

Clinical evaluation of Bio-Rad MRSASelect™ medium for the detection of livestock-associated methicillin-resistant *Staphylococcus aureus*

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Abstract Bio-Rad MRSASelect™ medium was evaluated for its ability to recover methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal samples of pig farmers and their household members. In total, 257 samples were inoculated on Bio-Rad MRSASelect™ medium with and without broth enrichment and on bioMérieux MRSA ID with broth enrichment. A sample was considered to be positive if at least one of the media grew MRSA. The sensitivity of Bio-Rad MRSASelect™ medium without broth enrichment was 63.9%. With broth enrichment, the sensitivity increased to 98.4%. The specificity was 95.4% both with and without broth enrichment. In conclusion, Bio-Rad MRSASelect™ medium as well as MRSA ID medium are reliable methods to detect MRSA carriage when used in combination with broth enrichment. The directly inoculated MRSASelect™ medium was statistically significantly less sensitive than the two media after broth enrichment.

First described in 1961, 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]. Since the 1990s, MRSA has also emerged in the community [3]. In addition, a new clone has been identified, which is related to an extensive reservoir in animals. Persons who are in direct contact with pigs frequently carry this livestock-associated MRSA (LA-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]. Methods to detect MRSA in clinical samples should ideally 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 [7]. The extension of MRSA beyond its previously known boundaries to livestock-related persons poses a challenge for microbiological laboratories to improve their screening strategies. The purpose of this study is to evaluate the in vivo sensitivity and specificity of a new selective medium for the detection of MRSA from nasal samples taken from people living or working on pig farms.

MRSASelect™ is a chromogenic medium for the identification of MRSA in human specimens and was supplied as preprepared culture plates from Bio-Rad, Hercules, California, USA. The composition of the chromogenic and selective mix is proprietary. On MRSASelect™, MRSA strains form distinctive pink colonies. The selective mixture inhibits methicillin-susceptible *S. aureus* (MSSA) strains, most bacteria not belonging to the genus *Staphylococcus*, and yeasts. Results can be read after 18–28 h, according to the manufacturer.

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Nasal samples were taken from pig farmers, their co-workers, and their household members as part of an ongoing study and sent by mail to the Laboratory for Microbiology and Infection Control at the Amphia Hospital, the Netherlands. Samples were inoculated onto *MRSASelect*TM medium and diluted with a sterile loop using a three-streak dilution method. In addition, a broth enrichment containing Mueller–Hinton broth with 6.5% NaCl was inoculated using the same swab, and this was incubated overnight at 35°C. Subsequently, 10 µl of the broth enrichment was inoculated onto both *MRSASelect*TM and *MRSA ID* (bioMérieux, La Balme Les Grottes, France). Both sets of plates were read after 18–28 h of incubation at 35–37°C. Growth of colonies on the *MRSASelect*TM agar plates showing pink coloration or growth of colonies with green coloration on the *MRSA ID* agar plates were considered to be indicative for MRSA. No growth or colonies with colors other than pink or green were considered to be negative. Presumptive *S. aureus* colonies were further identified by a latex agglutination test (SlidexTM Staph-Kit; bioMérieux, La Balme Les Grottes, France), and by the detection of DNase (Oxoid DNase agar; Thermo Fisher Scientific, Basingstoke, UK). When isolates were identified as *S. aureus*, the methicillin susceptibility was tested using the cefoxitin disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee on Clinical Laboratory Standards [NCCLS]) standards [8]. All cefoxitin-resistant isolates were confirmed to be MRSA using a duplex polymerase chain reaction (PCR) assay for the *mecA* gene and the coagulase gene as described previously [9, 10], and this was considered to be the “gold standard.” When MRSA was recovered from one or more of the media, the sample was considered to be positive.

A total of 257 freshly collected nasal samples originating from different individuals were analyzed in this study. When combined results for all media were analyzed, 61 of the 257 (23.7%) samples were identified as positive for MRSA. The results obtained with *MRSASelect*TM medium and *MRSA ID* medium are shown in Table 1. When used in combination with broth enrichment, the sensitivity of *MRSASelect*TM was comparable to *MRSA ID*. The directly inoculated *MRSASelect*TM was statistically significantly less sensitive than the two media after broth enrichment.

After broth enrichment, *MRSASelect*TM grew nine and *MRSA ID* grew ten false-positive strains. These false-positive strains were not further identified. This results in specificities of 95.4 and 94.9%, respectively ($p=1.0$). The specificity of the directly inoculated *MRSASelect*TM medium was also 95.4%. In combination with broth enrichment, the positive predictive values (PPV) of *MRSASelect*TM and of *MRSA ID* were 87.0 and 85.9%, respectively ($p=1.0$). The negative predictive values (NPV) were 99.5 and 100.0%, respectively ($p=1.0$). The PPV and NPV of directly inoculated *MRSASelect*TM were 81.3 and 89.5%, respectively.

We evaluated the in vivo performance of *MRSASelect*TM medium and *MRSA ID* medium with and without broth enrichment in a population with a high prevalence of MRSA carriage. Both media had high sensitivities and adequate specificities when used in combination with broth enrichment. However, without broth enrichment, the sensitivities were significantly lower. This confirms the importance of broth enrichment for the accurate detection of MRSA in clinical samples [11].

The sensitivity and specificity found in this clinical evaluation are significantly lower than recent findings in other studies [12, 13]. Stoakes et al. [12] compared *MRSASelect*TM medium with CHROMagar, mannitol-salt agar with oxacillin, and mannitol-salt agar with cefoxitin. In this study, nasal and perineal swabs were routinely collected from all patients admitted to the hospital who had been admitted to any health care facility in the previous 6 months. They processed a total of 2,125 (1,243 nasal and 882 perineal) consecutive swabs and found that three from the 111 confirmed MRSA strains were not detected by *MRSASelect*TM medium (sensitivity of 97.3%). Ben Nsira et al. [13] screened 666 samples using *MRSASelect*TM medium and three other selective media. Ninety-nine samples were positive for MRSA on at least one selective medium. Only one MRSA isolate was missed on *MRSASelect*TM medium (sensitivity of 99.8%). The discrepancy between the sensitivities obtained in these two studies in comparison to the direct plating in our study is most likely caused by the addition of broth enrichment in our culture procedure. This improves the sensitivity of the reference method substantially and,

Table 1 Analytical performance of *MRSASelect*TM and *MRSA ID* medium in a prospective clinical study of nasal swabs ($n=257$, including $n=61$ MRSA-positive samples)

Medium	Method	True-positive	False-positive	Sensitivity %	Specificity %	PPV %	NPV %
<i>MRSASelect</i> TM	Direct	39	9	63.9	95.4	81.3	89.5
	Enrichment	60	9	98.4	95.4	87.0	99.5
<i>MRSA ID</i>	Enrichment	61	10	100	94.9	85.9	100

PPV, positive predictive value; NPV, negative predictive value

thereby, reduces the sensitivity of the test that is evaluated (in this case, the direct plating method). 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., pig farmers and their household members, who carry a specific clone of MRSA, namely, LA-MRSA, and these strains all belong to multilocus sequence type 398 (ST398) [4, 5, 16, 17]. Therefore, our results do not necessarily apply to other types of MRSA.

A study that reported similar results to our findings was performed by van Loo et al. [11]. In total, they tested 3,000 samples from 409 patients for the presence of MRSA. Every sample was directly inoculated onto an MRSASelect™ medium. Fifty-five out of 70 true-positive samples (sensitivity 78.6%) were detected by the MRSASelect™ medium after 48 h of incubation. Subsequently, the investigators subcultured 225 samples from a broth enrichment containing Mueller–Hinton broth with 6 µg/ml oxacillin and 6 µg/ml aztreonam on the MRSASelect™ medium. This increased the yield of MRSA containing samples by 12%. Our increase was much larger, which may be explained by the choice of the type of broth enrichment. However, the present study used a less selective broth enrichment than van Loo et al. A recent study found that the broth enrichment as used by van Loo et al. resulted in suboptimal performance [14].

In another recent study, Nonhoff et al. [15] evaluated the performance of three chromogenic media, MRSA ID, MRSA Screen, and MRSASelect™, in combination with broth enrichment for the detection of MRSA in 1,002 mucocutaneous swabs from 639 hospitalized patients. A sample was considered to be positive when MRSA was isolated from any of the three media tested. MRSA strains were isolated from 68 (6.8%) specimens from 44 patients. The sensitivity of all chromogenic media was <50% after 18 h of incubation, but increased with prolonged incubation at 42 h to 75, 80.9, and 72.1% for MRSA ID, MRSA Screen, and MRSASelect™, respectively. The difference in sensitivity between MRSA Screen and MRSASelect™ after 42 h was significant. After enrichment, the sensitivity for all media was 85.3% at 24 h of incubation. The specificity was excellent for MRSA ID (99.9%) and MRSASelect™ (100%) after 18 h, and lower for MRSA Screen (97.2%), albeit not statistically significantly so. This study confirms the additional yield of broth enrichment and is comparable to our findings. Comparison of the results of all the above-mentioned studies with our current evaluation is not easy because of differences in the study design: swab type, body site sampled, direct inoculation versus prior homogenization in saline or broth, incubation time, use or not of broth enrichment, and, most importantly, the nature of the test chosen as the gold standard.

The results of our study have one important limitation. We sampled a specific population, i.e., pig farmers, who

carry a specific clone of MRSA, namely, LA-MRSA. Therefore, the performance may be different for other types of MRSA. There are several studies which reported that persons working with pigs frequently carry LA-MRSA [4, 16, 17]. van Loo et al. [4] performed a case–control study and showed that carriers of LA-MRSA were more often pig or cattle farmers. Thirty-four of 35 case-patients carried ST398; one had ST9. In addition, Wulf et al. [16] reported that veterinarians at an international conference on pig health frequently carried MRSA. Thirty-one of the 34 isolates were non-typeable by pulsed-field gel electrophoresis (PFGE) following *Sma*I digestion of chromosomal DNA. All of the non-typeable isolates belonged to *spa* types that correspond to ST398 (t011, t034, t108, t571, t567, and t899). Finally, van den Broek et al. [17] demonstrated that intensive and repeated exposure to pigs is an important factor in MRSA colonization and that this is almost always ST398. Although we did not perform molecular typing, it is beyond reasonable doubt that the vast majority of the MRSA strains recovered in our study belonged to ST398. The conclusion is that MRSASelect™ medium and MRSA ID medium in combination with broth enrichment are useful tools for the detection of LA-MRSA in nasal samples. In this study, the MRSASelect™ medium and MRSA ID medium were equivalent and can be used indifferently. The additional yield of the broth enrichment was comparable to what has been reported previously and is considered to be the standard for screening of MRSA. After direct inoculation or subculture of broth enrichment, typical MRSA colonies need to be confirmed by further testing.

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