

Improved Detection of Methicillin-Resistant *Staphylococcus aureus* Using Phenyl Mannitol Broth Containing Aztreonam and Ceftizoxime

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We tested a phenyl mannitol broth containing ceftizoxime and aztreonam (PHMB⁺) for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) with reference MRSA strains and, subsequently, with clinical samples ($n = 1,098$). All reference MRSA strains induced color change in PHMB⁺ after 24 to 72 h of incubation. In a clinical setting, 40 MRSA strains were detected with PHMB⁺, compared with only 23 detected with a routine method. Thus, this selective broth significantly ($P < 0.001$) improved the rate of MRSA detection.

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples continues to be important, since infections due to MRSA have high morbidity and mortality rates. Moreover, some MRSA strains have the potential to spread rapidly and colonize in other patients. In The Netherlands, therefore, patients who are suspected of being MRSA carriers are isolated until screening cultures are repeatedly negative for MRSA. Methods to detect MRSA in clinical samples ideally should have high sensitivity and a short time to reporting the results. To increase the sensitivity, one can simply take more screening samples on the same day or on consecutive days, but this method is more cumbersome and increase the time to reporting. Another way to increase the sensitivity is to use a broth in addition to agar plates, as was demonstrated previously (1, 3, 11; R. L. Sautter and L. W. Wells, Letter, J. Clin. Microbiol. **28**:2380–2381, 1990; M. L. Van Ogtrop, Letter, Antimicrob. Agents Chemother. **39**:2169, 1995). To increase the sensitivity of the detection of MRSA from a single sample and to improve laboratory efficiency, we developed a new selective broth containing phenol red, mannitol, aztreonam, and ceftizoxime (PHMB⁺). First, we tested the broth with laboratory reference strains. Subsequently, we compared our routine method of direct plating of specimens onto blood agar plates and mannitol salt agars with the new selective broth combined with a blood agar plate.

The PHMB⁺ was made by mixing 21 mg of dehydrated phenol red mannitol broth (Becton Dickinson, Le Pont de Claix, France) with 1,000 ml of distilled water, sterilizing the broth for 15 min at 121°C, and letting it cool to room temperature. We then mixed 5 mg of ceftizoxime (Yamanouchi) with 5 ml of distilled water and added the solution to the broth at a concentration of 5 µg of ceftizoxime per ml. Next, we mixed 75 mg of aztreonam (Bristol-Myers Squibb) with 5 ml of distilled

water, filtered the solution through an FP 030/2 filter (Schleicher & Schuell), and added it to the broth at a concentration of 75 µg of aztreonam per ml. Finally, we filled sterile tubes with 8 ml of PHMB⁺ and stored them at 4°C in the dark. (The broth has a shelf life of at least 4 weeks.)

We tested the PHMB⁺ with five different MRSA and five different methicillin-sensitive *S. aureus* (MSSA) strains isolated from patients. Methicillin resistance was confirmed by *MecA* PCR according to the method described by Murakami et al. (9). At first, all 10 strains were subcultured onto brucella blood agar and incubated for 18 h at 37°C. From each strain, a suspension was made in 0.9% NaCl with a density of 0.5 McFarland standard (10^8 CFU/ml), and dilution series of 10^8 to 10^0 CFU/ml were made. Five hundred microliters of each dilution was pipetted into five batches of PHMB⁺ (4.5 ml) of different production dates, each 1 week apart. Every batch of PHMB⁺ was prepared by the same person and stored at 4°C until use. One hundred microliters of the original solution of 0.5 McFarland standard was streaked onto a brucella blood agar plate as a control for the density of CFU. The broths were incubated for 14 days at 37°C and inspected daily basis for a color change from red to orange-yellow.

From June to December 1997, the Department of Medical Microbiology and Infectious Diseases of the Academic Hospital Rotterdam received 1,098 consecutive specimens for the detection of MRSA. These specimens originated from patients and employees and were either screening samples or samples taken during a putative MRSA outbreak. From employees, only the anterior nares were cultured. From patients, specimens were taken from the rectum, nose, throat, wounds, insertion sites of venous and arterial lines, and urine if a urine catheter was present. Samples were collected and transported with commercial swabs (Transwab; Medical Wire & Equipment Co. Ltd., Wiltshire, United Kingdom) to the laboratory and then stored for a maximum of 16 h at 4°C until inoculation. Only one swab was available per collection site.

For the routine culture of MRSA, the swabs were streaked onto 5% sheep blood agar (BA) plates (Becton Dickinson) and

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phenyl mannitol salt (7%) agar (PHMA) plates (Becton Dickinson). Subsequently, the swabs were submerged in PHMB⁺. All media were incubated for 3 days at 37°C and checked for growth of staphylococci each day. The broth was examined daily for color change from red to orange-yellow for 3 days. When the color of the broth had changed to orange-yellow, a loop of broth was subcultured onto the BA. If growth of a nonfermenter was observed on the primary BA, the broth was subcultured onto BA irrespective of the color of the broth. This subculture was examined for suspect colonies after incubation for 18 to 24 h at 37°C. Colonies suspected of growing *S. aureus* were identified with a Staphaurex Plus agglutination test (Abbott Murex, Chatillon, France) and tested with methicillin disk diffusion performed according to NCCLS guidelines (10). All morphologically different strains were tested. Staphaurex Plus-positive strains were confirmed with the Accu-Probe hybridization test (Gen Probe Inc., San Diego, Calif.) according to the guidelines of the manufacturer. Methicillin resistance was confirmed with *MecA* PCR (9). *MecA*-positive strains were sent to the laboratory of the National Institute of Public Health and the Environment (RIVM, Bilthoven, The Netherlands) for MRSA phage typing (unpublished method). The difference in proportion of detected MRSA strains between the two methods was statistically tested with the Sign test for paired samples using SPSS software; a *P* value of <0.05 was considered significant.

In the experimental setting, the MSSA strains did not produce any color change in the PHMB⁺ irrespective of the concentration of CFU, the incubation time, or the time of storage of the broth. All MRSA strains induced a distinct color change at the dilution step corresponding to approximately 10⁶ CFU/ml after incubation for 72 h. At densities of 10⁵ CFU/ml and higher, the color change was observed within 24 h. The storage life of the broth was at least 4 weeks at 4°C in the dark (data not shown).

In the clinical setting, 1,098 cultures were performed and were taken from nares (*n* = 466), perineum (*n* = 220), throat (*n* = 215), wounds (*n* = 101), exit sites of catheters (*n* = 43), urine (*n* = 22), and other sites (*n* = 31). A total of 136 (12%) of these cultures were positive for *S. aureus*, of which 40 (29%) were methicillin resistant (*MecA* PCR positive). The MRSA strains were cultured from eight different patients. Phage typing of the MRSA strains showed five distinct phage types, and one was untypeable. Twenty-three (57%) of the MRSA strains grew on both BA and PHMA and in PHMB⁺. Seventeen additional strains grew only in PHMB⁺ and not on BA or PHMA (Sign test, *P* < 0.001). Color changes occurred in 263 (24%) of the 1,098 cultures grown in PHMB⁺. (Table 1), and the most prevalent organisms, apart from MRSA, were coagulase-negative staphylococci (*n* = 107) and *Enterococcus* spp. (*n* = 33).

The results showed that by using the selective broth, we detected almost twice as many MRSA strains as we did with the routine technique. Furthermore, only a small fraction of the PHMB⁺ broths had to be subcultured, due to the presence of selective antibiotics. At the time of this study, our laboratory used methicillin agar diffusion instead of oxacillin agar diffusion to test for methicillin resistance. Since this test was used for both culture techniques, we do not believe it had a great effect on our results. This is the only study that presents a

TABLE 1. MRSA strain detection with the routine method versus PHMB⁺, in comparison with detection of other organisms

Species	No. of strains by method	
	Routine	PHMB ⁺
Coagulase-negative staphylococci ^a	NR ^e	111
MRSA ^b	23	40
<i>Enterococcus</i> spp. ^c	NR	37
Coagulase-negative staphylococci + <i>Enterococcus</i> spp.	NR	35
Other ^d	NR	40

^a In combination with other species (*n* = 4).

^b *P* < 0.001 by Sign test.

^c In combination with other species (*n* = 4).

^d Gram-positive rods, yeasts, and MSSA (*n* = 3).

^e NR, not registered.

selective broth with antibiotics inhibiting growth of both gram-positive and gram-negative bacteria for the selection of MRSA strains. Previous studies have used high concentrations of salt for selectivity, with or without aztreonam or oxacillin (1–7, 11, 14; Sautter and Wells, letter; Van Ogtrop, letter). By using only salt, one selects MRSA as well as MSSA strains, and salt has the disadvantage that some MRSA strains will not grow when concentrations exceed 2.5% (5). The rationale for using ceftizoxime and aztreonam in the selective broth instead of oxacillin and colistin was that earlier studies had shown that both oxacillin and colistin resulted in inhibited or slower growth of MRSA strains (data not shown). Furthermore, ceftizoxime is known to increase the phenotypic level of resistance to methicillin (8, 12, 13).

This study was designed to improve the efficiency and sensitivity of detecting MRSA, and in this respect, the need for speed remains important. The use of the BA plate is still necessary to detect nonfermenters that produce an alkaline environment in the broth, thereby prohibiting the phenol red from turning yellow. Therefore, broths should always be subcultured when a nonfermenter grows on BA. When there is an outbreak with a new MRSA strain, we suggest that its growth characteristics in the PHMB⁺ be determined immediately. This can be done by making a dilution series of the cultured strain, incubating the specimens, and checking the time required until a color change. From the results, one can choose the optimal incubation time for specimens from contact patients and health care workers. The present study clearly shows that MRSA screening with a selective phenyl mannitol broth including aztreonam and ceftizoxime is efficient and sensitive. This method is now implemented in the routine MRSA screening of our and other Dutch hospitals.

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