C-049

Screening of MRSA: A Comparison between One and Multiple Sites Sample Collection Using the New ESwab MRSA Collection Kit™

Suzane Silbert, Liana Carballo Menezes, Jussimara Monteiro, Martha Gabriela Celle Ribeiro, Susy Cristine Pereira, Antônio Carlos C. Pignatari
Special Clinical Microbiology Laboratory (LEMC), Federal University of São Paulo/UNIFESP, São Paulo, Brazil

ABSTRACT:

Background: A new ESwab MRSA Collection Kit™ (Copan, USA) was developed to collect and transport clinical specimens for the isolation of MRSA. This kit comprises of three regular size flocked swabs that allow collecting up to three specimens from different sites of the same patient and a tube with 1ml modified liquid Amies medium for the pooling of samples. The aims of this study were to evaluate the new ESwab MRSA Collection Kit™ for MRSA screening and to compare one site versus multiple sites sample collection for the screening of MRSA.

Methods: A total of 20 patients and 10 healthcare professionals from Hospital São Paulo (Brazil) were screened for MRSA colonization. The samples were collected from each patient/healthcare professional, using two different methods: 1) Single site collection (anterior nares) using one Amies Agar Gel swab (Copan, USA) and 2) Multiple sites collection (anterior nares, throat, axilla) using the ESwab MRSA Collection Kit™. After swabbing the patients, the Amies Agar Gel swabs were vortexed in saline while the 3 ESwabs from each patient were vortexed in the same Liquid Amies medium, resulting in a pool of samples from the same patient. Three aliquots of 200µl were collected from each medium and tested for MRSA by three different methods: real time PCR, direct culture using a CHROMagar™ MRSA agar plate (Paris, France) and pre-enrichment culture using TSB broth medium and a CHROMagar™ MRSA agar plate. Two MRSA clones previously identified and two ATCCs strains were used as controls.

Results: Among the 20 patients and 10 healthcare professionals collected, nine were MRSA-positive. Results from both collection methods and from the three MRSA isolation tests were concordant in 100% of the cases.

Conclusion: Our study corroborates the literature by proving that the essential site to sample for MRSA screening is the anterior nares. On the other hand it is common practice in countries outside of the USA to collect samples from multiple sites. For this purpose the new ESwab MRSA Collection Kit™ proved to be an excellent choice for MRSA screening, allowing the pool of multiple specimens, limiting the test costs and at the same time, broadening the screening capabilities.

INTRODUCTION:

The transmission of MRSA and the risk of MRSA infection can only be addressed effectively if measures are taken to identify MRSA carriers as potential sources and treating them to reduce the risk of transmission. This requires screening of patient populations for MRSA carriage either before or on admission, to identify carriers and implement a decolonization regimen.

The normal habitat of *S. aureus*, including MRSA, is human skin, particularly in the anterior nares, axilla, throat and perineum. Clinical infection with MRSA occurs either from the patient's own resident MRSA or by cross-infection from another person, who could be an asymptomatic carrier or have a clinical infection. Patients with a clinical infection caused by MRSA should, where possible, be cared for in a single-room isolation to minimize the risk of transmission.

The logical conclusion of risk factor assessments and the results of modeling studies is that the most appropriate approach to the reduction in MRSA carriage in the population, and resultant MRSA infections, is the universal screening of all admissions to hospital.

The essential patient site to sample is the anterior nares, as this is the most common carriage site for MRSA. Most patients positive at other sites have positive results from nose samples; However, a small proportion do not. For this reason, countries outside USA are recommending screening all patients admitted to the hospital by swabbing three different sites of the patient. This clearly had major resource implications for initial investment, mainly in terms of laboratory staff time and high costs of the laboratory tests.

In order to reduce these laboratory costs and follow the MRSA screening recommendations, a new ESwab MRSA Collection Kit™ (Copan, USA) was developed. This kit comprises of three regular size flocked swabs that allow collecting up to three specimens from different sites of the same patient and, a tube with 1ml modified liquid Amies medium for the pooling of samples.

The aims of this study were to evaluate the new ESwab MRSA Collection Kit™ for MRSA screening and to compare one site versus multiple sites sample collection for the screening of MRSA.

METHODS:

A total of 20 patients and 10 healthcare professionals from Hospital São Paulo (Brazil) were screened for MRSA colonization. The samples were collected from each patient/healthcare professional, using two different methods:

1. SINGLE SITE COLLECTION (anterior nares):

- 108C Amies Agar Gel Traditional Swab (Copan, USA)
- After swabbing the patients, the Amies Agar Gel Traditional Swab was vortexed in saline

2. MULTIPLE SITES COLLECTION (anterior nares, throat, axilla)

- ESwab MRSA Collection Kit™ (Copan, USA)
- After swabbing the patients, the three ESwabs from each patient were vortexed in the same Liquid Amies medium, resulting in a pool of samples from the same patient.

MRSA TESTS:

Three different tests were performed to detect MRSA. For all tests, the following strains were used as controls: *S. aureus* strain ATCC 43300, *S. aureus* NewYork-Japan clone, *S. aureus* Brazilian clone and *S. aureus* strain ATCC 25923.

First, an aliquot of 200µl were removed from each medium (Saline and Liquid Amies) and saved for the PCR Real Time MRSA protocol.

The remaining volume of each medium (approximately 700µl) was centrifuged and the pellets were reconstituted in 500µl of saline for direct and pre-enrichment culture.

1. DIRECT CULTURE:

- An aliquot of 200µl was inoculated directly onto a CHROMagar™ MRSA agar plate (Paris, France) and incubated at 35°C for 24-48h

2. PRE-ENRICHMENT CULTURE:

- Another 200µl aliquot was first cultured in an enrichment broth medium for 24h at 35°C and then cultured onto a CHROMagar™ MRSA agar plate for another 24-48h at 35°C.

Strains were identify as *S. aureus* when the colonies on CHROMagar™ MRSA agar plate were mauve. Identification from both, direct and pre-enrichment cultures, was confirmed using the Sthapy Test Kit (Probac, Brazil).

3. REAL TIME PCR FOR *nuc* AND *mecA* GENES:

The first 200µl aliquot removed from the medium was separated for Real Time PCR

The DNA extraction was performed using QIAamp DNA Mini Kit (QIAGEN, Germany), followed by an in house *nuc* and *mecA* Real Time PCR protocol.

16S rRNA gene was used as a internal control.

Primers for *nuc* gene (specific for *S. aures* strains):

R-5' GCCACGTCCATATTTATCAG 3'

F-5' TATGGTCCTGAAGCAAGTG 3'

Primers for *mecA* gene (methicilin resistant gene):

F-5' CTGGAACTTGTTGAGCAGAG 3'

R-5' TGGCTATCGTGTCACAATCG 3'

The Real Time PCR analysis were performed using the PCR SYBR Green Kit (QIAGEN, Germany) and the 7500 Real Time PCR System (Applied Biosystems, CA).

Cycling:

50°C - 2 min

95°C - 10 min

45 cycles:

95°C - 15 sec

60°C - 1 min

RESULTS:

TABLE 1.

Results of the samples collected from 30 patients using the ESwab MRSA kit and the 108C Amies Gel Traditional Swab

	ESwab MRSA Kit							108C Amies Agar Gel						
Sample	Direct Culture CFU /mL	Pre-enrichment culture CFU/mL	PCR for *mecA* gene	PCR for *nuc* gene	PCR 16S rRNA gene	Genotype Identification	CHROMagar Identification	Direct Culture CFU/mL	Pre-enrichment culture CFU/mL	PCR for *mecA* gene	PCR for *nuc* gene	PCR 16S rRNA gene	Genotype Identification	CHROMagar Identification
1	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S.aureus*	*S.aureus*
2	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
3	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
4	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S.aureus*	*S.aureus*
5	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
6	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
7	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
8	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
9	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
10	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
11	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
12	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	240	>300	positive	positive	positive	*S. aureus*	*S. aureus*
13	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
14	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
15	0	>300	positive	negative	positive	CNS	CNS	0	>300	positive	negative	positive	CNS	CNS
16	0	>300	positive	negative	positive	CNS	CNS	0	>300	positive	negative	positive	CNS	CNS
17	>300	>300	positive	negative	positive	CNS	CNS	32	>300	positive	negative	positive	CNS	CNS
18	0	>300	positive	negative	positive	CNS	CNS	0	>300	positive	negative	positive	CNS	CNS
19	0	>300	positive	negative	positive	CNS	CNS	0	>300	positive	negative	positive	CNS	CNS
20	0	0	negative	negative	positive	-	-	0	0	negative	negative	positive	-	-
21	0	0	negative	negative	positive	-	-	0	0	negative	negative	positive	-	-
22	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
23	>300	>300	positive	negative	positive	CNS	CNS	80	>300	positive	negative	positive	CNS	CNS
24	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
25	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
26	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
27	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
28	>300	>300	positive	negative	positive	CNS	CNS	40	>300	positive	negative	positive	CNS	CNS
29	>300	>300	positive	negative	positive	CNS	CNS	>300	>300	positive	negative	positive	CNS	CNS
30	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
ATCC 43300	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
ATCC 25923	0	0	negative	positive	positive	-	-	0	0	negative	positive	positive	-	-
Negative Control	0	0	negative	negative	negative	-	-	0	0	negative	negative	negative	-	-
S. aureus clone New York-Japan	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*
S. aureus clone Brazilian	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*	>300	>300	positive	positive	positive	*S. aureus*	*S. aureus*

*CNS: Coagulase Negative *Staphylococcus*

CONCLUSIONS:

1. Among the 20 patients and 10 healthcare professionals collected, nine were MRSA-positive.
2. Results from both collection methods and from the three MRSA detection tests were concordant in 100% of the cases.
3. Real Time PCR and CHROMagar™ MRSA agar plate were excellent methods for MRSA identification.
4. Our study corroborates the literature by proving that the essential site to sample for MRSA screening is the anterior nares. However, more samples should be tested for further conclusions.
5. It is common practice in countries outside of the USA to collect samples from multiple sites. For this purpose the new ESwab MRSA Collection Kit™ proved to be an excellent choice for MRSA screening, allowing the pool of multiple specimens, limiting the test costs and at the same time broadening the screening capabilities.