Journal of Medical Microbiology (2011), **60**, 905–908 DOI 10.1099/jmm.0.021964-0

Clinical evaluation of Oxoid *Brilliance* MRSA Agar in comparison with bioMérieux MRSA ID medium for detection of livestock-associated meticillin-resistant *Staphylococcus aureus*

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Received 4 May 2010
Accepted 10 March 2011

Oxoid *Brilliance* MRSA Agar and bioMérieux MRSA ID medium were evaluated for their ability to identify meticillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples. Nasal and throat samples ($n=629$) were taken from veterinarians and their household members. The sensitivities of *Brilliance* MRSA Agar and MRSA ID medium after 20 h of incubation were 63.6 and 64.5 %, and the specificities were 94.1 and 99.4 %, respectively. After an enrichment step, the sensitivities increased to 96.3 and 97.2 %, but the specificities decreased to 88.7 and 98.5 %, respectively. *Brilliance* MRSA Agar and MRSA ID medium are both sensitive methods for the screening of MRSA in combination with broth enrichment, but positive results require confirmation.

INTRODUCTION

Meticillin-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 (Archer, 1998; Noskin *et al.*, 2007). Recently, MRSA has emerged in the community (Kluytmans-Vandenbergh & Kluytmans, 2006). A new clone has also been identified that is related to an extensive reservoir in animals. Persons who are in direct contact with pigs frequently carry livestock-associated MRSA. These isolates belong mainly to multilocus sequence type 398 (ST398) (van Loo *et al.*, 2007; Voss *et al.*, 2005). The extension of MRSA beyond its known boundaries poses an additional challenge for microbiological laboratories to improve their screening strategies. Screening for MRSA among various human populations with increased risk for carriage is an essential component of MRSA control strategies. This includes both patients and healthcare workers (Muto *et al.*, 2003). In human healthcare settings, different procedures employed for the detection of MRSA from clinical samples have variable results (Brown *et al.*, 2005). Methods to detect 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 reliably, selective media have been developed (Merlino *et al.*, 2000).

The purpose of this study was to evaluate the sensitivity and specificity of a selective medium, Oxoid *Brilliance* MRSA Agar, for the identification of MRSA, using nose and throat samples taken from veterinarians and their household members in an ongoing study.

Abbreviations: MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-sensitive *S. aureus*.

METHODS

Oxoid *Brilliance* MRSA Agar is a chromogenic medium for identification of MRSA in human specimens and was supplied as prepared culture plates from Thermo Fisher Scientific. The composition of the chromogenic and selective mix is proprietary. MRSA isolates form distinctive denim-blue colonies on *Brilliance* MRSA Agar. The selective mixture inhibits meticillin-susceptible *S. aureus* (MSSA) strains, most bacteria not belonging to the genus *Staphylococcus* and yeasts. Results can be read after 18 h, according to the manufacturer.

Nose and throat samples were taken from veterinarians who worked mainly with pigs. The household members were also sampled and the samples were subsequently sent by mail in a transport container with Amies medium (Transwab; Medical Wire & Equipment) to the Laboratory for Microbiology and Infection Control in the Amphia Hospital, The Netherlands. A total of 629 freshly collected swabs from a total of 318 patients were analysed in this study. Samples were inoculated onto both *Brilliance* MRSA Agar and MRSA ID medium (bioMérieux), and diluted with a sterile loop using a three-streak dilution method. In addition, broth enrichment comprising Mueller–Hinton broth supplemented with 6.5 % NaCl was carried out using

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the same swabs. From the overnight broth enrichment, subcultures were inoculated onto *Brilliance* MRSA Agar and MRSA ID medium. The directly inoculated and overnight enriched inoculated plates were read separately after 18–24 h incubation at 35–37 °C by different laboratory technicians on different benches. The procedure was performed as recommended by the manufacturer. Growth of colonies showing blue coloration on *Brilliance* MRSA Agar plates or green coloration on MRSA ID agar plates was considered to be indicative of MRSA. No growth, or colonies with colours other than blue or green, were considered negative. Presumptive *S. aureus* colonies were initially identified by a latex agglutination test (Staphaurex Plus; Thermo Fisher Scientific) and by the detection of DNase (Oxoid DNase agar; Thermo Fisher Scientific). If the results of these tests were discordant, isolates were identified by detection of free coagulase using a tube coagulase test with rabbit plasma. When isolates were identified as *S. aureus*, their meticillin susceptibility was determined using a cefoxitin disc diffusion test according to Clinical and Laboratory Standards Institute standards (NCCLS, 1997). All cefoxitin-resistant isolates were confirmed to be MRSA using a duplex PCR for the *mecA* and coagulase genes, as described previously (Kluytmans *et al.*, 2002; van Griethuysen *et al.*, 1999), and this was considered the 'gold standard'. When MRSA was recovered from one or more of the media, the sample was considered positive.

RESULTS AND DISCUSSION

A total of 629 freshly collected nasal and throat samples were analysed in this study. In total, there were 318 nose samples and 311 throat samples. When combined results for all media were analysed, 107 of the 629 samples (17.0 %) harboured MRSA. The presence of MRSA in nose samples was comparable with that in throat samples [60/318 (18.9 %) vs 47/311 (15.1 %), respectively; $P=0.243$]. The prevalence of MRSA in persons with direct contact with pigs was significantly higher than in persons who had not had contact with pigs [52/86 (60.5 %) vs 18/232 (8.4 %); $P<0.001$]. Of the 168 samples obtained from veterinarians, 83 (49.4 %) harboured MRSA. In contrast, only 24 of the 461 (5.2 %) samples from the household members were positive for MRSA. There were no significant differences in the performance of the two chromogenic agar plates with regard to specimen type. The results obtained with direct inoculation of the media and after broth enrichment are shown in Table 1. The sensitivity of directly inoculated *Brilliance* MRSA Agar was comparable with that of directly inoculated MRSA ID medium [68/107 (63.6 %) vs 69/107 (64.5 %), respectively; $P=1.0$]. However, the specificity of directly inoculated *Brilliance* MRSA Agar was significantly lower than that of MRSA ID medium [491/522 (94.1 %) vs 519/522 (99.4 %), respectively; $P<0.001$]. The negative predictive values of directly inoculated *Brilliance* MRSA and MRSA ID medium were comparable [491/530 (92.6 %) vs 519/557 (93.2 %), respectively; $P=1.0$]. The positive predictive value of directly inoculated *Brilliance* MRSA Agar was significantly lower than that of MRSA ID medium [68/99 (68.7 %) vs 69/72 (95.8 %), respectively; $P<0.001$]. The addition of broth enrichment resulted in a substantial increase in the yield of MRSA for both agar plates. *Brilliance* MRSA Agar recovered 103 MRSA strains, resulting in a sensitivity of 96.3 %. This was comparable to the sensitivity of MRSA ID medium after broth enrichment [104/107 (97.2 %); $P=1.0$]. The highest specificity following enrichment was achieved by MRSA ID medium (98.5 %), which yielded only eight false-positive results. The number of false-positives was much higher for *Brilliance* MRSA Agar (59 false-positives), resulting in a specificity of 88.7 % ($P<0.001$). The colours of the colonies ranged from light blue to the specific denim-blue coloration considered typical for *Brilliance* MRSA Agar. In combination with broth enrichment, the negative predictive value of *Brilliance* MRSA Agar was comparable to that of MRSA ID medium [463/467 (99.1 %) vs 514/517 (99.4 %), respectively; $P=1.0$]. However, the positive predictive value of *Brilliance* MRSA Agar was significantly lower than that of MRSA ID medium following enrichment [103/162 (63.6 %) vs 104/112 (92.9 %), respectively; $P<0.001$].

Several studies have recently evaluated various novel chromogenic selective media for the detection of MRSA from clinical samples (Malhotra-Kumar *et al.*, 2010; Nonhoff *et al.*, 2009; Peterson *et al.*, 2010). Comparison of the results of these studies is not easy because of differences in the study designs: swab type, body site sampled, direct inoculation versus prior homogenization in saline or broth, incubation time and the use of broth enrichment. In the present study, we evaluated the performance of *Brilliance* MRSA Agar with and without

Table 1. Analytical performance of *Brilliance* MRSA Agar and MRSA ID medium in a prospective clinical study of nasal and throat samples ($n=629$, including $n=107$ MRSA-positive samples)

TP, True-positive; FP, false-positive; TN, true-negative; FN, false-negative; PPV, positive predictive value; NPV, negative predictive value.

Medium	Method	TP	FP*	TN	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Brilliance MRSA	Direct only	68	31	491	39	63.6	94.1	68.7	92.6
	Direct + enrichment	103	59	463	4	96.3	88.7	63.6	99.1
MRSA ID	Direct only	69	3	519	38	64.5	99.4	95.8	93.2
	Direct + enrichment	104	8	514	3	97.2	98.5	92.9	99.4

*False-positive indicates all colonies showing blue coloration on *Brilliance* MRSA Agar or colonies showing green coloration on MRSA ID medium that were not confirmed to be MRSA.

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broth enrichment in a population with a high prevalence of MRSA carriage. *Brilliance* MRSA Agar had a high sensitivity when used in combination with broth enrichment. However, without broth enrichment the sensitivity was significantly lower. This confirms the importance of broth enrichment for the accurate detection of MRSA in clinical samples (Malhotra-Kumar *et al.*, 2010; Nonhoff *et al.*, 2009).

It is difficult to compare our findings with those in other studies as there are few clinical studies that have used *Brilliance* MRSA Agar. However, other chromogenic media have been studied. For example, a study by Nonhoff *et al.* (2009) evaluated the performance of MRSA ID, MRSA-Screen and MRSA*Select* in combination with broth enrichment for the detection of MRSA in 1002 mucocutaneous swabs from 639 hospitalized patients. Swabs were plated on the three chromogenic media. Subsequently, broth enrichment subculture was carried out by overnight incubation on the different agar plates. MRSA strains were isolated from 68 (6.8 %) specimens from 44 patients. The sensitivity of all the chromogenic media was <50 % after 18 h incubation but increased with prolonged incubation of 42 h to 75, 80.9 and 72.1 % for MRSA ID, MRSA-Screen and MRSA*Select*, respectively. The difference in sensitivity between MRSA-Screen and MRSA*Select* after 42 h was significant. After enrichment, the sensitivity for all media was 85.3 % at 24 h incubation. The specificity was excellent for MRSA ID (99.9 %) and MRSA*Select* (100 %) after 18 h and lower for MRSA-Screen (97.2 %), although this was not statistically significant.

Another recently published study that reported similar findings was performed by Malhotra-Kumar *et al.* (2010). They evaluated and compared the potential for MRSA detection of five chromogenic media – *Brilliance* MRSA Agar (Oxoid), ChromID (bioMérieux), MRSASelect (Bio-Rad), CHROMagar (CHROMagar-Microbiology) and BBL-CHROMagar (BD Diagnostics). The media were tested on log serial dilutions of pure isolates of MRSA (n=60), non-MRSA (n=27) and defined mixtures simulating clinical samples (n=84). Further evaluations were carried out on pre-enriched nasal and groin screening swabs (n=213) from 165 hospitalized patients. The cumulative mean sensitivity on isolates, mixtures and clinical samples was highest for *Brilliance* (97 %) and similar for the other four media (⩾92 %). The cumulative mean specificity was highest for BBL-CHROMagar (99 %), followed by MRSASelect (98 %), CHROMagar (97 %), ChromID (89 %) and *Brilliance* (86 %). False-positive results were mainly due to meticillin-resistant *Staphylococcus epidermidis*. The two studies described above confirm the additional yield of broth enrichment and are comparable to our findings.

In contrast, Peterson *et al.* (2010) reported sensitivities that were different from our findings. They compared the performance of *Brilliance* MRSA Agar with traditional media in detecting MRSA. In this study, 767 nasal samples from a multi-centre study and 667 nasal samples from a tertiary hospital were screened for the presence of MRSA. After 24 h of incubation, the sensitivity and specificity were as follows: (i) multi-centre study: *Brilliance* MRSA Agar, 95.4 and 99.7 %; tryptic soy agar with 5 % sheep blood, 93.6 and 100 %; (ii) tertiary hospital: *Brilliance* MRSA Agar, 95.2 and 99.5 %; mannitol salt agar, 88.7 and 94.0 %, respectively. Extending the incubation to 48 h did not significantly improve the recovery of MRSA and resulted in decreased specificity. The higher sensitivity found in this study can easily be explained by the lack of broth enrichment in this comparison. However, this cannot explain the much higher specificity. The discrepancy between the sensitivities obtained in the above-mentioned study in comparison with the direct plating in our study is most probably caused by the addition of broth enrichment in our culture procedure. The differences in performance values may be predominantly due to the use of suboptimal media as the 'gold standard'. Another explanation for the discrepancy between the specificity found in our evaluation and in some other studies may be that we screened a specific population, i.e. veterinarians and their household members, who carry a specific clone of MRSA, named livestock-associated MRSA. Therefore, our results do not necessarily apply to other types of MRSA.

In the present work, the evaluation of the performance of chromogenic media for MRSA detection in screening swab samples in a high-prevalence population demonstrated a low sensitivity (64–65 %) of the two media after 20–24 h of incubation. However, the sensitivity of both media was increased significantly by using an enrichment step.

A selection of the false-positive colonies that grew on the *Brilliance* MRSA Agar were identified. Sixty per cent belonged to the genus *Bacillus*, 24 % were coagulase-negative staphylococci and 16 % were MSSA. After performing the additional identification tests recommended by the manufacturers, specificity was improved significantly.

The results of our study have one important limitation: we did not use a non-selective blood agar plate as the 'gold standard'. This could have increased the number of MRSA isolates recovered from the samples. However, this would have resulted in even lower sensitivities compared with the current evaluation.

Despite commercial efforts to develop new media with high sensitivity, the present study, as well as others (Malhotra-Kumar *et al.*, 2010; Nonhoff *et al.*, 2009), showed a substantial increase in sensitivity following enrichment compared with direct cultures. Therefore, broth enrichment remains necessary for reliable MRSA detection. Indeed, in our study, if screening samples had only been plated directly and inspected after 20–24 h, as suggested by the manufacturers, 29.9 % of the MRSA-positive samples detected would have been missed. In contrast, only one MRSA-positive sample (0.9 %) would have been missed if samples had been grown exclusively after enrichment.

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In summary, *Brilliance* MRSA Agar performed similarly to MRSA ID medium with respect to sensitivity but with a significantly lower specificity with and without broth enrichment. An advantage of *Brilliance* MRSA Agar is that this medium is less light sensitive than MRSA ID medium, making it easier to work with in the laboratory. The additional yield of the broth enrichment was comparable to what has been reported previously and has to be considered as the standard for screening of MRSA.

In conclusion, *Brilliance* MRSA Agar in combination with broth enrichment is a sensitive method for MRSA detection in people exposed to livestock. However, the specificity is relatively low and suspected colonies need to be confirmed by further testing.

ACKNOWLEDGEMENTS

The chromogenic medium Oxoid *Brilliance* MRSA Agar was kindly provided by Thermo Fisher Scientific (Basingstoke, UK). J. K. has received research grant support from Thermo Fisher Scientific and bioMérieux.

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