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Impact of a short pre-enrichment on detection and bacterial loads of methicillin-resistant *Staphylococcus aureus* from screening specimens

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Abstract

We compared the impact of direct plating on a chromogenic medium to plating after enrichment (4-hour and overnight) on detection of MRSA from 52 patient screening samples. MRSA colony counts for ≈70% samples were >20-fold higher after overnight pre-enrichment in comparison to the previous two time-points. Qualitative differences (sample MRSA positive/negative) were not significant between the direct-plating and 4-hour pre-enrichment time-points, indicating no advantage of the latter, while differences between overnight sample pre-enrichment and the previous two time-points were highly significant ($P < 0.005$, Score test).

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Text

Methicillin-resistant *Staphylococcus aureus* (MRSA) have emerged as major nosocomial pathogens in the last decade. Patients colonized with MRSA serve as reservoirs of self-infection or dissemination to other patients and to the hospital environment (1-3). Hence, screening for MRSA carriage and contact isolation of MRSA carriers is crucial for effective hospital infection control (4). Employing rapid and sensitive screening assays for MRSA detection could help to further improve infection control as well as decrease costs (5,6). While traditional culture-based methods tend to be slow and laborious, the next generation culture methods like the chromogenic media, which allow a direct colony color-based identification of MRSA from the primary culture, can reduce time to result from 48 to18-24 hours (6). Sensitivity of MRSA detection on chromogenic media is further enhanced by utilising an overnight enrichment prior to culture but at the cost of rapidity of the assay (7,8). A short pre-enrichment would enable next day results, however, whether this would offer any advantage in terms of increased sensitivities in comparison to a direct inoculation of media is not very clear. We compared the impact of direct plating on a chromogenic medium to plating after a short (4-hour) or overnight pre-enrichment on MRSA isolation from patient screening samples.

Samples were collected at the University of Geneva Hospital, a 2096-bed tertiary-care hospital, during August 2008 from 26 inpatients with known MRSA carriage detected within six months prior to sampling for this study. A total of 52 nasal and groin samples collected using saline-premoistened swabs were inserted into 1.5 ml of brain heart infusion broth (BHI) with 15% glycerol and immediately frozen at -80ºC till further analysis. Samples were thawed, vortexed for 25 seconds and 10 µl inoculated using a spiral plater (Eddy Jet, The Netherlands) directly on the following media: (i) a chromogenic medium, CHROMagar MRSA (BD Diagnostics, Erembodegem, Belgium), (ii) mannitol-salt agar with 4 µg/ml cefoxitin (MSAC; positive control for MRSA), and (iii) a blood agar plate (general sample control). Also, 10 µl of the sample was added to selective/enrichment broth (Tryptone soya broth with 2.5% salt, 20 µg/ml aztreonam, and 3.5 µg/ml cefoxitin; TSB-AC) (9), and incubated at 37ºC in air. 10 µl of the enriched sample was spiral plated on CHROMagar, MSAC and blood agar after 4

hours and further, after an overnight incubation. All plates were examined after overnight incubation at 37ºC 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), 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 the R statistical computing environment (10). Non-parametric 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 readings (MRSA positive/negative) at the three time-points were modelled using a logistic approach. Generalized estimating equation was used to account for repeated measures over time and the Score test to assess differences between the three time-points.

An intra-sample comparison of MRSA counts obtained on direct plating and after the two enrichments showed that counts at the overnight pre-enrichment time-point were significantly different from the other two time-points (Friedman's chi-square = 56.44, degrees of freedom = 2, $P < 0.0001$) (Table 1A; Figure 1). An overnight pre-enriched sample yielded >20 times more MRSA colonies than if plated directly or after a 4-hour pre-enrichment in approximately 70% of the samples. Nine of the 52 samples did not show presence of MRSA at any of the three time points studied. Identical MRSA counts were obtained for 26 of the 52 (50%) samples on direct plating and after the short pre-enrichment, 10 (19.23%) samples on direct plating and after overnight enrichment, and 11 (21.15%) samples after a short and an overnight pre-enrichment (Figure 2).

Qualitative differences in reading of plates for MRSA positivity after a direct plating or a 4-hour 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 ($P \approx 0.002$) or a short pre-enrichment ($P \approx 0.005$). Twenty-four samples were negative for presence of MRSA on direct plating, and the status of only 1 sample changed to MRSA positive after a 4-hour pre-enrichment. In contrast, 14 of these 24

samples gave positive results after an overnight enrichment, increasing the sensitivity of MRSA detection by 27%. A previous study also showed a 24% increase in sensitivity of MRSA detection on combining a pre-enrichment step with detection on CHROMagar MRSA (11). Overall, our results did not show any obvious advantage of a 4-hour pre-enrichment in comparison to direct sample plating.

Further analysis of the patient screening samples plated directly showed that average MRSA yields from nasal swabs were 7-fold higher than from groin swabs (Table 1B). Average MRSA nasal loads found in the present study (2.59×10^7 cfu/swab) were much higher than those reported by a US-based study (2.50×10^3 cfu/swab) that also screened patients with known MRSA carriage (12). These variations in abundance of carriage might in part be related to differences in colonization potential of the commonly prevalent MRSA clones (13). Furthermore, MRSA carriage in 12 of the 26 patients studied here was detected at only one body site, reiterating the importance of sampling multiple body sites to maximize detection of MRSA carriers.

In conclusion, we show here that a short 4-hour pre-enrichment does not offer any significant advantage for detection of MRSA in comparison to 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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Legends to table and figures

Table 1. MRSA colony counts at various time-points, and in nasal and groin swabs. DP, direct plating; Short-En, 4 hour pre-enrichment; ON-En, overnight pre-enrichment.

Figure 1. Sum of the ranks at each plating time and differences using Friedman's test. DP, direct plating; Short-En, 4 hour pre-enrichment; ON-En, overnight pre-enrichment.

Figure 2. Colony count profiles and trend after direct plating, short and overnight enrichment. DP, direct plating; Short-En, 4 hour pre-enrichment; ON-En, overnight pre-enrichment.

Time-point	CFU/ml*		
	Range	**Average**	**95%CI**
DP	0−3.89 x 10^8	9.84 x 10^6	4.75 x 10^5
Short-En	0−5.19 x 10^8	1.08 x 10^7	6.26 x 10^5
ON-En	0−6.39 x 10^8	7.10 x 10^7	1.28 x 10^6
Time-point/sample	**CFU/swab**		
	Range	**Average**	**95%CI**
DP/all swabs	0−5.84 x 10^8	1.48 x 10^7	7.13 x 10^5
DP/Nasal swab	0−5.84 x 10^8	2.59 x 10^7	1.42 x 10^6
DP/Groin swab	0−7.08 x 10^7	3.60 x 10^6	1.75 x 10^5

*Colony counts are expressed per ml to allow compariso between the three plating time-points

Table 1

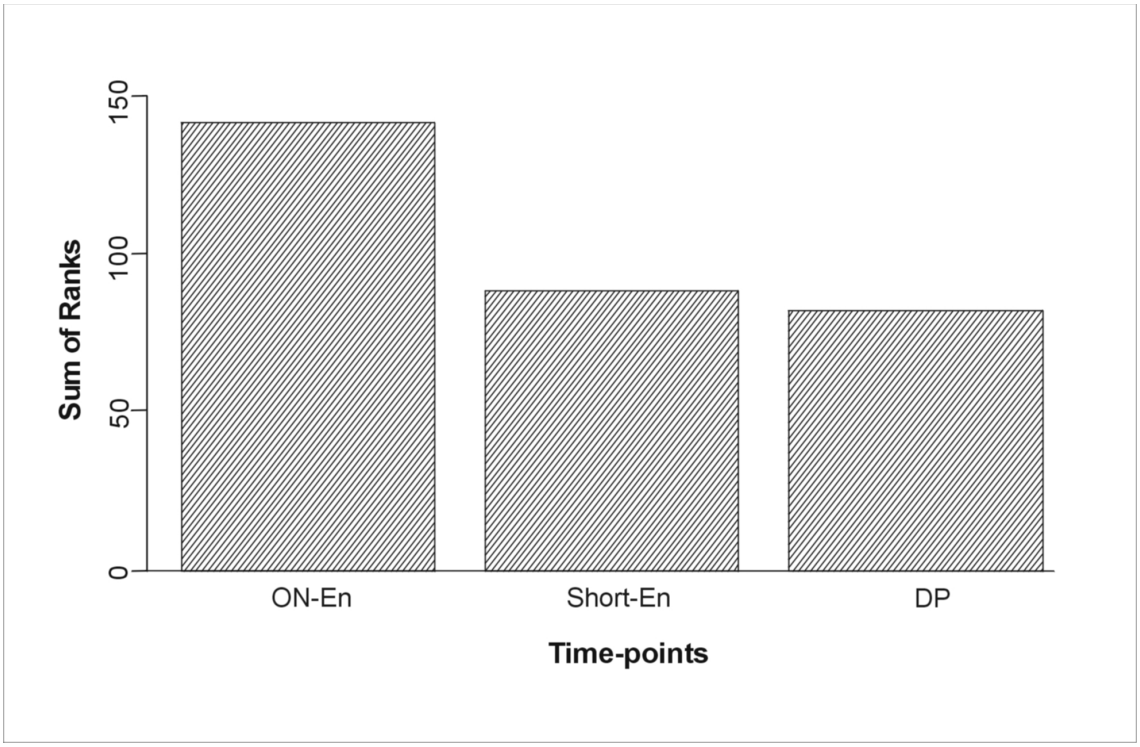

Figure 1

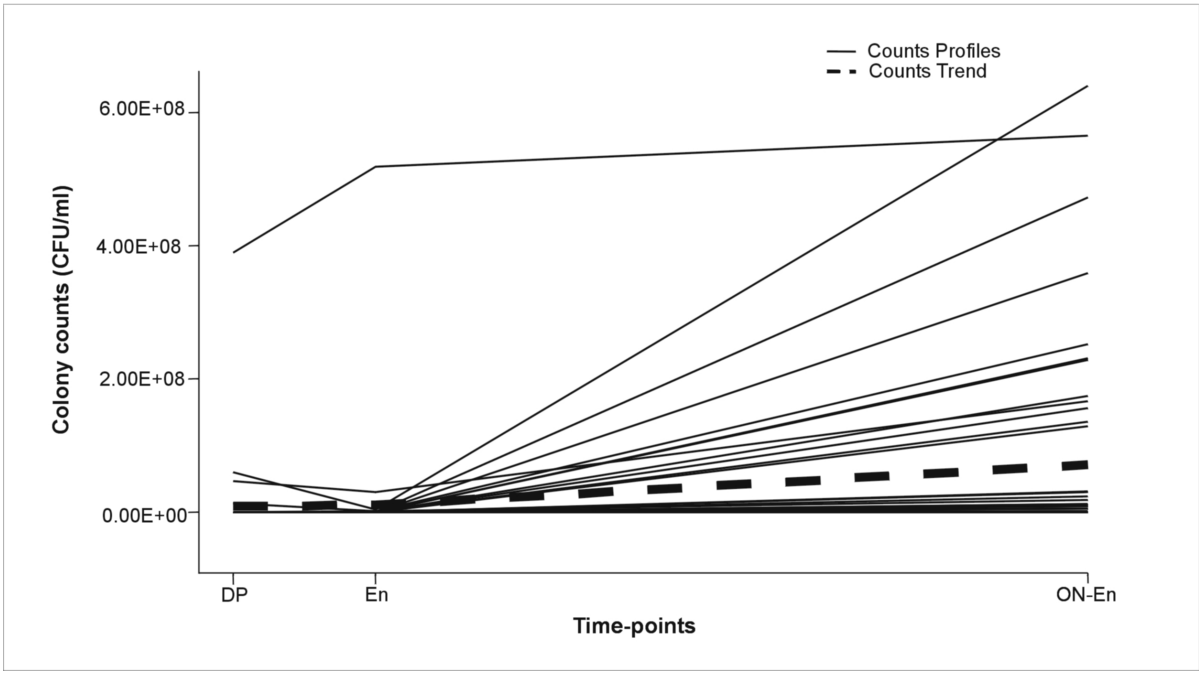
Counts Profiles
Counts Trend
6.00E+08
4.00E+08
2.00E+08
0.00E+00
Colony counts (CFU/ml)
DP
En
ON-En
Time-points

Figure 2