

EVALUATION OF “CHROMagar MRSA” FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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BACKGROUND

Methicillin-resistant *S. aureus* (MRSA) has emerged as a nosocomial pathogen of major worldwide importance and is an increasingly frequent cause of community-acquired infections. The main problem with MRSA is that they are resistant to all beta-lactam antibiotics and are usually co-resistant to other antibiotics too, limiting therapeutic options. The early isolation and identification of MRSA is essential for appropriate patient care. Methods used to detect MRSA in clinical samples ideally should be highly sensitive and specific and should report the results within a short time. “CHROMagar MRSA” (CHROMagar Microbiology, France) is a new chromogenic medium specially designed for the isolation and identification of MRSA. The composition of the chromogenic and selective mix is proprietary.

OBJECTIVE

The purpose of the present study was to evaluate the performance of “CHROMagar MRSA” in detecting MRSA in a well-defined collection of *S. aureus* and coagulase-negative staphylococci.

MATERIALS AND METHODS

We studied a total of 254 *Staphylococcus* spp.: 154 *S. aureus* (SAU) and 100 coagulase-negative staphylococci (CNS). The strains were identified to the species level by standard biochemical methods (Kloos W. E. et al., 1999, *Manual of Clinical Microbiology*, 7th ed. ASM). The isolates were collected between 1993 and 2004 from patients of 25 hospitals and 5 cities, and are part of the *Staphylococcus* collection of the National Institute of Infectious Diseases “Dr. Carlos Malbrán”, Argentina. Only one isolate per patient was included.

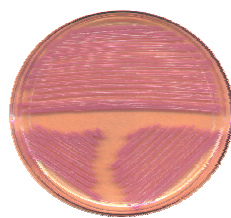
Of the 154 SAU, 80 were *mecA*-positive and 74 *mecA*-negative. Coagulase-negative staphylococci included 54 *mecA*-positive and 46 *mecA*-negative, representing nine species: 49 *S. epidermidis*, 14 *S. saprophyticus*, 13 *S. hominis*, 7 *S. haemolyticus*, 6 *S. simulans*, 4 *S. auricularis*, 3 *S. capitis*, 2 *S. cohnii* and 2 *S. warneri*.

PCR for *mecA* gene was made according previously described (J. Clin. Microbiol. 1995. 33: 2864-7) and used as reference method. Disk diffusion for oxacillin and cefoxitin and MIC for oxacillin (agar dilution method), were determined per CLSI guidelines (M2-A8 and M7-A6).

The “CHROMagar MRSA” medium was performed as recommended by the manufacturer. Isolates were inoculated on tryptic soy agar with 5% sheep blood and incubated for 24 h at 35°C. From the resulting cultures, isolates were streaked on a “CHROMagar MRSA” plate and incubated for 24 and 48 h at 35°C in darkness. Colonies showing mauve or pink color on “CHROMagar MRSA” medium were considered as positive, indicating MRSA. Absence of growing, colorless colonies or growing with a different color to mauve/pink were considered as a negative result, indicating methicillin-susceptible SAU (MSSA), methicillin-resistant CNS or methicillin-susceptible CNS.

RESULTS

FIGURE 1.
S. aureus mecA (+)



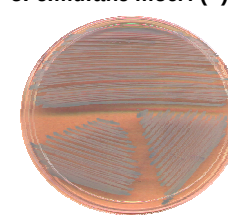
Mauve colonies

FIGURE 2.
S. epidermidis mecA (+)



Colorless colonies
(mauve background at the inoculum site)

FIGURE 3.
S. simulans mecA (+)



Grey colonies

TABLE 1. Results obtained with "CHROMagar MRSA" after 24 h of incubation

Color appearance of 254 staphylococci isolates streaked on "CHROMagar MRSA"				
Organism		n	Color of isolated colonies*	n
<i>S. aureus</i>	<i>mecA</i> +	80	mauve	80
	<i>mecA</i> -	74	absence of growth	70
			mauve	2
			pink	2
Coagulase-negative staphylococci	<i>mecA</i> +	54	colorless**	31
			colorless	7
			beige	13
			grey	3
	<i>mecA</i> -	46	absence of growth	46

* After 24 h of incubation at 35°C.

** mauve background at the inoculum site

- All 80 MRSA grew as mauve colonies (Figure 1)
- Most of MSSA (70/74) did not grow on the medium, but four strains gave false positive results and grew as mauve or pink colonies
- Methicillin-resistant CNS grew as colorless (Figure 2), beige or grey (Figure 3) colonies
- Methicillin-susceptible CNS did not grow on the plates
- None CNS grew as pink or mauve colonies

• Sensitivity (a) , specificity (b), positive (c) and negative (d) predictive values to detect MRSA at 24 h. from the total sample (SAU + CNS) or SAU alone, are listed in TABLE 2.

TABLE 2

CHROMagar MRSA at 24 h.				
	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
SAU	80/80 (100%)	70/74 (95%)	80/84 (95,2%)	74/74 (100%)
SAU + CNS		170/174 (97,7%)		174/174 (100%)

(a) [Number of positive results/number of true positive results] X 100

(b) [Number of negative results/number of true negative results] X 100

(c) [Number of true positive results/(number of true positive results + number of false positive results)] X 100

(d) [Number of true negative results/(number of true negative results + number of false negative results)] X 100

• Specificity to detect MRSA among SAU+CNS or SAU at 48 hs decreased to 94.2, and 86.5%, respectively. The sensitivity remained 100%.

• Strains producing false-positive results on "CHROMagar MRSA" at 24 and 48h are listed in TABLE 3.

• All false positive results were related to *mecA*-negative SAU (MSSA) that grew as pink or mauve color

TABLE 3

Strains producing false-positive results on "CHROMagar MRSA" after 24 and 48 h of incubation									
Organism	Nro.	<i>mec A</i>	Oxacillin			Cefoxitin		Color of colonies	Incubation
			disk diffusion (mm)	MIC (mg/l)		disk diffusion (mm)			
<i>S. aureus</i>	2836	NEG	16	S	1	S	19	R	mauve
<i>S. aureus</i>	2837	NEG	15	S	0,5	S	29	S	pink
<i>S. aureus</i>	4738	NEG	21	S	0,5	S	29	S	mauve
<i>S. aureus</i>	4940	NEG	22	S	0,5	S	28	S	pink
<i>S. aureus</i>	4200	NEG	20	S	1	S	34	S	pink
<i>S. aureus</i>	4092	NEG	18	S	0,25	S	30	S	pink
<i>S. aureus</i>	4096	NEG	16	S	0,12	S	29	S	pink
<i>S. aureus</i>	2834	NEG	17	S	0,5	S	27	S	pink
<i>S. aureus</i>	2835	NEG	14	S	2	S	28	S	pink
<i>S. aureus</i>	4950	NEG	21	S	0,5	S	26	S	mauve

CONCLUDING REMARKS

- "CHROMagar MRSA", after 24 h of incubation, is a highly sensitive and specific medium to differentiate MRSA from MSSA, methicillin-resistant CNS and methicillin-susceptible CNS.
- On the basis of its good performance and easy identification of positive colonies, further studies should be required to determine the benefit of using this chromogenic medium in isolation and identification of MRSA directly from clinical samples and in this way, reduce the time required for a final report.