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Evaluation of Four Selective Agars and Two Enrichment Broths in Screening for Methicillin-Resistant *Staphylococcus aureus*▽

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To evaluate methicillin-resistant *Staphylococcus aureus* detection, we tested in vitro four selective agars and two enrichment broths apart and in combination. Tryptone soya broth with salt, aztreonam, and cefoxitin appeared to be the most sensitive medium. This broth was superior to a phenol red mannitol broth with aztreonam and ceftizoxime.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of morbidity and mortality worldwide. Detection of MRSA in screening samples is an important part of the control of MRSA, but due to annex flora, the low level of MRSA often found in screening samples, and the emergence of heteroresistant MRSA strains (4, 8, 9, 12), this is laborious and often difficult. Further cocolonization with other staphylococcal species might lead to false-positive results, especially when using PCR-based methods (1). The use of an enrichment broth for MRSA detection is known to increase sensitivity but requires an extra day of incubation (13, 14, 16). Several brands of chromogenic agars supplemented with antibiotics have recently become available and have performed well by direct inoculation and 24-h incubation (2, 13, 14). However, studies that compare chromogenic agars show conflicting results (2). Most of the published evaluations were performed on samples with the traditional "health care-associated" MRSA strains and with no information on the clonal diversity of the investigated isolates. Clonal differences are known to be one of the reasons for discrepancies when MRSA selective media are studied (7).

We evaluated the performance in vitro of four different selective agars and two enrichment broths, separately and in combination, using low inocula of MRSA isolates.

Ninety-six well-characterized MRSA strains, representing 13 clonal complexes and all six main SCC*mec* types (6), including low-level-resistant isolates; 52 methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates; and 49 methicillin-resistant coagulase-negative staphylococcus (MRCNS) isolates were tested. The agars tested were ChromID MRSA (bioMérieux, Marcy l'Etoile, France); MRSA Select (Bio-Rad, Hercules, CA); a chromagar plate manufactured at Statens Serum Institut (SSI) on license from CHROMagar, Paris, France (MRSA SSI); a mannitol salt agar with 4 mg/liter cefoxitin (MSA); and a 5% blood agar (BA). Two enrichment broths were tested, (i) phenol red mannitol broth with 0.5% salt, 75 mg/liter aztreonam, and 5 mg/liter ceftizoxime (PHMB) (16) and (ii) tryptone soya broth with 2.5% salt, 20 mg/liter aztreonam, and 3.5 mg/liter cefoxitin (TSB) from SSI. The concentrations included in TSB were based on pilot investigations (data not shown). These tests showed that 3 mg/liter cefoxitin allowed breakthrough growth of MSSA and that 4 mg/liter cefoxitin and >20 mg/liter aztreonam resulted in loss of sensitivity. Approximately 20 CFU (20 μl of 10^3 CFU/ml vortexed suspension in 0.9% NaCl) of each strain taken from the same suspension, made from fresh overnight-incubated isolates, were inoculated directly onto agar plates and into the two broths. After 18 to 20 h and 40 to 48 h of incubation at 35°C (±1°C), colony color and counts were registered for each of the agar plates. For broths, turbidity and color change were registered after 18 to 20 h of incubation, followed by subculturing 20 μl of the broth suspension onto each of the agar plates. Plates were incubated at 35°C (±1°C) for another 18 to 24 h. Colony counts were read semiquantitatively. Specificity was defined as the percentage of plates without growth of MSSA and MRCNS exhibiting the same colony color as the MRSA isolates. MICs for MRSA isolates were determined using an Etest (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar with 2% NaCl as described by the manufacturer.

The performance of the plates, with and without enrichment, is shown in Table 1.

No difference was seen between the chromogenic agars irrespective of enrichment and enrichment broth. Optimal sensitivity was obtained after 18 to 24 h of incubation. Forty-eight hours of incubation only caused additional growth of MSSA and MRCNS, leading to reduced specificity.

The mannitol salt agar with cefoxitin required either 48 h of incubation or prior enrichment in TSB to achieve comparable sensitivity.

Preincubation in PHMB resulted in a lower sensitivity on all plates (Table 1), whereas TSB did not reduce the sensitivity of the chromogenic agars and increased the sensitivity of the MSA plate. Preincubation in either of the two broths resulted in breakthrough growth of MSSA. Table 2 shows the numbers of isolates with no growth or reduced growth (<1,000 colonies)

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TABLE 1. Sensitivity and specificity of MRSA selective media evaluated in vitro[a]

Medium	Direct inoculation (24-h incubation)			Enrichment broth plus agar (48-h incubation)					
				TSB			PHMB		
	Sensitivity (%)	Specificity (%)[b]		Sensitivity (%)	Specificity (%)[b]		Sensitivity (%)	Specificity (%)[b]	
	MRSA	MSSA	MRCNS	MRSA	MSSA	MRCNS	MRSA	MSSA	MRCNS
MRSA SSI	99	99	100	100	96	100	75	96	100
ChromID MRSA	98	100	94	100	92	96	73	92	96
MRSA Select	98	100	98	100	87	98	75	94	96
MSA	87/92[c]	100	70	100	87	65	71	98	73
BA	100			100	85	33	76	73	43

[a] Both direct inoculation and subculture in two different broths and subsequent plating were evaluated for 196 staphylococcal isolates in pure culture.
[b] Percentage of plates without additional workload due to growth of MSSA and MRCNS exhibiting the same colony color as the MRSA isolates.
[c] Following 24 and 48 h of incubation, respectively.

on the individual chromogenic agars and on the blood agar plate after incubation in the two enrichment broths.

To confirm the observed differences between the two broths, 43 MRSA strains, including the 24 MRSA strains which did not grow in PHMB at SSI, were blinded and sent to Erasmus University Center (EUC) for a second evaluation. Six of the isolates did not grow after incubation in PHMB (Table 2). In addition, 3 of the remaining 37 isolates produced <1,000 colonies when plated after enrichment. All isolates grew well in TSB. Using a best-case scenario for PHMB (growth in at least one of the two experiments), a sensitivity of only 93% was found for preenrichment in PHMB, compared to a sensitivity of 100% for TSB. The observed difference in sensitivity between the two evaluations (76% versus 94%) for PHMB could be due to a slightly higher inoculum in the second evaluation, as indicated by colony counts. The median inoculum in the first evaluation was 21 CFU (range of 10 to 42 CFU), and that in the second evaluation was 27 CFU (range of 9 to 100 CFU).

Our in vitro evaluation showed that three chromogenic MRSA agars all supported the growth of this diverse collection of MRSA strains at low inocula. Specificity was high, provided that a maximum of 24 h of incubation was used (Table 1). The PHMB enrichment displayed low sensitivity, suggesting that the antibiotic concentration was too high. TSB supported growth of all of the MRSA strains tested. Incubation of the chromogenic agars beyond 24 h led to increased breakthrough growth of MSSA isolates. The MSA plate showed sensitivity comparable to that of the chromogenic agars only after preenrichment.

Although our in vitro results implied that the chromogenic agars did not perform better when preceded by enrichment, two recent studies of clinical specimens both showed that an enrichment step in combination with chromogenic agars led to increased sensitivities of 14 to 26% and 12%, respectively (10, 15). These studies indicate that, under clinical conditions, sensitivity is significantly increased by the addition of an enrichment step even when chromogenic agars are used.

Our study shows that TSB with low-level cefoxitin and aztreonam is a better alternative than PHMB, as published by Wertheim et al. (16). Furthermore, TSB works well with a PCR-based method for MRSA detection (11).

Although more studies on the use of chromogenic media with and without enrichment are needed to clarify the best practice, it now seems quite clear that when designing enrichment broths one has to be careful with salt content and anti-

TABLE 2. Distribution of strains with reduced growth (RG) or no growth (NG) on the chromogenic MRSA selective media and on BA after incubation in enrichment broths[b]

MIC (μg/ml)	No. of MRSA isolates tested	No. of isolates with RG/no. of isolates with NG					No. of MRSA isolates tested[a]	No. of isolates with RG/no. of isolates with NG[a]	
		Chromogenic agar with direct inoculation			Broth plus BA				
		MRSA SSI	ChromID	MRSA Select	TSB	PHMB		TSB	PHMB
≤4	2		0/1	0/2		0/2	3		2/2
6	3	1/0	1/1	1/0		1/1	3		1/1
8	3					0/2	2		0/1
12	2					1/1	1		
24	4					0/1	1		
32	5					1/2	3		0/1
48	7					2/4	4		
64	10					2/5	6		
>64	60	0/1				3/5	20		0/1
Total	96	1/1	1/2	1/2	0/0	10/23	43	0/0	3/6

[a] Confirmatory evaluation of broth plus BA.
[b] All blank cells in the table indicate isolates for which there was full growth. Values are given only for isolates with RG or NG.

biotic concentrations, since either may inhibit the growth of some MRSA isolates (3, 5, 15).

In conclusion, delineation of the optimal phenotypic method is still important for epidemiological reasons like strain typing and resistance testing. Even in the new era of rapid molecular techniques, an enrichment step may still be advantageous.

REFERENCES

1. **Becker, K., I. Pagnier, B. Schuhen, F. Wenzelburger, A. W. Friedrich, F. Kipp, G. Peters, and C. von Eiff.** 2006. Does nasal cocolonization by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible *Staphylococcus aureus* strains occur frequently enough to represent a risk of false-positive methicillin-resistant *S. aureus* determinations by molecular methods? J. Clin. Microbiol. **44:**229–231.
2. **Brown, D. F., D. I. Edwards, P. M. Hawkey, D. Morrison, G. L. Ridgway, K. J. Towner, and M. W. Wren.** 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J. Antimicrob. Chemother. **56:**1000–1018.
3. **Bruins, M. J., P. Juffer, M. J. Wolfhagen, and G. J. Ruijs.** 2007. Salt tolerance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. J. Clin. Microbiol. **45:**682–683.
4. **Hartman, B. J., and A. Tomasz.** 1986. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29:**85–92.
5. **Jones, E. M., K. E. Bowker, R. Cooke, R. J. Marshall, D. S. Reeves, and A. P. MacGowan.** 1997. Salt tolerance of EMRSA-16 and its effect on the sensitivity of screening cultures. J. Hosp. Infect. **35:**59–62.
6. **Kondo, Y., T. Ito, X. X. Ma, S. Watanabe, B. N. Kreiswirth, J. Etienne, and K. Hiramatsu.** 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob. Agents Chemother. **51:**264–274.
7. **Merlino, J., M. Leroi, R. Bradbury, D. Veal, and C. Harbour.** 2000. New chromogenic identification and detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus*. J. Clin. Microbiol. **38:**2378–2380.
8. **Merlino, J., J. Watson, B. Rose, M. Beard-Pegler, T. Gottlieb, R. Bradbury, and C. Harbour.** 2002. Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in central Sydney, Australia. J. Antimicrob. Chemother. **49:**793–801.
9. **Mir, N., M. Sanchez, F. Baquero, B. Lopez, C. Calderon, and R. Canton.** 1998. Soft salt-mannitol agar-cloxacillin test: a highly specific bedside screening test for detection of colonization with methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **36:**986–989.
10. **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.
11. **Ornskov, D., B. Kolmos, H. P. Bendix, N. J. Nederby, I. Brandslund, and P. Schouenborg.** 2008. Screening for methicillin-resistant *Staphylococcus aureus* in clinical swabs using a high-throughput real-time PCR-based method. Clin. Microbiol. Infect. **14:**22–28.
12. **Rohr, U., C. Mueller, M. Wilhelm, G. Muhr, and S. Gatermann.** 2003. Methicillin-resistant *Staphylococcus aureus* whole-body decolonization among hospitalized patients with variable site colonization by using mupirocin in combination with octenidine dihydrochloride. J. Hosp. Infect. **54:** 305–309.
13. **Safdar, N., L. Narans, B. Gordon, and D. G. Maki.** 2003. Comparison of culture screening methods for detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*: a prospective study comparing 32 methods. J. Clin. Microbiol. **41:**3163–3166.
14. **Stoakes, L., R. Reyes, J. Daniel, G. Lennox, M. A. John, R. Lannigan, and Z. Hussain.** 2006. Prospective comparison of a new chromogenic medium, MRSASelect, to CHROMagar MRSA and mannitol-salt medium supplemented with oxacillin or cefoxitin for detection of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **44:**637–639.
15. **van Loo, I., S. van Dijk, 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.
16. **Wertheim, H., H. A. Verbrugh, C. van Pelt, P. de Man, A. van Belkum, and M. C. Vos.** 2001. Improved detection of methicillin-resistant *Staphylococcus aureus* using phenyl mannitol broth containing aztreonam and ceftizoxime. J. Clin. Microbiol. **39:**2660–2662.