Complete MRSA Nasal Screening Using a Single, New and Novel Swab Transport System

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AMENDED ABSTRACT:

A new patented Flocked Swab with Liquid Amies, Copan, Ca, USA (ESwab™) was designed to improve the sensitivity of the traditional transport swab systems. In this new swab the organism inoculum theoretically is released into 1mL of Amies Liquid and after that it should be possible to perform different tests on the collected sample. The purpose of this study was to evaluate the Eswab™ for MRSA nasal screening.

Methods: A total of 30 patients from a Nephrology Unit of Hospital Sao Paulo (Sao Paulo, Brazil) were screened for MRSA colonization. The samples were collected using one Eswab™ per patient and they were separated in two different groups (15 samples in each group). After swabbing the anterior nares, all the swabs were vortexed in the Liquid Amies medium and from the first group of samples three aliquots of 200*µ*l were collected from the medium and tested. For direct culture, the 200*µ*l aliquot was inoculated directly onto MRSA screening medium agar plate (Probac, Brazil) and incubated at 35°C for 24-48h. The other 200*µ*l aliquot was first cultured in a pre-enrichment broth medium for 24h at 35°C and then cultured onto MRSA screening medium agar for another 24-48h at 35°C. The third aliquot was used to screen MRSA by PCR. The DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Germany), followed by an *in house mec*A PCR protocol. *S. aureus* ATCC 43300 and two MRSA clinical strains previously identified were used as positive controls. On the second group of samples, after vortexing, the swab was removed to the enrichment broth. An aliquot of 200*µ*l of the medium was also removed for the PCR and another aliquot of 700*µ*l was centrifuged. The pellets were reconstituted in 100*µ*l of distillated water and the whole volume was cultured onto a selective plate for direct culture.

Results: Among the 15 patients screened on the first group, 12 were MRSA-colonized. Both, pre-enrichment culture and PCR were concordant in 100% of the cases. In contrast, only four out of 12 patients were positive by direct culture. On the second group, 13 were MRSA colonized with 100% concordance between pre-enrichment culture and PCR results. Eleven out of these 13 were also positive by direct culture. In both groups, results from PCR were obtained after 6h, while from cultures were obtained after 48-72h.

Conclusion: The new Eswab™ proved to be excellent for a complete MRSA nasal screening. It was possible to perform from a single swab three different tests and have the first results faster than by conventional tests. Although direct culture were less sensitive than the others two tests, we were able to improve the sensitivity of this test by increasing the initial volume inoculated.

INTRODUCTION:

Staphylococcus aureus is a leading cause of bloodstream and other invasive infections and has become increasingly resistant to first-line antimicrobial agents in health-care settings. Since the introduction of methicillin into clinical use, methicillin-resistant *S. aureus* (MRSA) strains have emerged worldwide as important nosocomial pathogen, and the prevalence of these strains in the community is now increasing substantially.

The rapid and accurate identification of MRSA in clinical specimens has important implications for the therapy and management of both colonized and infected patients. Numerous molecular approaches that reduce the time for identification of MRSA has been described. However, none of them had presented a system to collect the clinical samples that enable molecular and traditional culture tests.

A new Flocked Swab with Liquid Amies, Copan, Ca, USA (ESwab™) was designed to improve the sensitivity of the traditional transport swab systems. In this new swab the organism inoculum theoretically is released into 1mL of Amies Liquid and after that it should be possible to perform different tests on the collected sample. The purpose of this study was to evaluate the ESwab™ for a complete MRSA nasal screening.

MATERIAL AND METHODS:

A total of 30 patients from a Nephrology Unit of Hospital Sao Paulo (Brazil) were screened for MRSA colonization. The samples were collected using one Eswab™ per patient and they were separated in two different groups (15 samples in each group). After swabbing the anterior nares, all the swabs were vortexed in the Liquid Amies medium.

FIRST GROUP:

Three aliquots of 200µl were collected from the Liquid Amies medium, after vortexing with the swab.

- **Direct culture:** the 200µl aliquot was inoculated directly onto MRSA screening medium agar plate (Probac, Sao Paulo, Brazil) and incubated at 35°C for 24-48h.
- **Pre-enrichment culture:** another 200µl aliquot was first cultured in an enrichment broth medium for 24h at 35°C and then cultured onto MRSA screening medium agar for another 24-48h at 35°C.

PCR: the third aliquot was used to screen MRSA by PCR. The DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Germany), followed by an in house mecA PCR protocol. S. aureus ATCC 43300 and two MRSA clinical strains previously identified were used as positive controls.

MATERIAL AND METHODS

SECOND GROUP:

Eswab™ tubes were first vortexed with the swab inside

- **Pre-enrichment culture:** after vortexing, the swab was removed and placed into enrichment broth medium for 24h at 35°C and then cultured onto MRSA screening medium agar for another 24-48h at 35°C.
- **PCR:** an aliquot of 200ul of the medium was also removed to screen MRSA by PCR. The DNA extraction was performed using QIAamp DNA Mini Kit, followed by an in house mecA PCR protocol. S. aureus ATCC 43300 and two MRSA clinical strains previously identified were used as positive controls.
- **Direct culture:** the remaining volume of Liquid Amies medium (approximately 700µl) was centrifuged and the pellets were reconstituted in 100µl of distillated water. This whole re-suspended pellet was inoculated directly onto MRSA screening medium agar plate and incubated at 35°C for 24-48h.

New Swab Designed:

- Flocked Swab with Liquid Amies (Eswab™ - Copan, Ca, USA)

MecA PCR Protocol:

- DNA extraction: QIAamp DNA Mini Kit (Qiagen, Germany).

PCR:

• Go Taq Green Master Mix (Promega) 2x	10µM
• Primer F (CTGGAACTTGTTGAGCAGAG)	1µL
• Primer R (TGGCTATCGTGTCACAATCG)	1µL
• H2O	7µL
• Template	1µL
• Final volume	20µL

Cycling:

- 95°C 5 min

35 cycles:

- 95°C 1 min
- 53°C 1 min
- 72°C 1 min

Final:

- 72°C 10 min

PCR products are visualized by electrophoresis using:
Agarose gel 1,5% (45mL of gel + 2µL of ethidium bromide); 100V, 50mA, 20 min.

RESULTS:

Table 1: Results from the 15 nasal samples tested on the first group				
Sample	**Direct Culture CFU / mL**	**Pre-enrichment culture CFU / mL**	**PCR**	**Biochemical identification**
1	0	>300	+	*S. aureus*
2	>300	>300	+	*S. aureus*
3	0	0	-	-
4	0	>300	+	*S. aureus*
5	>300	>300	+	*S. aureus*
6	0	0	-	-
7	>300	>300	+	*S. aureus*
8	>300	>300	+	*S. aureus*
9	0	>300	+	*S. aureus*
10	0	>300	+	*S. aureus*
11	0	>300	+	*S. aureus*
12	0	>300	+	*S. aureus*
13	0	>300	+	*S. aureus*
14	0	>300	+	*S. aureus*
15	0	0	-	-

Table 2: Results from the 15 nasal samples tested on the second group				
Sample	**Direct Culture CFU / mL**	**Pre-enrichment culture CFU / mL**	**PCR**	**Biochemical identification**
1	0	0	-	-
2	7	>300	+	CNS
3	>300	>300	+	*S. aureus*
4	>300	>300	+	*S. aureus*
5	0	0	-	-
6	56	>300	+	*S. aureus*
7	0	>300	+	*S. aureus*
8	0	58	+	*S. aureus*
9	>300	>300	+	*S. aureus*
10	46	>300	+	*S. aureus*
11	3	>300	+	CNS
12	88	>300	+	CNS
13	13	>300	+	CNS
14	>300	>300	+	*S. aureus*
15	22	>300	+	*S. aureus*

CNS, coagulase-negative *Staphylococcus*.

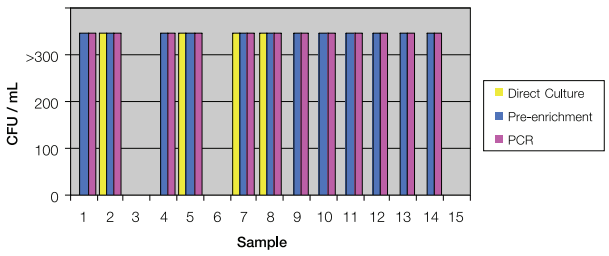


Figure 1: Graphic results from the 15 nasal samples tested on the first group.

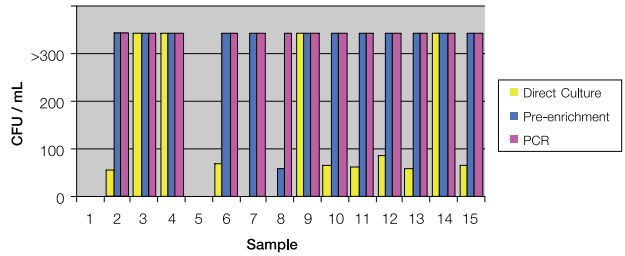
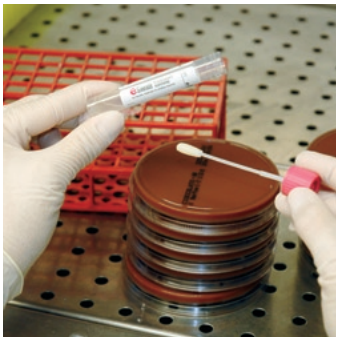
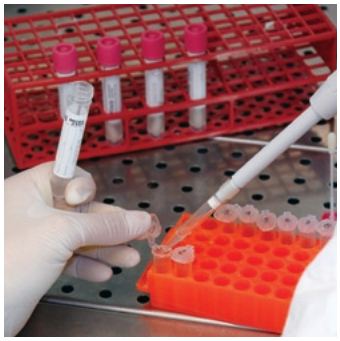


Figure 2: Graphic results from the 15 nasal samples tested on the second group.





CONCLUSIONS:

1. The results presented demonstrated the possibility to perform from a single swab three different tests;
2. Results from PCR were faster (6h) than results obtained from cultures (48-72h).
3. PCR and pre-enrichement culture results had 100% of concordance;
4. Although direct culture were less sensitive than the others two tests, it was possible to improve the sensitivity of this test by increasing the initial volume inoculated.
5. The new Eswab™ proved to be an excellent choice for a complete MRSA nasal screening;
6. Certainly this new design of swab will allow the microbiologist to perform different laboratory tests from a single sample collected.