs were then immediately placed on ice and frozen at −80°C within 4 h (7). In a central microbiology laboratory, each swab bud was placed into 1 ml of Butterfield's buffer (Pace Analytical Life Science Laboratory, Oakdale, MN) and subjected to a vortex procedure for 15 s. Using the same buffer, 4 serial 1:10 dilutions were carried out to achieve a 1-ml final volume. A spread-plate technique was used to inoculate 0.1 ml of the reaction mixture onto MRSA-selective chromogenic medium (CHROMagar plates; BDDS, Cockeysville, MD) for duplicate experiments. Using 0.2-ml aliquots of remaining buffer, 3 separate CHROMagar plates were inoculated and incubated at 35°C for 24 h. If no mauve colonies were present, plates were reincubated for another 24 h. Colonies of presumptive MRSA from plates containing 30 to 300 CFU/ml were subcultured onto sheep blood agar (SBA) plates (Becton Dickinson) and incubated for 24 h. The tube coagulase test (Becton Dickinson) was performed. Each swab bud was then placed into 10 ml of tryptic soy broth (TSB)–6.5% NaCl (BDDS) and incubated at 35°C for 24 h, and a loopful of TSB was inoculated onto a CHROMagar plate and processed as described above. Medical records of patients whose cultures grew MRSA were reviewed to assess risk factors for MRSA carriage as well as for current and past MRSA infection.

Colony counts per swab were converted to $\log_{10}$ counts to stabilize variance and to make the bacterial load data more normally distributed. Statistical analyses were conducted using $\log_{10}$ counts. For sensitivity and negative-predictive-value determinations, the standard for comparison was MRSA found at any sampled body site by primary culture or enrichment. Cultures that grew only after enrichment were given a value of "1" for the plate representing the lowest dilution. McNemar's test was used to determine differences in sensitivity. The Wilcoxon ranked-sum test was used to detect a correlation between the number of colonized body sites and the presence of an underlying illness. The Wilcoxon ranked-sum test and a *t* test were used to detect a correlation between the MRSA $\log_{10}$ colony count and the presence of an underlying illness. A mixed-model analysis of variance (ANOVA), with the patient representing a random variable, was used to test for differences between body sites in mean $\log_{10}$ counts. Ordinal logistic re-

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▽ Published ahead of print on 5 January 2011.

TABLE 1. Anatomic sites with MRSA

Anatomic site(s) sampled or MRSA test result	No. (%) of patients with MRSA detected at anatomic site
Nares only	13 (21.7)
Nares, groin, perineum, axilla	10 (16.7)
Nares, groin	8 (13.3)
Nares, groin, perineum	8 (13.3)
Negative	7 (11.7)
Nares, perineum	3 (5.0)
Nares, groin, axilla	3 (5.0)
Groin, perineum	2 (3.3)
Nares, axilla, perineum	2 (3.3)
Groin only	2 (3.3)
Nares, axilla	1 (1.7)
Axilla only	1 (1.7)

TABLE 2. Quantitative cultures of MRSA at various anatomic sites

Anatomic site sampled	No. of patients with MRSA detected at anatomic site	Mean $\log_{10}$ count of MRSA (SD)
Nares	48	1.95 (1.34)[a]
Axilla	17	0.87 (0.68)[b]
Groin	33	1.70 (1.52)
Perineum	25	1.65 (1.27)

[a] Mean $\log_{10}$ counts for the samples from the nares were greater than those for the samples from the axilla ($P < 0.001$).

[b] Mean $\log_{10}$ counts for the samples from the axilla were less than those for the samples from the groin ($P = 0.002$) and perineum ($P = 0.02$).

gression was used to test for a relationship between $\log_{10}$ counts in the nares and the number of colonized body sites as well as to test the relationship between patient age and the number of colonized body sites. A level of significance of alpha = 0.05 was used.

Samples from at least 1 body site of 53 of 60 adults exhibited growth of MRSA (Table 1). A total of 29 of 123 (24%) MRSA-positive cultures grew only by broth enrichment. The mean age of colonized patients was 55 (standard deviation [SD], 20) years; 34 (64%) of the patients were male; and 41 (77%), 10 (19%), and 2 (4%) were Caucasian, African American, and Hispanic, respectively. Of 53 patients, 40 (75%) were found to have MRSA colonization at extranasal sites. Sensitivity and negative predictive values for cultures of each body site were as follows: for the nares, 91% and 58%, respectively; for the groin, 63% and 26%; for the perineum, 47% and 20%; and for the axilla, 32% and 16%. The nares exhibited greater sensitivity than the axilla ($P < 0.001$), groin ($P = 0.003$), and perineum ($P < 0.001$). The groin was more sensitive than the axilla ($P = 0.002$). The greatest yields from any 2 body sites were those of the nares and groin, which exhibited a combined sensitivity value of 98% and a negative predictive value of 88%. Mean $\log_{10}$ counts for the nares were greater than those for the axilla ($P < 0.001$), and mean $\log_{10}$ counts for the axilla were less than those for the groin ($P = 0.002$) and perineum ($P = 0.02$) (Table 2). No other differences in mean $\log_{10}$ counts between different sites were significant. Using ordinal logistic regression, a relationship was found between $\log_{10}$ counts in the nares and the odds of a greater number of body sites found to be colonized with MRSA ($P < 0.001$; odds ratio [OR], 2.1 for a 1-log change; 95% confidence interval [CI], 1.44 to 3.03). Using Kendall's correlation coefficients, $\log_{10}$ counts determined for each cultured body site correlated with $\log_{10}$ counts determined for all other cultured sites ($P \leq 0.03$ for all comparisons).

There was no correlation between the number of body sites with MRSA and the presence of diabetes (16 patients [$P = 0.07$]) or other forms of immunosuppression (14 patients [$P = 0.09$]); there was no correlation between $\log_{10}$ colony count and diabetes ($P = 0.4$) or immunosuppression ($P = 0.9$). However, we found a correlation between diabetes and mean $\log_{10}$ counts in the perineum ($P = 0.02$). There was no correlation between the number of body sites that grew MRSA and the likelihood of MRSA infection at enrollment or during the year prior to enrollment. Of the patients who had MRSA in their nares at enrollment, the $\log_{10}$ colony count was lower in those patients who had an MRSA infection at enrollment or during the year prior to enrollment than in those who harbored MRSA but had no documented MRSA infection (mean $\log_{10}$ colony counts of 1.6 and 2.4, respectively [$P = 0.03$]).

We found that cultures of samples from the nares and cultures of combined samples from the nares and groin have similar sensitivities (91% and 98%, respectively) but that determinations based on the combined samples increased the negative predictive value from 58% to 88%, confirming other investigations suggesting that cultures of multiple body sites should be used to rule out MRSA colonization (2, 3, 5, 6, 12). The sensitivity determined in our study with cultures of samples from the nares and groin was higher than that previously reported (85% sensitivity) (9) but was similar to the sensitivities reported by others for studies using cultures of samples from the nares and groin (96% sensitivity) (21), nares and rectum (96% sensitivity) (5), and nares and perineum (87% to 90% sensitivity) (3, 9).

The likelihood of MRSA colonization of body sites other than the nares varies by strain type (11, 21). Although we did not subtype isolates, we investigated the quantity of MRSA at different sites. We demonstrated a relationship between the quantity of MRSA in the nares and the number of body sites colonized with MRSA ($P < 0.001$; OR, 2.1 for a 1-log change; 95% CI, 1.44 to 3.03), in agreement with a previous study performed with methicillin-susceptible *S. aureus* (17). We found a correlation between the quantities of MRSA colonizing different body sites. It is unclear whether the quantity of MRSA carriage is related to levels of antimicrobial peptides such as RNase 7 (22).

Although one study showed a correlation between increasing numbers of colonized body sites and increasing age (3), we did not find such a correlation. Some investigators found a correlation between *S. aureus* carriage and diabetes (1). We found no correlation between the number of body sites colonized or the quantities of MRSA colonizing body sites and diabetes or other forms of immunosuppression, but our study was underpowered. Surprisingly, the quantity of MRSA in the nares correlated inversely with the likelihood of MRSA infection at enrollment or over the year prior to enrollment. This finding is in contrast to those of previous studies of methicillin-susceptible *S. aureus* (20) and requires further investigation to determine whether our result is an anomaly or reproducible.

Regarding study limitations, we did not obtain serial cultures

and so we did not determine whether colonization was persistent (18). Differences in contact times or surface areas cultured may explain some of the differences in the observed $\log_{10}$ counts. We did not culture samples from the throat, an important site for methicillin-susceptible *S. aureus* colonization (10, 11). Since we did not determine the strain(s) of MRSA cultured, we were unable to correlate strain types with colonized body sites, and we did not perform molecular fingerprinting to prove the concordance of isolates colonizing different body sites. We did not test for the presence of the *mecA* gene or perform other MRSA confirmatory tests. Lastly, we did not correlate MRSA carriage and MRSA infections that may have developed after enrollment.

We thank John Perry and Michele Astphan for assistance in specimen collection.

This study was funded by 3M Health Care Products, St. Paul, MN.

Potential conflicts of interest: Gail Maxey, Dan Morse, and Jennifer Cartony are employed by 3M Healthcare and they have received 3M stock options. Leonard Mermel has served as a consultant to 3M Healthcare. Pauline Covington has no conflicts of interest.

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