Evaluation of different chromogenic media for the detection of methicillin-resistant *Staphylococcus aureus* CC398 in broilers

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Abstract Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has emerged in a wide variety of animal species, including poultry. The objective of this study was to evaluate three different chromogenic media for MRSA clonal complex (CC) 398 detection in broilers. On three Belgian poultry farms, 50 broiler chickens were sampled per farm from both nose shell and cloaca. All swab specimens were enriched and inoculated the following day on three chromogenic media: chromID MRSA (bioMérieux), *Brilliance* MRSA 2 Agar (Oxoid) and *MRSASelect* (Bio-Rad). ChromID had the highest isolation rates, yet, *Brilliance* MRSA 2 Agar demonstrated the highest relative sensitivity, while *MRSASelect* and *Brilliance* MRSA 2 Agar showed the highest relative specificity. A subset of MRSA isolates was confirmed to be CC398 by the polymerase chain reaction (PCR) targeting *sauI-hsdS1*. In conclusion, *Brilliance* MRSA 2 Agar outperformed *MRSASelect* and chromID MRSA for the detection of MRSA in broilers.

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Introduction

Since 2005, an increasing number of reports on livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been published, particularly in pigs, but also in cattle, horses and poultry. In Europe, the majority of LA-MRSA belongs to clonal complex (CC) 398 [1]. The few studies that investigated the prevalence of LA-MRSA in poultry flocks appear to show a low within-flock prevalence [2–5]. This is in contrast to the high within-herd prevalence described in pigs in numerous studies [6–8]. The performances of chromogenic media for MRSA detection have previously been studied in pigs [9, 10] and veal calves [9]. It is of importance also to know which medium is optimal for use in broiler populations. Because, so far, no comparative studies have been performed in broilers, the present study aimed at evaluating three different chromogenic media [chromID MRSA (bioMérieux), *Brilliance* MRSA 2 Agar (Oxoid) and *MRSASelect* (Bio-Rad)] for MRSA detection in broilers.

Materials and methods

Sample collection and processing

This study was conducted between March and July 2011 on three mixed poultry–pig farms (Farms A, B and C) in Belgium. Samples were collected from 150 broiler chickens (50 per farm) between 5 and 6 weeks of age from two anatomical sampling sites (nose shell and cloaca) with separate swabs. The selection of animals was done as follows: in each corner and in the middle of the poultry stable, ten broiler chickens were sampled by convenience.

Sampling was performed by using a rayon-tipped sterile dry swab (Copan Innovation, Brescia, Italy) inserted into

Mueller–Hinton broth (MHB; Oxoid, Basingstoke, UK) and supplemented with 6.5 % sodium chloride. After sampling, the swabs were transported within the hour to the laboratory under cooled conditions. After overnight (18–20 h at 37 °C) incubation of the swabs in the salt-enriched MHB, 1 µl was inoculated the following day using the four-way-streaking-plate-method on the three different chromogenic media (chromID MRSA, *Brilliance* MRSA 2 Agar and *MRSASelect*), following the instructions for use provided by the respective manufacturers. All media were allowed to warm to room temperature and were protected from light before inoculation. After 24 h of incubation at 37 °C, a total of 900 plates (150 broilers × 2 sampling sites × 3 different media) were read and interpreted, according to the manufacturers' instructions (technical files). Suspect colonies were identified by characteristic growth morphology and colour (pale to dark green and round colonies on chromID MRSA due to alpha-glucosidase production, denim-blue colonies due to phosphatase activity easy to read against the opaque background on *Brilliance* MRSA 2 Agar and small pink colonies on *MRSASelect*; the composition of the chromogenic mix is proprietary). The ATCC 43300 (MRSA) reference strain was included to verify the integrity of the media. One typical colony per suspected positive plate was purified for MRSA confirmation by plating on the corresponding MRSA-selective medium, followed by plating on tryptone soy agar (TSA; CM0131; Oxoid, Basingstoke, UK) and overnight incubation at 37 °C.

MRSA confirmation

DNA was extracted from a phenotypically suspected colony according to the method of Strandén et al. [11] and DNA lysates were stored at −20 °C until further use.

MRSA isolates were confirmed by multiplex polymerase chain reaction (PCR) targeting a *Staphylococcus*-specific 16S rRNA sequence, the *mecA* gene coding for methicillin resistance and the *S. aureus*-specific region of the thermonuclease (*nuc*) gene [12]. DNA amplification was performed using the Applied Biosystems StepOnePlus Real-Time PCR System.

To confirm the presence of MRSA CC398, a selection of 100 MRSA isolates were tested with a PCR targeting *sau1-hsdS1*, developed for the rapid detection of *S. aureus* CC398 by Stegger et al. [13]. This was an at-random selection of MRSA isolates evenly distributed over the different chromogenic media.

Data analysis and statistical analysis

When MRSA was isolated from an enriched swab specimen on at least one of the three media and confirmed by multiplex PCR, the swab was considered to be positive (gold standard). A true-positive result was defined as when an

isolate picked from the given plate had a typical morphology and was confirmed to be MRSA by PCR. A false-positive result was defined as when the isolate picked from the given plate had a typical morphology but proved not to be MRSA by PCR. A false-negative result was defined as a given plate without typical colonies and where no isolate was picked from but from the same sample MRSA was found on at least one other plate. Finally, a true-negative result was when no isolate was picked from the given plate and neither was MRSA detected on any of the other plates.

MRSA isolation rates (IR) per sampling site (nose shell, cloaca) and per chromogenic medium were calculated as the proportion of samples testing positive by phenotype (colony morphology and colour) on that sampling site and that medium, respectively.

For each sampling site and for each of the three media, the relative sensitivity, relative specificity and the 95 % confidence intervals (CI) for MRSA detection were calculated. The relative sensitivity was calculated as the ratio of MRSA positives on that specific medium and sampling site (= true-positive results) to the total number of MRSA confirmed by PCR (gold standard) that were recovered from all media on the specific sampling site (= sum of the true-positive and the false-negative results). The relative specificity of a medium for a specific sampling site was calculated as the ratio of the true-negatives to the sum of the true-negative and the false-positive results. The 95 % CI of the relative sensitivity and relative specificity was also calculated ($x \pm \frac{1.96\sqrt{p(1-p)}}{n}$) with p being the sensitivity or specificity, respectively.

Results

IR and performance characteristics (number of MRSA true-positives, false-negatives and false-positives, relative sensitivity and relative specificity with corresponding 95 % CI) per sampling site (nose shell, cloaca) and per chromogenic medium are shown in Table 1. In both sampling sites, the most MRSA-positive results by phenotype were found on chromID MRSA, yet, both the relative sensitivity and relative specificity of chromID MRSA were low compared with the other media. ChromID MRSA detected more false-positives than *Brilliance* MRSA 2 Agar and *MRSASelect*. Furthermore, chromID MRSA and *MRSASelect* detected more false-negatives than *Brilliance* MRSA 2 Agar. *Brilliance* MRSA 2 Agar demonstrated, overall, the highest relative sensitivity for both sampling sites compared to *MRSASelect* and chromID MRSA. Regarding the relative specificity, *MRSASelect* and *Brilliance* MRSA 2 Agar showed the highest values for both sampling sites compared to chromID MRSA.

Table 1 Isolation rates (IR) and performance characteristics [number of methicillin-resistant *Staphylococcus aureus* (MRSA) true-positives, false-negatives and false-positives, relative sensitivity and relative specificity with corresponding 95 % confidence intervals (CI)] of three chromogenic MRSA screening media in a study carried out on three

Belgian poultry farms for all broiler swab specimens per anatomical sampling site (nose $n=150$ and cloaca $n=150$), including $n=50$ MRSA-positive samples for nose samples (gold standard) and $n=48$ for cloaca samples (gold standard)

Sampling site	Chromogenic medium	Isolation rates (IR)	Number of MRSA true-positives	Number of MRSA false-negatives	Number of MRSA false-positives	Relative sensitivity, % (95 % CI)	Relative specificity, % (95 % CI)
Nose	<i>Brilliance</i> MRSA 2	32.0 % (48/150)	47	3	1	94.0 (83.5–98.7)	99.0 (94.6–100.0)
	MRSASelect	26.7 % (40/150)	40	10	0	80.0 (66.3–90.0)	100.0 (96.4–100.0)
	chromID MRSA	40.7 % (61/150)	34	16	27	68.0 (53.3–80.5)	73.0 (63.2–81.4)
Cloaca	<i>Brilliance</i> MRSA 2	32.0 % (48/150)	48	0	0	100.0 (92.6–100.0)	100.0 (96.5–100.0)
	MRSASelect	24.7 % (37/150)	37	11	0	77.1 (62.7–88.0)	100.0 (96.5–100.0)
	chromID MRSA	34.0 % (51/150)	39	9	12	81.3 (67.4–91.1)	88.2 (80.4–93.8)

The PCR targeting *sauI-hsdSI* confirmed that all of the 100 tested MRSA isolates belonged to *S. aureus* CC398.

Discussion

The present study is the first comparing the performances of chromogenic media for MRSA detection in broilers. ChromID MRSA had the highest IR, while both the relative sensitivity and relative specificity of chromID MRSA were low in comparison to the other media. This high IR is explained by the fact that the definition of IR pertains to the phenotype, indicating that several false-positive isolates were found on this medium, which also explains the lower relative specificity. The lower relative sensitivity can be explained by the fact that chromID MRSA yielded the highest number of false-negative results. When taking the relative sensitivity and relative specificity into account, *Brilliance* MRSA 2 Agar outperformed MRSASelect and chromID MRSA for the detection of MRSA in broilers on both sampling sites. Thus, in terms of detecting the correct amount of MRSA positives, not only is a low number of false-positives (= high relative specificity) important, but also a low number of false-negatives (= high relative sensitivity) is necessary, as a low relative sensitivity will give an underestimation of the MRSA prevalence.

In a previous study of Graveland et al. [9], no statistically significant differences were found between the different media used for MRSA analysis of veal calf nasal swabs, while chromID MRSA had significantly lower rates of positive samples compared to MRSASelect and MRSA Screen (Oxoid, predecessor of *Brilliance* MRSA) for the analysis of pig nasal swabs. The current study cannot be compared to that of Graveland et al. [9], as another methodology for enrichment including antibiotics was used. In the study of Pletinckx et al. [10], the same methodology was

used as in the present study, and chromID MRSA had the highest relative sensitivity and relative specificity for the detection of MRSA in pigs. On chromID MRSA, a rate of false-positives of only 1 % (relative specificity=96 %) was found in pigs [10], in comparison to a false-positive rate of 13 % (39/300) (relative specificity=73 and 88 %) in the current study. On MRSASelect (Bio-Rad), a false-positive rate of 10 % (relative specificity=64 %) was found in pigs [10], whereas in the present study, no false-positives (relative specificity=100 %) were seen. *Brilliance* MRSA Agar used in the porcine study [10] can not, however, be compared to *Brilliance* MRSA 2 Agar in the present study, as Oxoid improved the latter medium by adding new inhibitory components and a new counter-stain, reducing the number of false-positives. Comparison of the performance characteristics of chromID MRSA and MRSASelect for pigs and broilers, respectively, indicates that the best MRSA screening method might be host-dependent, as differences in microbial flora could give rise to false-positives on the different media. Microbial flora might also differ according to the sampling site; however, in the current study, no difference was observed in the number of false-positives between nose and cloaca. The observer could represent a source of subjectivity, but all the plates in this study and in the porcine study [10] were read and interpreted by the same person, who was accustomed to working with chromogenic plates for the identification of MRSA. Differences in selective and elective components in the media may explain why some microorganisms give rise to false-positives on one medium and not on another.

The isolation of staphylococcal and non-staphylococcal organisms on chromogenic media designed for MRSA has been reported in a human MRSA study by Compennolle et al. [14]. Coagulase-negative staphylococci were a major cause of false-positive results, but, also, *Enterobacter* spp. resulted in false-positives on chromID MRSA [14]. In a

former study in broilers [5], coagulase-negative staphylococci and enterococci were isolated from chromID MRSA (data not shown). Those species can harbour the *mecA* gene [15, 16], encoding beta-lactam resistance, which may partly explain how they could give rise to false-positives on chromID MRSA (which contains cefoxitin, a beta-lactam antibiotic). Beta-lactam resistance could, however, also be mediated by *blaZ*, typically carried by a plasmid [17]. This and other factors that could contribute to yielding false-positives should be examined further. A high number of false-positives leads to extra costs, as more plates are used, and because all suspect MRSA isolates need to be confirmed by multiplex PCR.

Conclusion

The results show that screening with chromID MRSA, a chromogenic media that had the highest relative sensitivity and relative specificity in detecting livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in pigs in a previous study [10], is less useful for LA-MRSA screening in poultry, due to a higher number of false-positives and false-negatives. *Brilliance* MRSA 2 Agar outperformed MRSASelect and chromID MRSA for the detection of MRSA in broilers.

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Conflict of interest The authors declare that they have no conflict of interest and no financial or personal relations with people or organisations that could have inappropriately influenced this work.

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