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# Early Screening of Oxacillin-Resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* from Blood Culture

The timely detection of blood-borne pathogens is one of the most important functions of the microbiology laboratory. Recently, methicillin-resistant staphylococci have become the most important pathogens seen by the laboratory. The purpose of this study was to evaluate Staphy agar, a novel screening medium, for the detection methicillin-resistant *Staphylococcus aureus*, *S. epidermidis*, or other coagulase-negative staphylococci (CNS) from positive blood cultures showing Gram-positive cocci in clusters. Eighty-six blood cultures that yielded Gram-positive cocci in clusters were included in this study. The organisms were finally identified by the Vitek system, and oxacillin resistance was confirmed by polymerase chain reaction (PCR)-based *mecA* gene detection. The identification and oxacillin resistance of all *S. aureus* strains showed complete agreement with the Vitek and PCR results. The presumptive detection of *S. epidermidis* and other CNS were consistent with the Vitek system in 94.7%, and the screening of oxacillin resistance was consistent with the result of PCR in 92.1% of 38 strains. The Staphy agar method is reliable and rapid for differentiating Gram-positive cocci in clusters in blood and for determining their methicillin resistance.

Key Words : *Oxacillin; Staphylococcus aureus; Staphylococcus epidermidis*

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## INTRODUCTION

Bloodstream infection is one of the most serious situations in infectious disease, and the timely detection and identification of blood-borne pathogens is one of the most important functions of the microbiology laboratory. By the currently used blood culture methods, it usually takes at least 2 days to get complete identification and susceptibility results from blood culture isolates after a positive signal is obtained from the Vital system. Staphylococci are among the bacteria most frequently isolated from the blood. When Gram-positive cocci in clusters are found in blood cultures, they are staphylococci most probably. Of these, *Staphylococcus aureus* is well-documented as a human opportunistic pathogen (1). Although the coagulase-negative staphylococci (CNS) are considered normal flora, there has been a 500% to 700% increase in the number of significant bloodstream infections caused by *S. epidermidis* and other CNS in the last 10 yr in the United States (2). At present, methicillin-resistant *S. aureus* (MRSA) is a serious clinical and epidemiological problem in hospitals of all sizes, and these organisms should be considered cross-resistant to all $\beta$-lactam antibiotics (3). Furthermore, the prevalence of methicillin-resistant strains is as high as 60-66% of all *S. epidermidis* clinical isolates (4, 5). Therefore, it is very important to identify staphylococci from the blood and to determine their resistance to oxacillin in a timely manner. Commercially available kits for rapid identification of *S. aureus* or MRSA, such as the latex agglutination tests targeting PBP 2a or other proteins (6-9), thermonuclease test (10), lysostaphin sensitivity test (11), fluorescent staphylocoagulase test (12), the DNA-RNA-DNA chimeric probe-based *mecA* detection test (13), and BBL Crystal MRSA ID test kit (13) focus on either identifying *S. aureus* or detecting methicillin resistance. These tests show excellent results, but most of them are too expensive to be used in clinical laboratories in Korea. Yoshida's medium has an advantage in detecting MRSA in a single medium rapidly and inexpensively because it contains mannitol, 4-MUPA, and oxacillin (14). However, it cannot differentiate *S. epidermidis* from other CNS. This study used a modified Yoshida's MRSA-detecting medium, named Staphy agar, designed for early detection of MRSA and methicillin-resistant *S. epidermidis* (MRSE). We evaluated this agar as a means of screening MRSA, MRSE, and other methicillin-resistant CNS from blood cultures yielding Gram-positive cocci in clusters within a day.

## MATERIALS AND METHODS

Eighty-six consecutive blood cultures that yielded Gram-

positive cocci in clusters were tested over a period of three months. Blood culture was performed using the Vital system (bioMérieux, Marcy-l'Etoile, France). The medium for selecting and differentiating staphylococci was prepared as Yoshida's MRSA-detecting medium, with a modification of using oxacillin disk instead of oxacillin-containing medium (14). Briefly, 20 g of heart infusion agar, 20 g of NaCl, 5 g of mannitol, 1.25 mg of phenylethyl alcohol, and 12.5 mg of phenol red were dissolved in 500 mL of distilled water and autoclaved. After the medium was cooled to 50℃, 50 mg of 4-methylumbelliferyl phosphoric acid (4-MUPA) (Sigma, St. Louis, MO, U.S.A.) was added. The medium was dispensed in 100-mm plates, stored at 2-8℃, and used within 1 month. We named this medium Staphy agar. Half of the surface of each Staphy agar plate was inoculated by streaking the swab dipped in the positive blood culture on the surface to achieve confluent growth of colonies. The remaining half was streaked with a sterile loop to isolate single colonies. Disks containing 1 μg of oxacillin, 5 μg of novobiocin, and 300 U of polymyxin B (all from Becton Dickinson, Cockeysville, MD, U.S.A.) were placed on the first half of the agar. The agar plate was incubated for 18 to 24 hr at 37℃ under room air. If colonies were found on the Staphy agar plate after overnight incubation, the organism was considered to be able to grow in a 4% NaCl environment. Yellow color indicated the ability to ferment mannitol. Colonies that fluoresced under a Wood's lamp were considered alkaline phosphatase positive. The catalase test was performed with 3% $H_2O_2$. The coagulase or clumping factor test was performed to identify *S. aureus* by a Slidex Staph-Kit (bioMérieux Vitek, St. Louis, MO, U.S.A.). If the inhibition zone around the novobiocin disk was at least 16 mm, the organism was considered susceptible. If the inhibition zone around the polymyxin B disk was at least 10 mm, the organism was considered susceptible (1). We identified an organism as *S. aureus* if yellow colonies were catalase and clumping factor positive, susceptible to novobiocin and resistant to polymyxin B and fluoresced. Pink colonies that were catalase positive, clumping factor negative, and susceptible to novobiocin and resistant to polymyxin B were identified as *S. epidermidis*. Colonies that were catalase positive and did not have the above characteristics of *S. aureus* or *S. epidermidis* were classified as "other CNS." The resistance to antibiotics was interpreted using the criteria of the disk diffusion method recommended for staphylococci (15). Also, a standard disk diffusion test was performed using Muller-Hinton agar (MHA) (15). The organisms were finally identified with a Vitek GPI card (bioMérieux Vitek). The *mecA* gene was detected by PCR as the gold standard (16).

## RESULTS

The Vitek identification of 86 strains was as follows: 48 *S. aureus*; 20 *S. epidermidis*; four each of *S. hemolyticus* and *S. intermedius*; three *S. hominis*; two each of *S. auricularis*, *S. capitis*, and *S. sciuri*; and one *S. simulans*. Among the 48 *S. aureus* strains, 23 were *mecA* positive by PCR. Also, 15 of the 38 CNS strains were *mecA* positive. The identifications of *S. aureus* by Staphy agar and Slidex Staphy kit showed complete agreement with the Vitek results. The presumptive differentiation of *S. epidermidis* and other CNS by Staphy agar was consistent with the Vitek identification in all but two cases (94.7%) (Table 1). The two discrepant cases were presumed to be *S.*

Table 1. Comparison between presumptive and final identification of staphylococci

| Reactions in Staphy agar | | | | | Catalase | Clumping factor | No. of isolates | Presumptive identification | Final identification |
|---|---|---|---|---|---|---|---|---|---|
| Growth in 4% NaCl | Mannitol fermentation | Phosphatase | NB 5μg | PB 300U | | | | | |
| + | + | + | S | R | + | + | 48 | *S. aureus* | *S. aureus* (48) |
| + | - | + | S | R | + | - | 12 | *S. epidermidis* | *S. epidermidis* (12) |
| + | - | - | S | R | + | - | 10 | *S. epidermidis* | *S. epidermidis* (8)<br>*S. auricularis* (1)*<br>*S. hominis* (1)* |
| + | + | + | S | S | + | - | 6 | Other CNS | *S. capitis* (1)<br>*S. intermedius* (3)<br>*S. sciuri* (2) |
| + | + | + | S | R | + | - | 2 | Other CNS | *S. auricularis* (1)<br>*S. simulans* (1) |
| + | + | - | S | S | + | - | 6 | Other CNS | *S. capitis* (1)<br>*S. hemolyticus* (4)<br>*S. hominis* (1) |
| + | - | + | S | S | + | - | 2 | Other CNS | *S. intermedius* (1)<br>*S. hominis* (1) |

Abbreviations: NB, novobiocin; PB, polymyxin B; +, positive reaction; -, negative reaction; S, susceptible; R, resistant; CNS, coagulase-negative staphylococci.

*Two strains were presumed as *S. epidermidis*.

Table 2. Results of presumed oxacillin susceptibility in Staphy agar as comparing with the presence of *mecA* gene

| Presence of *mecA* gene | Oxacillin inhibition zone in Staphy agar | | |
|---|---|---|---|
| *S. aureus* | ≤10 mm (Resistant) | 11-12 mm (Intermediate) | ≥13 mm (Susceptible) |
| *mecA* positive | 23 | 0 | 0 |
| *mecA* negative | 0 | 0 | 25 |
| CNS | ≤17 mm (Resistant) | | ≥18 mm (Susceptible) |
| *mecA* positive | 14 | | 1† |
| *mecA* negative | 2* | | 21 |

*The species of these two strains were *S. epidermidis* and *S. intermedius*,
†The species of this strain was *S. intermedius*.

*epidermidis*, whereas the Vitek identifications were *S. auricularis* and *S. hominis*. All 23 *mecA*-positive *S. aureus* strains showed no inhibition zones around oxacillin disks on either MHA or Staphy agar, and all 25 *mecA*-negative strains showed inhibition zones of ≥13 mm on both agars (Table 2). For 14 of the 15 *mecA*-positive and 21 of the 23 *mecA*-negative CNS strains (92%), the interpretations of oxacillin inhibition zones in both agars were consistent with the *mecA* PCR results. One *mecA*-positive CNS showed an inhibition zone of 18 mm on Staphy agar; a very major error, contrary to the result of MHA, which showed 17 mm of inhibition. Two *mecA*-negative strains showed inhibition zones of 17 mm in Staphy agar; again, a major error, the same as in MHA.

## DISCUSSION

The most significant staphylococci in human bacteremia are *S. aureus* and *S. epidermidis*. *S. aureus* is an important cause of both community-acquired and nosocomial bacteremia. It is clear that *S. epidermidis* is an important cause of bacteremia and has been correlated with the increase in the use of prosthetic and indwelling devices and the growing number of immunocompromised patients in hospitals (1). Although other CNS are commonly isolated from blood cultures, they are more likely to be contaminants and are not associated with increased morbidity and mortality, even if therapy is delayed. Therefore, the allocation of staphylococcal strains to *S. aureus*, *S. epidermidis*, and other CNS is of great value in determining the significance of isolates from blood cultures.

Traditionally, staphylococci isolated from blood have been subcultured onto solid media for identification and susceptibility testing. However, this strategy requires overnight incubation just for subculturing. Therefore, many investigators have addressed the early and direct detection of MRSA as well as *S. aureus* from blood cultures (17-20). In this study, Staphy agar was designed to grow staphylococci selectively using the Yoshida formula modified to identify both *S. aureus* and *S. epidermidis* with addition of the novobiocin disk, polymyxin B disk, and a few tests such as catalase and latex coagulase. While oxacillin resistance was determined by growth on agar containing oxacillin 4 μg/mL in Yoshida's medium, the oxacillin disk diffusion method was applied for Staphy agar. Compared with the original formula, this method using Staphy agar has the advantage of detecting methicillin-resistant as well as methicillin-susceptible strains and of differentiating *S. epidermidis* (14). In this study, we simply omitted oxacillin and put disks of oxacillin, novobiocin, and polymyxin B to detect methicillin-susceptible strains as well as to identify the species presumptively.

All *S. aureus* strains grow in 4% NaCl, produce alkaline phosphatase, ferment mannitol, and are susceptible to novobiocin and resistant to polymyxin B. Some strains of CNS, however, have the same characteristics, as shown in Table 1. So, clumping factor test could be reserved for the strains that show the same phenotypes as above. Presumptive identification of *S. epidermidis* was consistent with the Vitek identification in 94.7% of 38 CNS strains, even though there is a debate about whether the Vitek system is perfect in identifying CNS. This result showed that this method is useful for detecting *S. aureus* and *S. epidermidis* within a day after a blood culture appears positive.

The single examples of *S. auricularis* and *S. hominis* were identified presumptively as *S. epidermidis* because of their resistance to polymyxin B on the Staphy agar plate. This discrepancy illustrates some limitations. One is that the size of the bacterial inoculum might cause an error in the polymyxin B susceptibility test on a Staphy agar plate. The other is an exceptional phenotype of strain itself. However, more than 97% of *S. epidermidis* are resistant to polymyxin B, and more than 97% of other CNS are susceptible to polymyxin B (21). So it is reasonable to consider that polymyxin B-resistant CNS should be *S. epidermidis* and to use the current method as a presumptive identification system.

At present, both MRSA and methicillin-resistant CNS cause problems in patient care. It is expected that as many as 60% to 90% of *S. epidermidis* and other CNS from clinical isolates will be resistant to methicillin (4, 5, 22, 23). The oxacillin resistance deduced from the Staphy agar plate was consistent with that of the *mecA* PCR method in all *S. aureus* and 92% of the CNS strains, resulting in only one very major error. It is well known that the depth of the agar plate, the inoculum size, and the constituents of the medium are critical factors in disk diffusion susceptibility tests. In this study, the depth of the agar plate was checked, but the inoculum size was not standardized. Thus, Staphy agar itself as well as the inoculum size might cause some errors. Also, we identified two samples showing a major error in both media. All three discrepant cases were at the threshold for differentiating resistant strains from susceptible ones. Even though the possibility of PCR failure in these cases of major error was not completely excluded, we think that an observational error of 1 mm is inherent in a disk diffusion method, revealing

another limit of this method.

Another point that should be considered is borderline or heteroresistant MRSA. Because MRSA strains were homogeneously resistant, the performance to detect borderline or heteroresistant MRSA was not evaluated in this study. However, MHA with 2% NaCl and mannitol salt agar has been reported to detect heteroresistant *S. aureus* more accurately than MHA (24). We therefore think Staphy agar probably detects heteroresistant MRSA, and further evaluation is necessary to confirm whether it can detect heteroresistant MRSA.

Conventional biochemical or kit-based methods are often used for the identification and susceptibility testing of staphylococci in clinical microbiology laboratories, and it usually takes 2 days after a positive signal is seen in a blood culture to obtain the final result. We designed a simple method, the Staphy agar plate test, combined with some other tests to differentiate staphylococci and their oxacillin susceptibility. By this method, we could detect *S. aureus* and *S. epidermidis* and determine their oxacillin susceptibilities presumptively without subculturing blood isolates, which shorten the reporting time for reliable results to 1 day. We think that this medium can also be used as a primary selective medium in the surveillance culture of MRSA. Further examinations including variable species of staphylococci and strains with variable degree of resistance will be necessary to confirm the value of this new method.

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