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Impact of a Short Period of Pre-Enrichment on Detection and Bacterial Loads of Methicillin-Resistant *Staphylococcus aureus* from Screening Specimens▽

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We compared the impacts of direct plating on a chromogenic medium and of plating after enrichment (4 h and overnight) on the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from 52 patient screening samples. MRSA colony counts for ≈70% of samples after overnight pre-enrichment were >20-fold higher than the counts after the other two treatments. The qualitative differences (sample was MRSA positive/negative) between the results of the direct plating and 4-h pre-enrichment treatments were not significant, indicating no advantage of the latter; however, the number of samples positive for MRSA increased significantly after an overnight sample pre-enrichment ($P < 0.005$).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a major nosocomial pathogen in the last decade. Patients colonized with MRSA serve as reservoirs of self-infection or dissemination to other patients and to the hospital environment (2, 12, 6). Hence, screening for MRSA carriage and contact isolation of MRSA carriers is crucial for effective hospital infection control (3). Employing rapid and sensitive screening assays for MRSA detection could help to further improve infection control, as well as decrease costs (4, 7). While traditional culture-based methods tend to be slow and laborious, the next-generation culture methods, such as the use of chromogenic media, which allow a direct colony color-based identification of MRSA from the primary culture, can reduce the time to result from 48 to 18 or 24 h (7). The sensitivity of MRSA detection on chromogenic media is further enhanced by utilizing an overnight enrichment prior to culture but at the cost of rapidity of the assay (5, 13). A short pre-enrichment would enable next-day results; however, whether this would offer any advantage in terms of increased sensitivity in comparison to that of a direct inoculation of the medium remains unclear. We compared the impacts of direct plating on a chromogenic medium and of plating after a short (4 h) or overnight pre-enrichment on MRSA isolation from patient screening samples.

Samples were collected at the University of Geneva Hospitals, a 2,096-bed tertiary-care institution, during August 2008 from 26 inpatients with known MRSA carriage detected within 6 months prior to sampling for this study. A total of 52 nasal and groin swabs collected using swabs premoistened with saline were inserted into 1.5 ml of brain heart infusion broth with 15% glycerol and immediately frozen at −80°C until further analysis. Samples were thawed and vortexed for 25 s, and 10-μl amounts were inoculated, using a spiral plater (Eddy Jet, The Netherlands), directly onto the following media: (i) a chromo-

TABLE 1. MRSA colony counts after various treatments

Treatment	No. of CFU/ml[a]			No. of positive samples
	Range	Average	95% confidence interval	
Direct plating	1.00×10^2 to 3.89×10^8	1.83×10^7	1.68×10^2 to 1.67×10^8	28
4-h pre-enrichment	2.00×10^2 to 5.19×10^8	2.07×10^7	2.65×10^2 to 2.01×10^8	27[b]
Overnight pre-enrichment	4.00×10^2 to 6.39×10^8	9.23×10^7	5.95×10^2 to 5.67×10^8	40[c]

[a] Colony counts are expressed per ml to allow comparison between the three plating treatments.
[b] Two samples showed growth after direct plating but not after 4 h of pre-enrichment.
[c] Two samples showed growth after direct plating, of which one also grew after 4 h but not after 24 h of pre-enrichment.

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TABLE 2. MRSA colony counts in nasal and groin swabs

Sample source(s)[a]	No. of CFU/swab			No. of positive samples
	Range	Average	95% confidence interval	
All swabs	1.50×10^2 to 5.84×10^8	2.74×10^7	2.51×10^2 to 2.50×10^8	28
Nasal swab	3.00×10^2 to 5.84×10^8	6.13×10^7	4.50×10^2 to 4.60×10^8	11
Groin swab	1.50×10^2 to 7.08×10^7	5.51×10^6	2.10×10^2 to 5.06×10^7	17

[a] All samples were plated directly.

genic medium, BBL CHROMagar MRSA II (BD Diagnostics, Erembodegem, Belgium); (ii) mannitol-salt agar with 4 μg/ml cefoxitin (MSAC; positive control for MRSA); and (iii) blood agar (general sample control). Amounts of 10 μl of the samples were also added to a selective enrichment broth (Tryptone soya broth with 2.5% salt, 20 μg/ml aztreonam, and 3.5 μg/ml cefoxitin) (1) and incubated at 37°C in air. Amounts of 10 μl of the enriched samples were spiral plated on CHROMagar, MSAC, and blood agar after 4 h and after an overnight incubation. All plates were examined after overnight incubation (18 h) at 37°C in air, and colony counts were made from the CHROMagar plates. Five putative MRSA colonies were picked from the CHROMagar plates, purified on blood agar, and subjected to confirmatory tests. Confirmation was done using latex agglutination (Pastorex Staph Plus; Bio-Rad, Belgium), the tube coagulase test, and a subculture on MSAC. A sample was considered negative for MRSA if (i) characteristic colonies were not observed on the primary agar cultures or (ii) the putative MRSA colonies did not give characteristic results on confirmatory tests.

Statistical analysis was done using R version 2.6.2 (The R Foundation for Statistical Computing). Nonparametric comparisons were made using Friedman's test (a distribution-free test used to compare observations repeated on the same subjects, based on ranks). Qualitative differences in observations (MRSA positive/negative) at the three treatments were modeled using a logistic approach. A generalized estimating equation was used to account for repeated measures over time, and the Score test to assess differences between results from the three treatments.

An intrasample comparison of MRSA counts obtained after direct plating and after the two enrichments showed that counts after an overnight pre-enrichment were significantly different from counts after the other two treatments (Friedman's chi-square = 56.44, degrees of freedom = 2, $P < 0.0001$) (Table 1). A sample that was pre-enriched overnight yielded >20 times more MRSA colonies than if plated directly or after a 4-h pre-enrichment in approximately 70% of the samples. Ten of the 52 samples did not show the presence of MRSA after any of the three treatments studied.

The qualitative differences in reading of plates for MRSA positivity after a direct plating or a 4-h enrichment were also not significant ($P = 0.317$, Score test), while clear differences were observed after an overnight enrichment in comparison to either direct plating ($P = 0.002$) or a short pre-enrichment ($P = 0.005$). Twenty-four samples were negative for the presence of MRSA after direct plating, and the status of only one sample changed to MRSA positive after a 4-h pre-enrichment. In contrast, 14 of these 24 samples (13 samples plus 1 sample already positive after the 4-h treatment) gave positive results after an overnight enrichment, increasing the sensitivity of MRSA detection by 25%. A previous study also showed a 24% increase in the sensitivity of MRSA detection on combining a pre-enrichment step with detection on CHROMagar MRSA (10). Overall, our results did not show any obvious advantage of a 4-h pre-enrichment in comparison to direct sample plating.

Further analysis of the patient screening samples plated directly showed that the average MRSA yields from nasal swabs were sevenfold higher than from groin swabs (Table 2). The average MRSA nasal loads found in the present study (2.59×10^7 CFU/swab) were much higher than those reported in a United States-based study (2.50×10^3 CFU/swab) that also screened patients with known MRSA carriage (8). These variations in the abundance of carriage might in part be related to differences in the colonization potential of the commonly prevalent MRSA clones (11). Interestingly, a majority of the MRSA strains isolated in the present study belonged to sequence type 228-staphylococcal cassette chromosome *mec* I, which was also recently found to be the most persistent and frequently isolated clone from cystic fibrosis patients showing long-term bronchial colonization with MRSA (9). Furthermore, MRSA carriage in 12 of the 26 patients studied here was detected at only one body site, emphasizing the importance of sampling multiple body sites to maximize detection of MRSA carriers.

In conclusion, we show here that a short, 4-h pre-enrichment does not offer any significant advantage for detection of MRSA in comparison to detection with direct plating on chromogenic media and cannot replace an overnight enrichment, at least when culture-based methods are used for downstream processing.

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