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# Characterization of coagulase-negative staphylococci and macrococci isolated from cheese in Germany

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## ABSTRACT

Cheese, especially ripened varieties, harbor a very complex and heterogeneous microbiota. In addition to the desired microorganisms (starter cultures) added during cheese production, potentially harmful bacteria may also enter the production chain. Regarding the latter, the focus of this study was on coagulase-negative staphylococci (CNS) and *Macrococcus caseolyticus*. Both are known to harbor a variety of genes coding for antibiotic resistance, including *mec*A, *mec*B, *mec*C, and *mec*D. Coagulase-negative staphylococci or macrococci carrying such genes or other virulence factors should not be present in cheese. Cheese samples (101 in total) were collected from retail sources. Coagulase-negative staphylococci and *M. caseolyticus* were isolated utilizing selective agars, and species were identified by phenotypical tests and partial sequencing of the *sod*A gene. The results allowed identification of 53 CNS strains and 19 *M. caseolyticus* strains. Among the CNS, 11 isolates of *Staphylococcus saprophyticus* and one *Staphylococcus epidermidis* isolate were obtained. Both species are potential human pathogens and may thus adversely affect the safety of these food products. Screening for antimicrobial resistance was performed by application of disc diffusion tests, a gradient strip-test, and 14 different PCR tests. Evidence for methicillin resistance (by either positive disc diffusion assay for cefoxitin or by *mec* PCR) was found in CNS isolates and *M. caseolyticus* (9 isolates each). Regarding other virulence factors, no genetic determinants for coagulase or the most common staphylococcal enterotoxins *sea*, *seb*, *sec*, *sed*, and *see* were detected in any of the CNS or *M. caseolyticus* isolates by PCR testing. In conclusion, the presence of facultatively pathogenic CNS and carriers of genes for antibiotic resistance in both groups of microorganisms, especially *mec* genes, and the respective food safety issues need further evaluation and surveillance.

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## INTRODUCTION

Cheese, especially the ripened varieties, harbor a very complex and heterogeneous microbiota. In addition to the desired microorganisms, such as the starter cultures added during cheese production, which stem from the brining bath or the ripening process (Bockelmann and Hoppe-Seyler, 2001; Mounier et al., 2006; Montel et al., 2014; Stavropoulou et al., 2018), detrimental bacteria may also enter the processing chain (Stavropoulou et al., 2018; Hammer et al., 2019). This study focused on CNS and *Macrococcus caseolyticus*, which can be opportunistic pathogens if they carry or express genes for virulence factors and antibiotic resistance.

Regarding the CNS, the presence of *Staphylococcus saprophyticus* in cheese as a potential human pathogen needs attention (Hammer et al., 2019). Further, while *M. caseolyticus* is not currently considered a human pathogen, it appears to be an emerging pathogen in veterinary medicine (Schwendener et al., 2017; Li et al., 2018; MacFayden et al., 2018; Schwendener et al., 2019; Ramos et al., 2021).

Coagulase-negative staphylococci and *M. caseolyticus* are known to harbor a variety of genes encoding antibiotic resistance, including *mec*A, *mec*B, *mec*C, and *mec*D (Baba et al., 2009; International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements, 2009; Fontes et al., 2013; Chajęcka-Wierzchowska et al., 2015; Schwendener, et al., 2017; Schwendener, et al., 2019; Becker, 2021; da Silva Abreu et al., 2021). In addition, it has been shown that bacteria are able or at least have the potential to exchange genes with another species of the same genus or even a species of another genus (Alonso et al., 2001; Resch et al., 2008; Becker et al., 2018; Schwendener and Perreten, 2018). Therefore, when CNS or macrococci (as well as other bacteria) carrying genes coding for antibiotic resistance are present in cheese or any other foods, they can potentially cause harm to the consumer.

As shown in the literature cited in an earlier study (Hammer et al., 2019), CNS are capable of producing staphylococcal enterotoxins. In terms of *M. caseolyticus*, however, 10 strains from Turkish fermented sausage (Çetin and Tuncer, 2016) and 1 from camel milk (Njage et al., 2013) were previously shown to be incapable of enterotoxin production owing to a lack of respective genes.

The objective of this study was to characterize CNS and *M. caseolyticus* strains isolated from cheeses sold in Germany in terms of virulence factors and potential antibiotic resistance.

## MATERIALS AND METHODS

No animals were used in this study, and ethical approval for the use of animals was thus deemed unnecessary.

### Cheese Samples and Isolation of Staphylococci and Macrococci

Cheese samples (101 in total) were obtained from retail sources, focusing on producers (15 in total) for which brine bath samples were examined in a previous study (Hammer et al., 2019) and staphylococci and macrococci were found to be abundant. From each sample, 10 g (ratio rind/curd 1:10) was homogenized in double buffered peptone water [peptone water 20.0 g/L (wt/vol) (Oxoid), 3.5 g/L (wt/vol) $Na_2HPO_4$, 1.5 g/L (wt/vol) $KH_2PO_4$, (both Merck)] prewarmed to 37°C. A 0.1-mL aliquot as well as 4 × 0.25 mL aliquots were spread-plated onto either rabbit plasma fibrinogen Baird-Parker agar (start of the study) or Brilliance MRSA 2 agar (after change of laboratory practice owing to availability of agar) (both Oxoid) and incubated for 24 h at 37°C. Presumptive staphylococcal and macrococci colonies (one of each morphological type from each plate) were streaked onto blood agar [Columbia blood agar base supplemented with 5% (vol/vol) defibrinated sheep blood (both Oxoid)] and incubated for 24 h at 37°C.

### Species Identification

Presumptive macrococci or CNS strains from cheese were isolated on blood agar and were identified as described earlier by phenotypical and genotypical testing (Hammer et al., 2019). Briefly, Gram staining, catalase test, hemolysis, coagulase test (PCR), API 32 ID STAPH (bioMérieux), and partial sequencing of the *sod*A gene (429 bp) were used to identify the isolates as CNS or micrococci, using described previously methods (Poyart et al., 2001; Hammer et al., 2019). The proposal for reassignment of *Staphylococcus fleurettii* and *Staphylococcus sciuri* to a new genus *Mammaliicoccus* (Madhaiyan et al., 2020) was not considered in this study.

### Random Amplified Polymorphic DNA PCR

Random amplified polymorphic DNA (**RAPD**)-PCR was done to detect clonal relationships and thus avoid multiple isolation of strains with identical fingerprints for further study. Furthermore, RAPD-PCR fingerprinting was performed to compare strains obtained from cheese in this study to isolates obtained from brine baths used to salt the cheeses from the same producers in an earlier study (Hammer et al., 2019). The RAPD-PCR amplifications were performed with oligonucleotide primers OLP11 (5′-ACGATGAGCC-3′), OLP13 (5′-ACCGCCTGCT-3′), OPR-1 (5′- TGCGGGTCCT-3′), and OPR 16 (5′-CTCTGCGCGT-3′), as described by Williams et al. (1990). The PCR amplifications were carried out with 20 ng of DNA [extracted as described in Hammer et al. (2019)] in a total volume of 15 µL containing 0.3 µmol of primers using Eppendorf Hot Master Mix. After thermal cycling (95°C for 3 min; 35 cycles of 95°C for 30 s, 40°C for 30 s, and 72°C for 120 s; 72°C for 10 min; 4°C), DNA fragments were separated by electrophoresis in 1.5% agarose gel containing 0.1 µg/mL ethidium bromide. Fingerprint patterns were then visualized and documented under UV light.

### Antibiotic Resistance Testing

Screening for antibiotic resistance of strains was performed by application of the disc diffusion assay as described earlier (Hammer et al., 2019). Antibiotic compounds and loads were as follows: cefoxitin (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin G (10 U), and tetracycline (30 µg) (Oxoid). For evaluation, breakpoints provided by the Clinical and Laboratory Standards Institute (2014) or the European Committee on Antimicrobial Susceptibility Testing (2020) were used as far as available (neither organization provided breakpoints for all isolated bacterial species).

To estimate the MIC for cefoxitin, a gradient strip-test (Etest, bioMérieux) was applied according to the manufacturer's instructions. *Staphylococcus aureus* ATCC 29213 with an expected MIC of 1 to 4 µg/mL was used as a cefoxitin-resistant positive control. For the specific lot of test strips used in this study, the actual MIC for this control strain was 3 µg/mL, which was subsequently used as the breakpoint value indicating resistance to this compound.

### Genetic Determinants for Antibiotic Resistance, Enterotoxin, and Coagulase Production

Isolates were additionally screened for genes associated with aminoglycoside, β-lactam, erythromycin, methicillin, tetracycline, and vancomycin resistance (*aa6′-apH2″*, *bla*DHA, *bla*Fox, *erm*A, *erm*B, *mec*A, *mec*C, *tet*M, *tet*K, *tet*L, and *van*B) and virulence determinants including genes for enterotoxin (*sea*, *seb*, *sec*, *sed*, and *see*) and coagulase production. Details for all methods applied here including reference strains, references for primers, DNA extraction, and PCR conditions for all PCR amplifications conducted for the detection of the genetic determinants as listed above were previously described by Hammer et al. (2019). In addition, PCR amplifications were performed for the additional methicillin-resistance genes *mec*B and *mec*D genes. Primers for both of these genes were designed using primer-blast (NCBI) and included primers MecB2-r 5′-ACTACACAGAAACGGGATTGAT-3′, 5′-TCGTCGGAAATGCCGAACAT-3′, Macro-MecD-r 5′-AGGAGAGGAAACGCCTTCTG-3′, and Macro-MecD-f 5′-ACCCACAAACCATCCAATTTGT-3′. Reference strains used as positive controls were *Macrococcus canis* DSM 101690 (*mec*B) and *M. caseolyticus* IDM0819 (*mec*D) (kindly provided by Vincent Perreten, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland). *Staphylococcus aureus* ATCC 29213 was used as the negative control for both these PCR tests. For *mec*B, the PCR conditions were as follows: 95°C for 5 min; 35 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 45 s; 72°C for 7 min; and finally 10°C. For *mec*D, the PCR conditions were as follows: 98°C for 30 s; 35 cycles of 98°C for 30 s, 57°C for 30 s, and 72°C for 30 s; 72°C for 10 min; and finally 4°C.

## RESULTS AND DISCUSSION

In total, 101 cheese samples from retail were tested for the presence of potentially methicillin-resistant bacteria. Presumptive CNS and macrococci strains (n = 72), including 1 of each morphotype isolated from the respective agars (partly more than 1/sample), were isolated from soft cheese with red smear surface (n = 27), Camembert (n = 21), blue-veined cheese (n = 5), Tilsiter type cheese (n = 12), hard cheese (n = 2), and acid curd cheese (n = 5) (Table 1). The isolates obtained were further characterized using phenotypical and genotypical tests.

### Species Identification

All strains were gram-positive and coccus-shaped and showed a positive catalase reaction. None were coagulase PCR positive or exhibited hemolysis on blood agar. Partial sequencing of the *sod*A gene resulted in BLAST identifications between 99 and 100%, which confirmed the discriminatory power of this gene for species identification (Poyart et al., 2001; Hammer et al., 2019). Testing resulted in the identification of 53 CNS strains and 19 *M. caseolyticus* strains. The CNS strains included the following *Staphylococcus* species (no. of isolates in parentheses): *S. capitis* (1), *S. carnosus* (2), *S. epidermidis* (1), *S. equorum* (16), *S. fleurettii* (2), *S. saprophyticus* (17), *S. sciuri* (3), and *S. xylosus* (11). The same species were previously identified in cheese, especially *S. epidermidis*, *S. equorum*, *S. saprophyticus*, and *S. xylosus* (Coton et al., 2010; Kürekci, 2016; Mounier et al., 2006; Casaes Nunes et al., 2016; Organji et al., 2018). As reviewed by Stavropoulou et al. (2018), the dominant species in spontaneously fermented cheeses were *S. equorum*, *S. saprophyticus*, and *S. xylosus*. The

**Table 1.** Origin, number, and summarized resistance pattern of isolates of CNS and *Macrococcus caseolyticus*[1]

| | | CNS | | | | *M. caseolyticus* | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cheese type | No. of samples | No. of isolates | % | n-r | %-r | No. of isolates | % | n-r | %-r |
| Soft cheese, red smear | 42 | 18 | 42.9 | 16 | 88.9 | 9 | 21.4 | 7 | 77.8 |
| Camembert | 25 | 16 | 64.0 | 13 | 81.3 | 5 | 20.0 | 4 | 80.0 |
| Blue-veined cheese | 6 | 5 | 83.3 | 3 | 60.0 | 0 | — | — | — |
| Tilsiter type cheese | 8 | 7 | 87.5 | 6 | 85.7 | 5 | 62.5 | 3 | 60.0 |
| Semihard cheese | 5 | 0 | — | — | — | 0 | — | — | — |
| Hard cheese | 5 | 2 | 40.0 | 2 | 100 | 0 | — | — | — |
| Acid curd cheese | 10 | 5 | 50.0 | 5 | 100 | 0 | — | — | — |

[1]n-r = number of isolates showing resistance to antimicrobial agents either phenotypically or carrying respective genes. %-r = percentage of isolates showing resistance to antimicrobial agents either phenotypically or carrying respective genes.

latter species is used in industrial practice as a starter culture for surface ripening of cheese (Irlinger et al., 2015). In contrast to results reported by Mounier et al. (2006), identical strains compared with those isolated earlier from brine bathes of the same producers (Hammer et al., 2019) could not be identified in our study. Although strains belonged to the same species in some cases, they did not show any obvious clonal relationship on the basis of RAPD-PCR fingerprint patterns (data not shown).

As reported and discussed previously (Poyart et al., 2001; Hammer et al., 2019), the discriminatory power of *sod*A sequencing is superior to phenotypical testing utilizing the API 32ID STAPH miniaturized test kit. None of the *M. caseolyticus* strains could be identified by the API test, and only 1 of 16 strains of *S. equorum* was correctly identified by this test. Mismatches in identification when comparing the API 32ID miniaturized test kit results with *sodA* sequencing-based identification (numbers in parentheses) also occurred for *S. carnosus* (2), *S. fleurettii* (2), and *S. sciuri* (1); whereas, 100% congruent results were obtained for *S. saprophyticus* (n = 17) and *S. xylosus* (n = 11).

The high number of *S. saprophyticus* isolates still needs further consideration. As reported earlier (Hammer et al., 2019), *S. saprophyticus* ssp. *saprophyticus* is a biosafety-level 2 microorganism and should not be present in cheese. Discrimination of the 2 proposed subspecies, where subspecies *S. saprophyticus* ssp. *bovis* is classified as a biosafety level 1 microorganism, remains difficult. As already reported, phenotypic tests for nitrate reductase, pyrrolidonyl arylamidase, and D-ribose fermentation as distinguishing characteristics between the subspecies according to Hájek et al. (1996) and the respective UK standard (Public Health England, 2014) did not perform as well as *sod*A sequencing and *rpo*B sequencing (Hammer et al., 2019). The proposal of Madhaiyan et al. (2020) for unification of both subspecies is thus supported by our observations as well. *Staphylococcus epidermidis* is also classified as a biosafety-level 2 microorganism. Though well known as a colonizer of human skin, its significance for nosocomial infections in terms of biofilm formation in catheters or on implants is still emerging (Otto, 2012; Oliveira et al., 2018). In addition, its capability of forming biofilms is also a cause for hygienic problems in the food industry (Zou and Liu, 2020).

## *Antimicrobial Resistance Testing*

All CNS and *M. caseolyticus* isolates were tested for antibiotic resistance by applying disc diffusion assays and PCR tests (Table 2). For strains showing evidence

**Table 2.** Summary of results for detection of genes for antibiotic resistance and phenotypic tests for CNS and *Macrococcus caseolyticus* isolates from cheese in Germany[1]

| Species | | FOX | | *mecA* | | *mecB* | | *mecD* | | TET | | *tetK* | | *tetL* | | PEN | | *bla*TEM | | *blaZ* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| *Staphylococcus capitis* | 1 | 0 | — | 0 | — | 0 | — | 0 | — | 0 | — | 1 | 100 | 1 | 100 | 1 | 100 | 0 | — | 1 | 100 |
| *Staphylococcus carnosus* | 2 | 0 | — | 0 | — | 0 | — | 0 | — | 0 | — | 2 | 100 | 0 | — | 0 | — | 0 | — | 0 | — |
| *Staphylococcus epidermidis* | 1 | 0 | — | 0 | — | 0 | — | 0 | — | 0 | — | 1 | 100 | 1 | 100 | 1 | 100 | 0 | — | 1 | 100 |
| *Staphylococcus equorum* | 16 | 2 | 12.5 | 0 | — | 0 | — | 0 | — | 0 | — | 9 | 56.3 | 3 | 18.8 | 1 | 6.3 | 1 | 6.3 | 0 | — |
| *Staphylococcus fleurettii* | 2 | 0 | — | 2 | 100 | 0 | — | 0 | — | 0 | — | 1 | 50.0 | 0 | — | 1 | 50.0 | 1 | 50.0 | 0 | — |
| *Staphylococcus saprophyticus* | 17 | 0 | — | 0 | — | 1 | 5.9 | 1 | 5.9 | 0 | — | 16 | 94.1 | 3 | 17.6 | 0 | — | 1 | 5.9 | 0 | — |
| *Staphylococcus sciuri* | 3 | 0 | — | 0 | — | 0 | — | 0 | — | 0 | — | 1 | 33.3 | 0 | — | 1 | 33.3 | 0 | — | 0 | — |
| *Staphylococcus xylosus* | 11 | 0 | — | 0 | — | 0 | — | 1 | 9.1 | 11 | 100 | 11 | 100 | 0 | — | 1 | 9.1 | 0 | — | 0 | — |
| *Macrococcus caseolyticus* | 19 | 7 | 36.8 | 0 | — | 0 | — | 7 | 63.6 | 1 | 5.3 | 12 | 63.2 | 5 | 26.3 | 7 | 36.8 | 3 | 15.8 | 0 | — |

[1]Disk diffusion assay: FOX = cefoxitin; PEN = penicillin G; TET = tetracycline. Genes are in italics.

**Table 3.** Isolates of CNS from cheese in Germany showing multiple phenotypic and genotypic indications for antibiotic resistance and evidence for methicillin resistance (positive tests for either FOX, *mec*A, *mec*B, or *mec*D)[1]

| MRI[2] | Species | MIC, µg/mL | FOX | *mec*A | *mec*B | *mec*D | PEN | *bla*TEM | TET | *tet*K | *tet*L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | *Staphylococcus xylosus* | 3.0 | − | − | − | + | + | − | + | + | − |
| 14 | *Staphylococcus fleurettii* | 2.0 | − | + | − | − | + | + | − | − | − |
| 19 | *Staphylococcus sciuri* | 3.0 | + | − | − | − | − | − | − | + | − |
| 46 | *S. fleurettii* | 0.5 | − | + | − | − | − | − | − | + | − |
| 47 | *Staphylococcus saprophyticus* | 2.0 | − | − | − | + | − | − | − | + | + |
| 51 | *S. saprophyticus* | 3.0 | + | − | − | − | − | − | − | + | − |
| 56 | *S. saprophyticus* | 2.0 | + | − | + | − | − | − | − | + | − |
| 68 | *Staphylococcus equorum* | 0.3 | + | − | − | − | + | − | − | − | + |
| 72 | *S. equorum* | 3.0 | + | − | − | − | − | + | − | − | − |

[1]Disc diffusion assays: FOX = cefoxitin; PEN = penicillin G; TET = tetracycline. MIC for FOX determined with Etest. Genes are in italics.

[2]MRI = strain number Max Rubner-Institute.

of methicillin resistance, the Etest was also applied to determine the MIC values for cefoxitin.

In total, 14 PCR tests were applied for testing for the presence of genes encoding antibiotic resistance. None of the following genes were detected in isolates of CNS and *M. caseolyticus*: *aa6′-apH2″*, *bla*DHA, *bla*Fox, *erm*A, *erm*B, *mec*C, *tet*M, and *van*B. Of the CNS strains, 42 showed a positive PCR result for *tet*K, and 8 were positive for *tet*L. The *tet*K and *tet*L genes encode efflux pumps responsible for pumping tetracycline out of the bacterial cell and have also been determined to occur in CNS from foods of animal origin in previous studies (Resch et al., 2008; Chajęcka-Wierzchowska et al., 2015). However, only the *S. xylosus* (n = 11) strains showed resistance against tetracycline in the disk diffusion assay. In contrast, 14 CNS isolates were phenotypically resistant to penicillin G, but only 1 of these carried the *bla*TEM and 2 the *bla*Z gene. Coagulase-negative staphylococci showing evidence for methicillin resistance (being positive for *mec*A, *mec*B, or *mec*D or in the cefoxitin disk diffusion assay) are listed in Table 3. Interestingly, both *S. fleurettii* strains positive for *mec*A and 1 isolate each of *S. saprophyticus* and *S. xylosus* positive for *mec*D, were negative in the disk diffusion assay for cefoxitin. The MIC values were 2.0 and 0.5 µg/mL for *S. fleurettii*, 2.0 µg/mL for *S. saprophyticus*, and 3.0 µg/mL for *S. xylosus*; these values were below or directly at the breakpoint of 3.0 µg/mL. Carriage of the *mec*A gene by *S. fleuretti* is in accordance with phylogenetic work identifying this species as having an ancient origin and being the origin of the spread of the gene to at least other staphylococcal species (Tsubakishita et al., 2010; Rolo et al., 2017).

Screening for methicillin resistance by use of the cefoxitin disc diffusion assay showed positive results for 5 CNS (none of these carried a *mec* gene) and 7 strains of *M. caseolyticus* (6 of these carried the *mec*D gene). In addition, all of these *M. caseolyticus* isolates showed resistance to penicillin G in the disc diffusion assay. Genetic determinants for antibiotic resistance in *M. caseolyticus* (number in parentheses) were detected for *bla*TEM (3), *mec*D (7), *tet*K (12), and *tet*L (5). Although 12 strains carried genes for *tet*K, with 5 of these also carrying genes for *tet*L, only one exhibited phenotypic resistance, which was similar to the results for CNS. Several strains showed resistance to multiple antibiotics (Table 4). One isolate carried genes for *mec*D, *tet*K, and *tet*L and phenotypically showed resistance to cefoxitin, penicillin G, and tetracycline. Three isolates showed a positive disc diffusion assay result for cefoxitin, with a MIC for cefoxitin of 16.0 µg/mL, and the *mec*D gene was consistently detected. For 3 other isolates, results for cefoxitin disc diffusion assay and presence of *mec*D gene were consistent, the MIC for these, however, were varying: 8.0, 4.0, and 1.5 µg/mL, respectively. One strain showed a MIC of 8.0 and a positive cefoxitin test but no *mec*D gene, and another had a MIC of 2.0, a negative cefoxitin test, and the presence of the gene. To overcome these inconsistencies, it could be helpful to define an own reference strain of *M. caseolyticus* for determination of breakpoints for MIC and disc diffusion assay. Interestingly, no *mec*B gene was detected in the isolates, although Becker et al. (2018) postulated that *M. caseolyticus* was the ancient source of this gene. According to Schwendener et al. (2017), frequent detection of *mec*D genes can be expected, which the results of this study confirmed. These authors identified 2 genomic resistance islands for *mec*D in *M. caseolyticus*, both containing an integrase gene. The potential for broad host dissemination

**Table 4.** *Macrococcus caseolyticus* isolates from cheese in Germany showing multiple phenotypic and genotypic indications for antibiotic resistance[1]

| MRI[2] | MIC, μg/mL | FOX | *mec*D | PEN | *bla*TEM | TET | *tet*K | *tet*L |
|---|---|---|---|---|---|---|---|---|
| 2 | 16.0 | + | + | + | − | − | + | − |
| 3 | 16.0 | + | + | + | − | − | + | − |
| 4 | 16.0 | + | + | + | − | − | + | − |
| 10 | 8.0 | + | − | + | − | − | − | − |
| 11 | 8.0 | + | + | + | − | − | − | − |
| 12 | 4.0 | + | + | + | − | − | − | − |
| 13 | 1.5 | + | + | + | − | + | + | + |
| 41 | ND[3] | − | − | − | + | − | + | − |
| 69 | 2.0 | − | + | − | + | − | − | − |

[1]Disc diffusion assays: FOX = cefoxitin; PEN = penicillin G; TET = tetracycline. MIC for FOX determined with Etest. Genes are in italics.

[2]MRI = strain number Max Rubner-Institute.

[3]ND = not done. Etest was done only for strains showing evidence for methicillin resistance.

of the resistance gene was shown in this connection (Schwendener and Perreten, 2018). Accordingly, the *Macrococcus* group needs continuous surveillance owing to its emergence as a potential carrier and vector of *mec* genes (Becker, 2021) on the one hand, and its application as starter culture (Ramos et al., 2021) on the other.

### *Genetic Determinants for Enterotoxin Production*

Genetic determinants for the most common staphylococcal enterotoxins *sea*, *seb*, *sec*, *sed*, and *see* were not detected in any CNS or *M. caseolyticus* isolates. As discussed earlier (Hammer et al., 2019), Rodrigues et al. (2017) found an absence of the above-mentioned staphylococcal enterotoxin genes in several CNS species, whereas the presence of such genes was reported by other authors (Vernozy-Rozand et al., 1996; Oliveira et al., 2010; Casaes Nunes et al., 2016). For *M. caseolyticus*, only strains isolated from meat products (n = 10, from Turkish sausages) and 1 sample of camel milk have been tested for the presence of staphylococcal enterotoxin genes, showing no evidence to date for staphylococcal enterotoxin production capability by this species (Njage et al., 2013; Çetin and Tuncer, 2016).

## CONCLUSIONS

Cheese microbiota should not contain organisms that have pathogenic potential or carry antibiotic resistance. The genes encoding common staphylococcal enterotoxins were not present in the CNS group of bacteria and the *M. caseolyticus* species investigated in this study. Among the CNS, a high number of *S. saprophyticus* was isolated, which needs further consideration in terms of this microorganism's potential as a human pathogen. Genes coding for antibiotic resistance, including *mec*A and *mec*D, were detected in several isolates of the CNS group, while *mec*D was shown to be present in *M. caseolyticus*. These findings imply a potential food safety issue because a potential for broad-host dissemination of the *mec*D gene exists with *M. caseolyticus*.

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