Evaluation of a chromogenic agar (MRSASelect) for the detection of meticillin-resistant *Staphylococcus aureus* with clinical samples in The Netherlands

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A novel chromogenic medium for the detection of meticillin-resistant *Staphylococcus aureus* (MRSA), MRSASelect (Bio-Rad), was evaluated with clinical samples in a public health laboratory in The Netherlands. In total, 3000 samples were tested in the period January to March 2005, including 972 nose, 972 throat, 968 perineum, and 88 wound or urine samples. Presumptive MRSA colonies appeared pink/mauve on the MRSASelect medium. The performance of MRSASelect medium was compared with the routine screening method. Evaluation of the colony morphology showed that all confirmed MRSA isolates grew as pink/mauve colonies. None of the white colonies were MRSA strains. The number of false-positive pink/mauve colonies increased after prolonged incubation from 20 to 48 h. The specificity decreased from 92 % after 20 h incubation to 89 % after 48 h incubation. In total 70 MRSA strains were isolated, 55 of which were detected by the MRSASelect medium and 55 were detected by the routine screening method. Sensitivity was 78.6 % for both test procedures, and specificities were 99.5 and 100 %, respectively for the MRSASelect medium and the routine screening method. The addition of an enrichment broth to the MRSASelect medium increased the number of MRSA strains detected by 12 %. In total, 18 patients were MRSA positive, 4 of these were detected by the MRSASelect medium only and 1 was detected by the routine screening method only. Sensitivity on patient level was 94.4 and 77.8 % for the MRSASelect medium and the routine screening method, respectively, while specificities were 99.7 and 99.0 %.

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INTRODUCTION

The prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) in The Netherlands is among the lowest in Europe; below 1 % in both the community and in hospitals (Tiemersma *et al.*, 2004; Wertheim *et al.*, 2004). The Dutch search and destroy policy is part of the strategy to keep prevalence of MRSA low. Patients who are at risk for MRSA carriage are screened for colonization with it and isolated until screening cultures are negative, according to the guidelines established by the Dutch Working Group on Infection Prevention (<http://www.wip.nl/UK/>).

The routine screening procedure, used in this laboratory, takes at least 72 h to obtain a definitive result. Rapid detection of MRSA is essential to optimize therapy, to minimize patients' discomfort and to reduce costs. Chromogenic media are being developed to detect MRSA strains in one single step. Growth of non-staphylococcal strains is repressed by high salt concentration and enzymic substrate is used to achieve a specific colour reaction to

detect *S. aureus*. Growth of meticillin-susceptible *S. aureus* strains is repressed by the addition of various combinations of antibiotics (Merlino *et al.*, 2000). Various studies have been performed to evaluate different chromogenic media with clinical samples, two of which included the MRSASelect medium (Bio-Rad) (Becker *et al.*, 2002; Ben Nsir *et al.*, 2006; Blanc *et al.*, 2003; Merlino *et al.*, 2000; Perry *et al.*, 2004; Simor *et al.*, 2001; Stoakes *et al.*, 2006). In this study, we evaluated the performance of a chromogenic MRSA agar, MRSASelect in a low prevalence population. We evaluated the MRSASelect medium by comparing it with our routine screening method on clinical samples. In the period January to March 2005 samples from known MRSA patients or patients at risk for MRSA carriage or contacts of known MRSA carriers were included in this study.

METHODS

Clinical samples. In total, 3000 samples from 409 patients were tested for the presence of MRSA in the Public Health Laboratory Tilburg, St Elisabeth Hospital, in the period January to March 2005. The laboratory provides diagnostics for two hospitals in Tilburg, and for physicians and nursing homes in the region. Samples were

Abbreviation: MRSA, meticillin-resistant *Staphylococcus aureus*.

from patients who were at risk for MRSA carriage were followed up samples from known MRSA-positive patients or were samples from contacts of MRSA-positive patients to evaluate the spread of MRSA within the hospital. Cultures were taken from nose, throat, perineum, wounds, sputum and urine according to the guidelines of the Dutch Working Party on Infection Prevention. In total, 972 nose, 972 throat, 968 perineum and 88 wound or urine samples were included in the study.

Screening samples. Every sample was cultured on an MRSASelect plate (Bio-Rad) (composition of the medium is proprietary), a Columbia agar plate (Oxoid) supplemented with 5% sheep blood and 2 µg ciprofloxacin ml⁻¹ (BA-cipro) (Bayer), an MRSA screening agar, containing mannitol salt agar (Oxoid) supplemented with 4 µg oxacillin ml⁻¹ (MSA-oxa) (Sigma), and an enrichment broth containing Mueller–Hinton broth with 6 µg oxacillin ml⁻¹ and 6 µg aztreonam ml⁻¹ (MH-oxa-aztr) (ICN). For each sample the inoculated plates were analysed after 20 h and after 48 h. The enrichment broth was subcultured after 20 h on a Columbia agar plate supplemented with 5% sheep blood, and analysed after 20 h and after 48 h. The nose, throat and perineum samples from known MRSA patients were put into an enrichment broth separately, while unknown patients' nose, throat and perineum samples were pooled in one enrichment broth. If the pooled enrichment broth was positive the three samples were counted once. For the calculation of the sensitivity and specificity the samples from the solid media and the non-pooled enrichment broth were used.

Testing of colonies on MRSASelect medium. Presumptive MRSA colonies on the MRSASelect agar were pink/mauve and were tested with a latex agglutination test (Staphaurex plus, Murex Diagnostics) and subcultured on a Columbia agar plate supplemented with 5% sheep blood. If colony morphology was suspect for *S. aureus* and the latex agglutination test was positive further identification was performed (see below).

A total of 205 white colonies were tested with a latex agglutination test and subcultured on a Columbia agar plate supplemented with 5% sheep blood. Latex agglutination and catalase tests (Isenberg, 2004) were performed to exclude growth of *S. aureus*.

Confirmation of MRSA. Presumptive MRSA colonies (any colony on the Columbia agar plate and the MRSA screening agar, and pink/mauve colonies on the MRSASelect plate) were identified by latex

agglutination test, coagulase tube test (Isenberg, 2004), DNase (Isenberg, 2004), growth on a Mueller–Hinton plate supplemented with 6 µg oxacillin ml⁻¹, growth on a Mueller–Hinton plate supplemented with 2% NaCl, AccuProbe (Gen-Probe) and identification with the Vitek2 system (bioMérieux).

Susceptibility testing was performed by Vitek2 system. Isolates resistant to oxacillin, quinolones or aminoglycosides were further evaluated, including disc diffusion of oxacillin (1 µg) and meticillin (5 µg) (Sigma) on a Mueller–Hinton plate supplemented with 2% NaCl, disc diffusion of ciprofloxacin (5 µg), ofloxacin (5 µg), mupirocin (5 µg), gentamicin (10 µg) and tobramycin (10 µg) (Sigma) on a Mueller–Hinton plate and an E-test for oxacillin (AB Biodisk) on a Mueller–Hinton plate supplemented with 2% NaCl. Final confirmation was performed by *mecA* gene PCR (Del Vecchio *et al.*, 1995).

RESULTS AND DISCUSSION

In this study a chromogenic medium, MRSASelect, was evaluated with clinical samples. First we evaluated the colonies that grew on the MRSASelect medium. After 20 h incubation 33 (89%) MRSA strains were isolated and 4 (11%) samples generated false-positive pink/mauve colonies (Table 1). Other studies evaluating MRSASelect medium found a lower number of false-positive samples, 3 (3%) and 1 (1%), respectively (Stoakes *et al.*, 2006; Ben Nsira *et al.*, 2006). We found that prolonging incubation from 20 to 48 h increased the yield of confirmed MRSA strains by 38% (20 strains) (Table 1). Ben Nsira *et al.* (2006) found an increase of 3%. Stoakes *et al.* (2006) did not evaluate MRSASelect medium after 48 h. However, 97% of the MRSA strains were recovered after 18 h incubation, compared to 79% after 48 h in our study. For other chromogenic media the yield also increased after prolonged incubation. For example, Perry *et al.* (2004) found an increased yield of 11%, 19% and 20% for MRSA-ID, CHROMagar MRSA and ORSAB, respectively. Simor *et al.* (2001) also found an increase in recovery (23%) of MRSA on ORSAB plates after prolonged incubation.

Table 1. Number and identification of pink/mauve and white colonies growing on MRSASelect after 20 and 48 h incubation

	No. of colonies growing after 20 h*	No. of colonies growing after 48 h†	Total
Pink/mauve colonies			
MRSA	33	20	53
CNS	1	2	3
<i>Corynebacterium</i> spp.	3	4	7
Enterococci	0	6	6
White colonies			
CNS	48	58	106
<i>S. aureus</i>	0	1	1

CNS, coagulase-negative staphylococci.

*Number of pink/mauve or white colonies growing after 20 h on MRSASelect.

†Number of pink/mauve or white colonies growing after 48 h on MRSASelect that did not grow after 20 h incubation.

Table 2. Number of MRSA-positive samples for the different media

	No. of MRSA-positive samples	Sensitivity	Specificity
MSA-oxa/BA-cipro	37	52.9 %	100 %
MSA-oxa/BA-cipro + MH-oxa-aztr	55	78.6 %	100 %
MRSASelect	55	78.6 %	99.5 %
MSA-oxa/BA-cipro + MH-oxa-aztr + MRSASelect	70	100.0 %	100 %

In total, 69 samples generated pink/mauve colonies after 48 h incubation, 53 (77 %) of which were true positives and 16 (23 %) were false positives (Table 1). Specificity decreased from 92 % after 20 h incubation to 89 % after 48 h. False positives were enterococci or coagulase-negative staphylococci (Table 1). Ben Nsira *et al.* (2006) found four (4 %) false positives after 48 h. Compared to other chromogenic media (e.g. ORSAB medium) MRSASelect generated less false-positive results (Becker *et al.*, 2002; Blanc *et al.*, 2003; Simor *et al.*, 2001). Other chromogenic media also show a decrease in specificity after 48 h incubation (Perry *et al.*, 2004).

The colony morphology of false positives in our study showed less pigmentation compared to confirmed MRSA strains, an observation that was also noted by Ben Nsira *et al.* (2006). Similar observations were found in studies evaluating other chromogenic media (Becker *et al.*, 2002; Blanc *et al.*, 2003; Perry *et al.*, 2004; Simor *et al.*, 2001).

In total, 107 white colonies were evaluated, 106 of which were determined as coagulase-negative staphylococci and one of which as methicillin-susceptible *S. aureus* (Table 1). The number of white colonies doubled after prolonging incubation from 20 to 48 h (Table 1).

We compared MRSASelect medium with the routine screening method (MSA-oxa/BA-cipro + MH-oxa-aztr) (Table 2). Seventy confirmed MRSA isolates were found in eighteen patients (Table 2). Fifty-five MRSA isolates were detected by the screening method and fifty-five by the MRSASelect medium after 48 h incubation. Fifteen MRSA isolates were not detected by the MRSASelect medium and fifteen MRSA isolates were not detected by the routine screening method. Sensitivity of the MRSASelect medium was 78.6 % and specificity was 99.5 %, compared to sensitivity of 78.6 % and specificity of 100 % for the routine screening method. The number of discrepancies between the MRSASelect medium and the routine screening method is unexpectedly high. We cannot explain the lower sensitivity in our study in comparison to the studies of Stoakes *et al.* (2006) and Nsira *et al.* (2006). Stoakes *et al.* (2006) found that 3 from the 111 confirmed MRSA strains were not detected by MRSASelect (sensitivity of 97.3 %) and Ben Nsira *et al.* (2006) reported 1 missing MRSA isolate (sensitivity 99.8 %). Differences in sampling, in MRSA prevalence and in circulating clones in the different countries may account for differences in sensitivity. The

sensitivity we found was similar to that of other chromogenic media evaluated with clinical samples (Perry *et al.*, 2004). To increase the yield we added an enrichment broth (MH-oxa-aztr) in a pilot study. From 225 samples we subcultured the enrichment broth on MRSASelect medium. We found 12 % more MRSA isolates. We have now used the MRSASelect medium in combination with the enrichment broth (MH-oxa-aztr) in our laboratory for more than a year. Thirty percent of all the MRSA isolates were yielded from the enrichment broth, indicating an increase in yield that is higher than in the pilot study.

Analysis at the patient level showed that from a total of 408 patients screened for MRSA carriage 18 were MRSA carriers. MRSA isolates of 13 patients were detected by both methods. MRSA isolates of 4 carriers were detected by MRSASelect medium only, while 1 patient was detected by the routine screening method only. Sensitivity at the patient level was 94.4 and 77.8 % for the MRSASelect medium and the routine screening method, respectively, while specificities were 99.7 and 99.0 %. The number of patients with MRSA that were missed in our study was 1/18 (5 %) for MRSASelect and 4/18 (22 %) for the routine screening method. Perry *et al.* (2004) found similar figures for other chromogenic media: 2/43 (5 %), 7/43 (16 %) and 4/43 (9 %) for MRSA-ID, CHROMagar and ORSAB, respectively, and 12/43 (28 %) for mannitol salt agar.

In conclusion, in our hands MRSASelect is a useful medium for the detection of MRSA, although in this study it was less sensitive than in other studies. The MRSASelect medium generated a lower number of false positives compared to other chromogenic media. The increase in true-positive MRSA isolates after a prolonged incubation in our study is higher than for other chromogenic media. Optimization of screening was achieved by the addition of an enrichment broth next to primary culture on the MRSASelect medium. In a country with low MRSA prevalence (e.g. The Netherlands) the main advantage of MRSASelect medium compared to the routine screening procedure is that the time to obtain a negative culture result is reduced from 72 to 48 h.

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