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ARTICLE

Performance of Oxoid Brilliance™ MRSA medium for detection of methicillin-resistant *Staphylococcus aureus*: an in vitro study

E. Verkade · S. Elberts · C. Verhulst · J. Kluytmans

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Abstract Oxoid Brilliance™ MRSA was evaluated for its ability to identify methicillin-resistant *Staphylococcus aureus*. A well-defined collection of staphylococci was used (n=788). After 20 h incubation, the sensitivity was 99.6% and the specificity was 97.3%. This new medium is a highly sensitive method of screening for MRSA.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged worldwide as a nosocomial pathogen of major importance, and the incidence of infections caused by MRSA continues to increase [1, 2]. Recently, MRSA has emerged in the community [3]. Also a new clone has been identified that is related to an extensive reservoir in animals. Persons who are in direct contact with pigs frequently carry this animal-related MRSA [4, 5]. Laboratory-based screening for MRSA colonization of patients and health care workers remains a cornerstone of infection control measures to limit the spread of this organism [6]. The extension of MRSA beyond its known boundaries poses an additional challenge for microbiological laboratories to improve their screening strategies. Methods of detecting MRSA in clinical samples ideally should have a high sensitivity and specificity combined with a short time to reporting of the results. To identify *S. aureus* from contaminated samples more easily and more reliably, selective media have been developed [7].

E. Verkade (✉) · S. Elberts · C. Verhulst · J. Kluytmans
Laboratory for Microbiology and Infection Control,
Amphia Hospital,
P.O. Box 90158, 4800 RK, Breda, The Netherlands
e-mail: everkade@amphia.nl

The purpose of this study was to evaluate the in vitro sensitivity and specificity of a new selective medium, called Oxoid Brilliance™ MRSA for the identification of MRSA, using a well-defined collection of strains.

Materials and methods

A collection consisting of 266 MRSA strains, 257 methicillin-susceptible *S. aureus* (MSSA) strains, and 265 coagulase-negative staphylococci (CNS) was used. The collection was described previously [8–10]. In short, the MRSA isolates were collected in the Netherlands between 1989 and 1998 and were obtained from the MRSA strain collection of the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Identification of strains as *S. aureus* and as being methicillin-resistant was performed by duplex PCR for the *mecA* gene and the coagulase gene [11]. Bacteriophage typing was performed as described previously [12, 13], by using the international set of phages at 1 and 100 times the routine test dilution concentrations, an additional set of Dutch phages, and a set of experimental MRSA phages. The 266 MRSA isolates included in the study comprised 247 different phage types. Three isolates were not typable. All clonal lineages were represented with the exception of the recently emerged animal-related strains, ST398 [4]. CNS strains were confirmed as coagulase-negative using both DNase test and Remel Staphaurex® Plus. The MSSA and CNS strains were isolated from cultures of blood from patients who were admitted to the following hospitals between January 1996 and May 1999: St. Elisabeth Hospital and Tweesteden Hospital, Tilburg, the Netherlands; Pasteur Hospital, Oosterhout, the Netherlands; Tweesteden Hospital, Waalwijk, the Netherlands; and

Amphia Hospital, Breda, the Netherlands. Only one isolate per patient was included. Isolates were identified by a latex agglutination test (Staphaurex Plus; Thermo Fisher Scientific, Dartford, UK), by the detection of free coagulase using a tube coagulase test with rabbit plasma, and by the detection of DNase (Oxoid DNase agar; Thermo Fisher Scientific, Basingstoke, UK). If the results of these tests were discordant, an AccuProbe culture identification test (Gen-Probe, San Diego, CA, USA) was performed according to the manufacturer's instructions, and this was considered the "gold standard." The *S. aureus* blood culture isolates were classified as methicillin-susceptible at the time of collection by broth microdilution susceptibility testing performed according to CLSI (formerly NCCLS) standards [14]. The isolates were stored at −70°C until they were tested.

Oxoid Brilliance™ MRSA is a new chromogenic medium for identification of MRSA in human specimens and was supplied as prepoured culture plates from Thermo Fisher Scientific (Basingstoke, UK). The composition of the chromogenic and selective mix is proprietary. On Oxoid Brilliance™ MRSA, MRSA strains form distinctive denim blue colonies (Fig. 1). The selective mixture inhibits MSSA strains, most bacteria not belonging to the genus *Staphylococcus*, and yeasts. Results can be read after 18 h, according to the manufacturer. The isolates were inoculated onto Columbia agar plates with 5% sheep blood and incubated for 24 h at 35°C. From the resulting cultures, a suspension of 0.5 McFarland was made, and subsequently, 10 µl was streaked onto an Oxoid Brilliance™ MRSA plate with a sterile loop using a three-streak dilution method. The results were read after 20 h of incubation at 35°C. Growth of colonies showing blue coloration was considered to be indicative of MRSA. No growth or colonies with colors other than blue was considered negative. The procedure was performed as recommended by the manufacturer. Discordant results of MRSA strains (no growth on Oxoid Brilliance™ MRSA) were confirmed by *mecA* gene and *S. aureus* PCR as described previously [11]. If the *mecA* PCR was negative the strain was removed from the analysis.

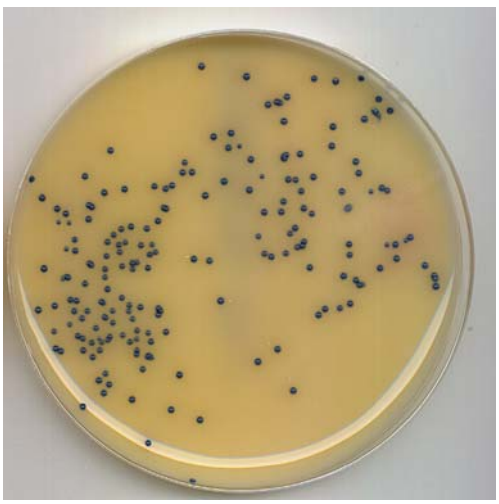

Fig. 1 Typical color and morphology of MRSA strains on Oxoid Brilliance™ MRSA plates

Results and discussion

The results obtained with Oxoid Brilliance™ MRSA are shown in Table 1. In the current evaluation, 29 of the 266 MRSA strains gave discordant results (10.9%). Subsequently, a PCR for the *mecA* gene was performed on these isolates and 28 strains had a negative result. These 28 MRSA strains were removed from the analysis, according to the protocol. After 20 h incubation the sensitivity of Oxoid Brilliance™ MRSA was 237 out of 238 (99.6% [95% CI 98.2%–99.9%]) and the specificity was 508 out of 522 (97.3% [95% CI 96.4%–98.2%]). The one MRSA strain that did not grow on the Oxoid Brilliance™ MRSA agar plate was tested for its cefoxitin susceptibility using disk diffusion. The cefoxitin zone diameter of this strain was 28 mm, which is considered susceptible according to the CLSI criteria [14].

Five of the MSSA strains and nine of the CNS strains also grew blue colonies ranging from light blue through the typical denim blue color and were reported as positive on Oxoid Brilliance™ MRSA agar. The five MSSA strains had cefoxitin zone diameters ranging from 24 to 30 mm, which is considered susceptible and falls within the susceptible range of the wild type strains [15]. The false-positivity rate of Oxoid Brilliance™ MRSA due to MSSA strains was lower than for CNS (Table 1), but these results were not statistically significant (P=0.42).

Oxoid Brilliance™ MRSA was a highly reliable screening tool for the detection of MRSA. All but one MRSA strain included in the analysis was detected after 20 h of incubation. In a recent study, the in vitro sensitivity and specificity of MRSA ID (bioMérieux, La-Balme-Les-Grottes, France) was evaluated using the same collection of strains [9]. A total of 251 MRSA strains, 249 MSSA strains, and 478 CNS strains

Table 1 Results for Oxoid Brilliance™ MRSA medium after 20 h of incubation

Isolate	Number of strains with a positive test result/total number of strains (%)
MSSA	5/257 (1.9)
CNS	9/265 (3.4)
Total (CNS + MSSA)	14/522 (2.7)
MRSA	237/238 (99.6)

were tested. The sensitivity of Oxoid Brilliance™ MRSA used in the present evaluation was significantly higher than MRSA ID in the previous evaluation (99.6% [237 out of 238] vs 96.4% [242 out of 251] respectively, P=0.021). The specificity of Oxoid Brilliance™ MRSA was lower than that of MRSA ID (97.3% [508 out of 522] and 98.2% [714 out of 727] respectively), but this difference was not significant (P=0.33). In another study, Diederen et al. evaluated the in vitro sensitivity and specificity of CHROMagar MRSA (CHROMagar Microbiology, Paris, France), using the same collection of strains [8]. A total of 216 MRSA strains and 241 MSSA strains were tested. Oxoid Brilliance™ MRSA medium used in the present evaluation has a higher sensitivity at 24 h of incubation than CHROMagar MRSA medium (99.6% [237 out of 238] and 95.4% [206 out of 216] respectively, P=0.004). After 48 h of incubation the sensitivity of CHROMagar MRSA raised to 100%. The specificity of Oxoid Brilliance™ MRSA medium, considering MSSA, was lower at 24 h of incubation than that of CHROMagar MRSA medium (98.1% [252 out of 257] and 100% [241 out of 241] respectively), but this difference was not statistically significant (P=0.06).

A remarkable finding is the absence of the *mecA* gene in 28 MRSA strains. One might speculate that these strains were not the original strains in the collection. However, we have shown in a previous evaluation that this was due to loss of the *mecA* gene in the freezer. In that study, 36 (14.4%) of 250 methicillin-resistant *Staphylococcus aureus* isolates had lost the *mecA* gene [16]. In the current evaluation, 28 (10.5%) of 266 previously confirmed MRSA strains no longer harbored the *mecA* gene. These findings have important implications for the management of strain collections as well as the use of strain collections for an in vitro evaluation. In order to avoid an underestimation of the sensitivity of the MRSA screening test under evaluation, discordant test results must be checked for the presence of the *mecA* gene at that moment in time.

The strength of this study is that we used a well-defined collection of strains with many different strains. Therefore, we can conclude that this medium is able to detect almost all MRSA strains that can be found in clinical samples. There are also several limitations to this study. First, a relatively high inoculum was used of isolates in pure culture. In clinical samples there will usually be a relatively low amount of MRSA and there are other species present as well. This could lead to more false-negative results in a clinical evaluation, compared with our results. Also, the growth of species other than CNS was not evaluated. This may lead to more false-positive results when Oxoid Brilliance™ MRSA medium is used on clinical samples. The performance on patient samples has to be determined in a prospective clinical evaluation. Kilgour et al. evaluated Oxoid Brilliance™ MRSA, CHROMagar MRSA and MRSA ID for the detection of MRSA [17]. A total of 300 freshly collected swabs from a total of 99 patients were analyzed in this study. When combined results for all media were analyzed, 269 of the 300 clinical samples were identified as negative for MRSA. Oxoid Brilliance™ MRSA medium correctly identified the highest number of samples positive for MRSA (28 out of 31) within 24 h, yielding a sensitivity of 90.3%. CHROMagar and MRSA ID required 48 hours incubation and both media yielded sensitivities of 83.9% (26 out of 31). The highest specificity was also achieved by Oxoid Brilliance™ MRSA (97.4%), which yielded only 7 false-positive results. CHROMagar and MRSA ID yielded 30 and 33 false-positives, providing specificities of 88.8% and 87.7% respectively. This small scale clinical study confirms the results of our study.

In conclusion, Oxoid Brilliance™ MRSA is a sensitive and a specific tool for differentiation between MSSA and CNS/MRSA in vitro. Oxoid Brilliance™ MRSA is able to detect a large number of different MRSA strains after only 20 h of incubation. Therefore, with Oxoid Brilliance™ MRSA, optimal results can be obtained within a day. In addition, further clinical studies will be performed to determine the utility of Oxoid Brilliance™ MRSA for the detection of MRSA directly from clinical samples.

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