

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

Peer-reviewed author version

Van Heirstraeten, L.; CORTINAS ABRAHANTES, Jose; Lammens, C.; Lee, A.;  
Harbarth, S.; MOLENBERGHS, Geert; AERTS, Marc; Goossens, H. &  
Malhotra-Kumar, S. (2009) Impact of a Short Period of Pre-Enrichment on Detection  
and Bacterial Loads of Methicillin-Resistant *Staphylococcus aureus* from Screening  
Specimens. In: JOURNAL OF CLINICAL MICROBIOLOGY, 47(10). p. 3326-3328.

DOI: 10.1128/JCM.01088-09

Handle: <http://hdl.handle.net/1942/9993>

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

L. Van Heirstraeten<sup>1,3</sup>, J. Cortiñas Abrahantes<sup>2,3</sup>, C. Lammens<sup>1,3</sup>, A. Lee<sup>4</sup>, S. Harbarth<sup>4</sup>, G. Molenberghs<sup>2,3</sup>, M. Aerts<sup>2,3</sup>, H. Goossens<sup>1,3</sup>, S. Malhotra-Kumar<sup>1,3,5\*</sup> on behalf of the MOSAR WP2 study group

<sup>1</sup>University of Antwerp, Antwerp, Belgium, <sup>2</sup>University of Hasselt, Hasselt, Belgium, <sup>3</sup>Vaccine and Infectious Disease Institute, Belgium, <sup>4</sup>University of Geneva, Geneva, Switzerland,

<sup>5</sup>Research Foundation-Flanders (FWO-V), Belgium.

## 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  $\approx 70\%$  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).

\*Corresponding author. Mailing address: Department of Medical Microbiology, Campus Drie Eiken, University of Antwerp, S3, Universiteitsplein 1, B-2610 Wilrijk, Belgium. Phone: 32-3-820-25-51. Fax: 32-3-820-26-63. E-mail: [surbhi.malhotra@ua.ac.be](mailto:surbhi.malhotra@ua.ac.be)

## 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 to 18-24 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 -80°C 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 37°C 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 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), 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 ( $2.59 \times 10^7$  cfu/swab) were much higher than those reported by a US-based study ( $2.50 \times 10^3$  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.

## Reference List

1. **Coello, R., J. R. Glynn, C. Gaspar, J. J. Picazo, and J. Fereres.** 1997. Risk factors for developing clinical infection with methicillin-resistant *Staphylococcus aureus* (MRSA) amongst hospital patients initially only colonized with MRSA. *J.Hosp.Infect.* **37**:39-46.
2. **Rohr, U., A. Kaminski, M. Wilhelm, L. Jurzik, S. Gatermann, and G. Muhr.** 2009. Colonization of patients and contamination of the patients' environment by MRSA under conditions of single-room isolation. *Int.J.Hyg.EnvIRON.Health* **212**:209-215.
3. **Leclercq, R.** 2009. Epidemiological and resistance issues in multidrug-resistant staphylococci and enterococci. *Clin.Microbiol.Infect.* **15**:224-231.
4. **Harbarth, S.** 2006. Control of endemic methicillin-resistant *Staphylococcus aureus*--recent advances and future challenges. *Clin.Microbiol.Infect.* **12**:1154-1162.
5. **Harbarth, S., C. Masuet-Aumatell, J. Schrenzel, P. Francois, C. Akakpo, G. Renzi, J. Pugin, B. Ricou, and D. Pittet.** 2006. Evaluation of rapid screening and pre-emptive contact isolation for detecting and controlling methicillin-resistant *Staphylococcus aureus* in critical care: an interventional cohort study. *Crit Care* **10**:R25.
6. **Malhotra-Kumar, S., K. Haccuria, M. Michiels, M. Ieven, C. Poyart, W. Hryniewicz, and H. Goossens.** 2008. Current trends in rapid diagnostics for methicillin-resistant *Staphylococcus aureus* and glycopeptide-resistant enterococcus species. *J.Clin.Microbiol.* **46**:1577-1587.
7. **Ieven, M., Michiels, M, Jansens, H, Breughelmans, J, and Goossens H.** 2007. MRSASelect applied on enrichment broths significantly increases the sensitivity of MRSA detection in screening samples. 2007. 17th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), March 31-April 3, 2007.
8. **van, L., I, D. S. van, I. Verbakel-Schelle, and A. G. Buiting.** 2007. Evaluation of a chromogenic agar (MRSASelect) for the detection of methicillin-resistant *Staphylococcus aureus* with clinical samples in The Netherlands. *J.Med.Microbiol.* **56**:491-494.
9. **Bocher, S., R. Smyth, G. Kahlmeter, J. Kerremans, M. C. Vos, and R. Skov.** 2008. Evaluation of four selective agars and two enrichment broths in screening for methicillin-resistant *Staphylococcus aureus*. *J.Clin.Microbiol.* **46**:3136-3138.

10. **Ihaka R and Gentleman R. R.** 1996. A Language for data analysis and graphics. *Journal of Computational and Graphical Statistics* 5, 299-314.
11. **Nahimana, I., P. Francioli, and D. S. Blanc.** 2006. Evaluation of three chromogenic media (MRSA-ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus*. *Clin.Microbiol.Infect.* **12**:1168-1174.
12. **Mermel L, Lonks J, Gordon S, Perl T, Fadem S, Muralidhar A, Dacus D, Maxey GE, Cartony JM, Morse D, and Mach P.** 2008. Quantitative Analysis Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Clinical Nasal Swab Samples Collected From US Patient Populations. Society for Healthcare Epidemiology of America (SHEA) 18th Annual Scientific Meeting Orlando, Florida.
13. **Peacock, S. J., S. de, I, and F. D. Lowy.** 2001. What determines nasal carriage of *Staphylococcus aureus*? *Trends Microbiol.* **9**:605-610.

## Acknowledgements

Financial support: LVH and this work are supported by funding from the European Community (MOSAR network contract LSHP-CT-2007-037941; TheraEDGE network contract FP7-216027). SM-K is funded by the Research Foundation-Flanders (FWO-V), Belgium. GM, MA, and JCA gratefully acknowledge support from FWO-V, Research Grant G.0151.05, and Belgian IUAP/PAI network P6/03 "Statistical Techniques and Modelling for Complex Substantive Questions with Complex Data" of the Belgian Government (Belgian Science Policy).

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

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

Table 1

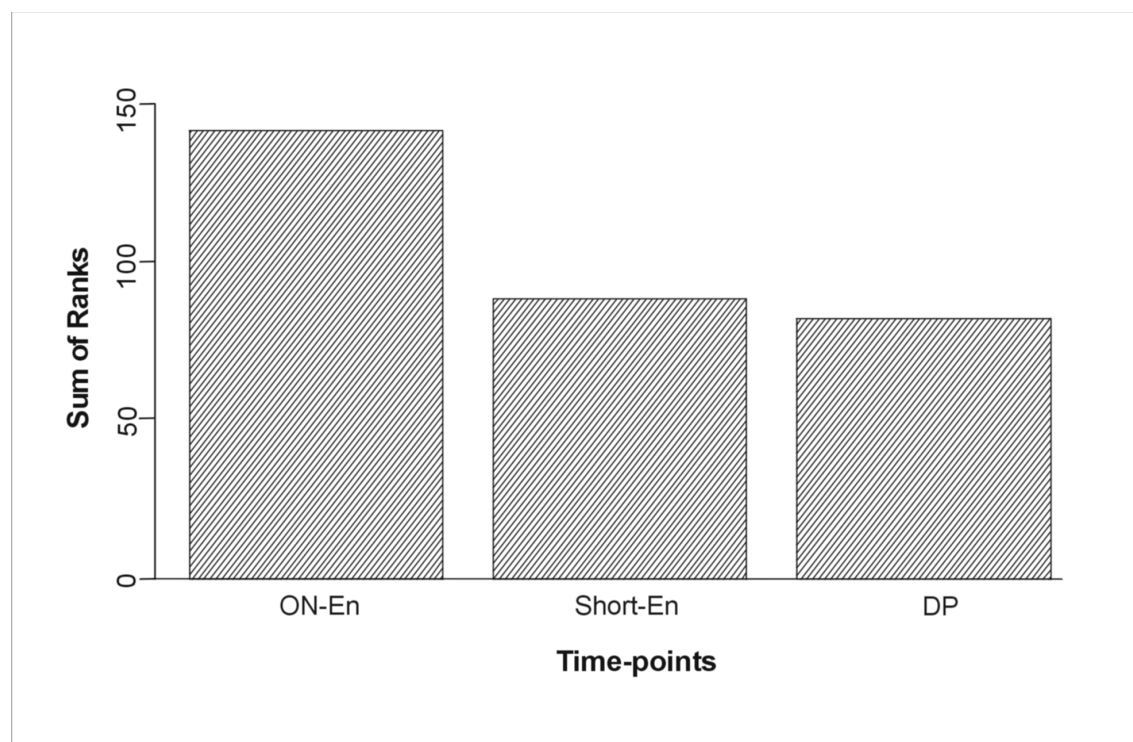


Figure 1

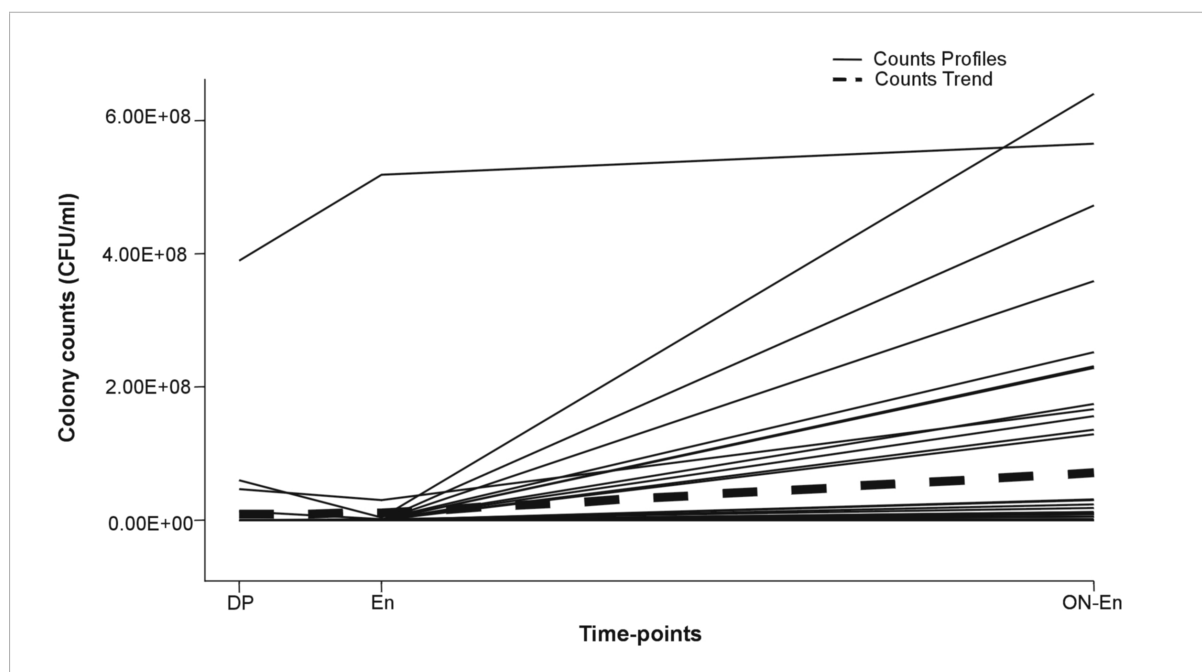


Figure 2