Poster Number

355/79

PARIS 2004

Detection of nasal colonization methicillin-resistant *Staphylococcus aureus* (MRSA): a prospective study comparing IDI-MRSA™ real-time PCR assay *versus* chromogenic CHROMagar™ MRSA and ORSAB media.

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BACKGROUND

Nosocomial infections caused by MRSA increase the lengh of hospital stay, are responsible for rising health care costs and have a high attribution mortality. Reliable and rapid detection of MRSA carriage is essential as part of epidemiologic investigation but is also necessary for the prompt implementation of barrier isolation of colonized patients. In contrast to « classical » PCR, real-time PCR can be performed in every laboratory without dedicated clean area. Moreover, real-time PCR allows an early detection of MRSA colonization, 2 hours compared to 1 or 2 days for culture and represents a rapid and powerful tool which can be used for the prevention of the spread of this antibiotic resistant bacteria in healthcare setting.

OBJECTIVES

In order to evaluate and to assess the feasibility of the IDI-MRSA™ real-time PCR amplification assay on the Smartcycler, a prospective study has been initiated in routine since June 2004 at Saint-Joseph Hospital in hospitalized patients at high risk for MRSA carriage : nasal swabs were taken from patients entering the ICU, vascular surgery, diabetology and geriatry wards during a 4 months period.

METHODS

Swabs were carefully inserted into each nostril so that the tip is entirely at the nasal ostium level (about 2.5cm from the edge of the nare) and gently rolled 5 times.

Real time PCR: We used IDI-MRSA™ Assay based on the detection of an *S. aureus*-specific target and the *mecA* gene. In specimens containing MRSA, amplification of the target (sequence near the insertion site of Staphyloccocal Cassette Chromosome mec (SCCmec) will occur. Amplification of the internal control, a DNA fragment of 335-bp including a 277-bp sequence not found in MRSA, take place unless there are PCR inhibitory subtances. In case of unresolved assay, we repeated testing with frozen lysates of the specimens.

Chromogenic media:

1.CHROMagar™ MRSA was provided by the CHROMagar Microbiology (Paris, France).
2.ORSAB was provided by Oxoid (Dardilly, France).
3.MRSA*Select* was provided for by Bio-Rad (Marnes la Coquette, France).

To optimise and increase the sensitivity a broth preenrichment (2 hours) before plating on solid media has been undertaken for all specimens.

The criteria for presumptive identification of *Staphylococcus aureus* were defined as follows:

- on CHROMagar™MRSA, well-separated mauve colonies after 24h of incubation without further testing.
- on ORSA, blue colonies after 24 or 48h of incubation & positive with a latex agglutination assay or a tube coagulase test.
- on MRA*Select*, well-separated pink colonies after 24h of incubation without further testing. The evaluation of this media began in the middle of August, so only 443 MRSA*Select* were tested.

MRSA isolates were confirmed by standard antibiogram and identification.

A true MRSA culture positive specimen was defined as a specimen where MRSA was identified by any of the culture techniques used.

In case of positive PCR and negative culture, a broth enrichment (24h) before plating on solid media has been undertaken.

RESULTS

682 specimens have been obtained from 508 patients.

Amplification failure was observed initially for 82 (12%) specimens; after retesting, 44 (6.4%) were considered as failure while 5 were true positive and 33 true negative.

Table 1. Results obtained with IDI-MRSA™ assay in reference to the culture techniques

		IDI- MRSA™		
		Positive	Negative	Total
CULTURE TECHNIQUES	Positive	45	2	47
	Negative	19	572	591
	Total	64	574	638

Clinical sensitivity and specificity

64 (9.3%) specimens were positive by PCR and selective agar, 19 (2.9%) were positive by PCR only (3 of these patients were under antibiotic treatment). 572 specimens remained negative by both methods.

The sensitivity and specificity of this assay were 95.7 % and 96.8% respectively with a positive predictive value of 70.3% and negative predictive value of 96.6 %.

RESULTS

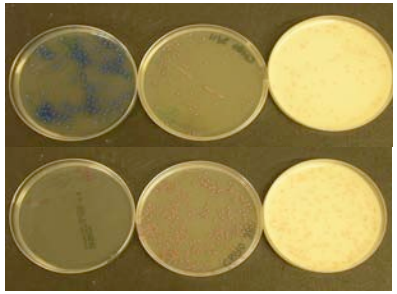
Table 2. Comparison for the positive cultures between CHROMagar™ MRSA, ORSAB and MRSA*Select* media

Media	ORSAB	CHROMagar ™ MRSA	MRSA*Select*
Number of tests	N = 682	N = 682	N = 443
Positive in 24h	20	38	25
Positive in 48h	14	1	0
Positive after 24h broth enrichment	3	3	3
Negative	8	3	1
Total	45 (7%)	45 (7%)	29 (7%)

CHROMagar™ MRSA and MRSA*Select* allowed a much better detection of MRSA than ORSAB medium within 24h. Furthermore, CHROMagar™MRSA and MRSA*Select* are more accurate than ORSAB because of a significantly higher specificity.

Homogeneous MRSA strain after 24 hours

Heterogeneous MRSA strain after 24 hours



ORSAB CHROMagar™ MRSA MRSA *Select*

Table 3. PCR failure and swab

	IDI- MRSA™					
	Negative	Positive	Failure then negative	Failure then positive	Failure 2 fold	N (682)
Copan Venturi transystem ®	182	19 (8.6%)	12	0	8 (3.9%)	221
Conventionnal swab	360	39 (8.4%)	21	5	36 (8%)	461

No difference for positive results was observed using conventional swab or Copan Venturi Transsystem but a significant failures (2 fold) were found with conventional swab. So, to limit unresolved assay, we strongly recommanded as the manufacturer do the use of dedicated swab.

Copan Venturi Transsystem ®

Conventional swab

DISCUSSION- CONCLUSION

In this study, the positive predictive value was 70.3%. However, 19 samples from 17 patients were found to be PCR-positive and culture- negative. Further studies are in progress (sequencing on PCR products) in order to determine these 19 « false positive » are true positive increasing significantly the positive predictive value of the PCR. These « false positive » could be for example related to patients treated with antibiotics.

•These premilary results obtained by the early real-time PCR for the detection of MRSA are promising. Despite 6.4% (44/682) amplification failure, we consider that IDI-MRSA™ real-time PCR assay with an excellent sensitivity represents at Saint-Joseph hospital a significant breakthrough in the detection of MRSA colonization.