Prevalence and characterization of methicillin-resistant *Macrococcus* spp. in food producing animals and meat in Switzerland in 2019

Jennifer Eleonora Keller[1], Sybille Schwendener[1], Jil Neuenschwander[1], Gudrun Overesch[1], Vincent Perreten[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

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Prävalenz und Charakterisierung von Methicillin-resistenten *Macrococcus* spp. bei Nutztieren und Fleisch in der Schweiz im Jahr 2019

Die Prävalenz von Methicillin-resistenten *Macrococcus* spp. bei Kälbern und Schweinen wurde anhand von Proben aus dem Schlachthof und Fleisch aus dem Einzelhandel bestimmt. Für die Studie wurden Proben verwendet, welche im Rahmen der nationalen Überwachung auf Methicillin-resistenten *Staphylococcus aureus* bei Lebensmittelliefernden Tieren sowie Fleisch entnommen wurden. Die Isolate wurden einer antimikrobiellen Empfindlichkeitsprüfung für 19 Antibiotika unterzogen. Mittels molekularen Techniken (z. B. PCR, Microarray, WGS) wurden Sequenztyp (ST), Resistenzgene und die Elemente, welche ein Methicillin-Resistenzgen *mec* tragen, identifiziert.

Methicillin-resistente *Macrococcus* spp. (*M. caseolyticus* (n=38), *M. bohemicus* (n=4) und *Macrococcus* spp. (n=2)) konnten aus 40 von 299 Nasenabstrichen von Kälbern isoliert werden, was einer Prävalenz von 13,38 % (95 % CI, 9,98 % – 17,70 %) entspricht, und aus vier von 303 Nasenabstrichen von Schweinen [1,32 % (95 % CI, 0,36 % – 3,35 %)]. Eine von 311 Schweinefleischproben aus der Schweiz enthielt ein *Macrococcus* sp. [0,32 % (95 % CI, 0,01 % – 1,78 %)], und vier von 309 Rindfleischproben (260 inländische und 49 importierte) enthielten *M. caseolyticus* [1,29 % (95 % CI, 0,35 % – 3,28 %)].

Die *M. caseolyticus* Stämme gehörten verschiedenen STs an, wobei ST21 sowohl bei Schweinen als auch bei Kälbern am häufigsten vorkam. Das *mecD* Gen befand sich bei 42 Stämmen auf *Macrococcus* resistance island *mecD* (McRI$_{mecD}$) und bei drei Stämmen auf staphylococcal cassette chromosome *mec* (SCC*mecD*) Elementen, während *mecB* bei vier Stämmen ausschliesslich auf Plasmiden gefunden wurde. Neben der Resistenz gegenüber β-Laktame wiesen die Stämme auch Resistenzen gegen-

Summary

The prevalence of methicillin-resistant *Macrococcus* spp. in calves and pigs at slaughterhouses and in retail beef and pork meat was determined using samples taken in 2019 within the framework of the national monitoring of methicillin-resistant *Staphylococcus aureus* in food producing animals in Switzerland. The isolates were submitted to antimicrobial susceptibility testing of 19 antibiotics and to molecular techniques (e.g. PCR, microarray, WGS) for the identification of resistance genes, elements containing the methicillin resistance genes *mec* and sequence type (ST).

Methicillin-resistant *Macrococcus* spp. (*M. caseolyticus* (n=38), *M. bohemicus* (n=4) and *Macrococcus* spp. (n=2)) were isolated in 40 of 299 nasal swabs from calves representing a prevalence of 13,38 % (95 % CI, 9,98 % – 17,70 %), and in four of 303 nasal swabs from pigs [1,32 % (95 % CI, 0,36 % – 3,35 %)]. One of 311 samples of Swiss pork meat contained a *Macrococcus* sp. [0,32 % (95 % CI, 0,01 % – 1,78 %)], and four of 309 beef meat samples (260 domestic and 49 imported) contained *M. caseolyticus* [1,29 % (95 % CI, 0,35 % – 3,28 %)].

The *M. caseolyticus* strains belonged to diverse STs, with ST21 being the most common in both pigs and calves. The *mecD* gene was located on *Macrococcus* resistance island *mecD* (McRI$_{mecD}$) in 42 strains and on staphylococcal cassette chromosome *mec* (SCC*mecD*) in three strains, while *mecB* was found on plasmids in four strains. In addition to resistance to β-lactams, the strains also exhibited resistance to tetracycline (n=17; *tet*(L), *tet*(K), *tet*(M)), streptomycin (n=13; *str*, *ant(6)-Ia*, *rpsL* mutation [K56R in ribosomal protein S12]), kanamycin (n=10; *aac(6')-Ie – aph(2'')-Ia*, *aph(2')-Ib*, *aph(2')-Ic*, *ant(4')-Ia*), clindamycin (n=9; *erm*(B), *erm*(45)), erythromycin (n=9; *erm*(B), *msr*(G), *erm*(45)), fusidic acid (n=9; *fusC*) and gentamicin (n=1; *aac(6')-Ie – aph(2'')-Ia*).

über Tetracyclin (n=17; *tet*(L), *tet*(K), *tet*(M)), Streptomycin (n=13; *str*, *ant(6)-Ia*, *rpsL*-Mutation [K56R im ribosomalen Protein S12]), Kanamycin (n=10; *aac(6')-Ie–aph(2'')-Ia*, *aph(2')-Ib*, *aph(2')-Ic*, *ant(4')-Ia*), Clindamycin (n=9; *erm*(B), *erm*(45)), Erythromycin (n=9; *erm*(B), *msr*(G), *erm*(45)), Fusidinsäure (n=9; *fusC*) und Gentamicin (n=1; *aac(6')-Ie–aph(2'')-Ia*) auf.

Diese Studie stellt die erste nationale Prävalenzstudie von Methicillin-resistenten *Macrococcus* spp. bei Schweinen, Kälbern, sowie Rind- und Schweinefleisch in der Schweiz dar und offenbart ein Reservoir von genetisch vielfältigen Stämmen, die mehrere Resistenzmerkmale tragen.

Schlüsselwörter: Antibiotikaresistenz; *Macrococcus* spp.; McRI*mecD*; Monitoring; SCC*mec*; WGS

This study represents the first national prevalence study of methicillin-resistant *Macrococcus* spp. in pigs, calves, pork and beef meat in Switzerland and revealed a reservoir of genetically diverse strains carrying several resistance traits.

Keywords: antibiotic resistance; *Macrococcus spp.*; McRI*mecD*; monitoring; SCC*mec*; WGS

Introduction

The genus *Macrococcus* belongs to the family of *Staphylococcaceae* and is closely related to the genera *Staphylococcus* and *Mammaliicoccus*, the latter containing the recently reclassified species of the *Staphylococcus sciuri* group.[1] Currently, the genus *Macrococcus* is composed of eleven species: *Macrococcus (M.) caseolyticus, M. bovicus, M. equipercicus, M. carouselicus,*[2] *M. brunensis, M. hajekii, M. lamae,*[3] *M. canis,*[4] *M. goetzii*, *M. epidermidis* and *M. bohemicus*.[5] Macrococci are gram-positive, coagulase-negative, and catalase-positive coccoid bacteria, which can be found on the skin and mucosa of animals as part of the commensal flora. In rare cases, they have been associated with diseases in animals, *M. caseolyticus* has been isolated from abscesses of slaughtered lambs,[6] cranial cavities of diseased broiler chicken[7] and bovine mastitis milk.[8] *M. caseolyticus*, *M. canis* and *M. bohemicus* were also detected in infection sites in dogs.[4,9–11]

In the last twenty years, more attention has been paid to macrococci, especially to *M. caseolyticus* because of its potential application in the food industry (*caseolyticus* = casein dissolving), but also because of the emergence of methicillin-resistant strains and their potential of disseminating resistance genes to other commensals or pathogenic bacteria.[12] Methicillin resistance in *Macrococcus* is specified by acquired *mecB* and *mecD* genes which encode for an alternative transpeptidase (or PBP2a, penicillin binding protein 2a) conferring resistance to all β-lactam antibiotics.[13,14] The *mecB* gene is found in *Macrococcus* spp. on large plasmids or on staphylococcal cassette chromosome *mec* (SCC*mec*) elements.[5,9,11,13,15,16] The *mecD* gene is usually carried on a *Macrococcus* resistance island (McRI$_{mecD}$), which is site specifically integrated at the 3' end of the 30S ribosomal protein S9 gene in the methicillin-resistant strains.[8] It has also been recently reported to be present on SCC*mec* elements in *M. bohemicus*.[17] While the *mecD* gene has never been detected in staphylococci, the *mecB* gene was also found in a methicillin-resistant *Staphylococcus (S.) aureus* (MRSA) on a plasmid which resembles a *mecB*-containing plasmid of a *M. canis* suggesting exchange of genetic elements between *Macrococcus* and *Staphylococcus*.[16,18]

Every two years, food producing animals like calves and pigs are routinely screened during one year at slaughterhouses for the presence of MRSA within the national surveillance monitoring of antibiotic resistance in Switzerland.[19] During the same year of monitoring, fresh beef and pork meat at retail is also routinely screened for the presence of MRSA to assess the risk for transmission to humans, since carcasses may be contaminated with bacteria during the slaughter process.[19] In this study, we used the same samples as those of the national surveillance monitoring of MRSA of 2019 to determine whether methicillin-resistant macrococci were present in the nasal cavities and meat of these animals and in which proportion. The resistance characteristics of the isolates were further analyzed using phenotypic and genotypic methods.

Materials and Methods

Sample collection, bacterial growth conditions and species identification

Methicillin-resistant *Macrococcus* spp. were isolated at the Center for Zoonosis Bacterial Animal diseases and Antimicrobial Resistance (ZOBA) at the Institute or Veterinary Bacteriology (IVB), Bern using the samples destined to the national surveillance monitoring of MRSA in food-producing animals and in beef and pork meat in 2019. Samples consisted of nasal swab samples

taken from calves (n=299) and pigs (n=303) using a representative sampling strategy at the seven largest pig and cattle slaughterhouses in Switzerland. Fresh, prepacked beef (n=309) and pork (n=311) meat were collected at retail from cantonal laboratories, considering the population density of the cantons and market share of retailers.[19] The nasal swabs and five grams of meat were placed into Mueller-Hinton broth (Becton, Dickinson and Company, Franklin Lake, New Jersey, United States) containing 6,5 % NaCl and incubated aerobically at 37°C for 24h, respectively. Then, a loopful (10µl) was spread onto BD BBL™ CHROMagar™ MRSA II (Becton, Dickson and Company) and incubated aerobically at 37°C for 24h. All colonies exhibiting coloration from light pink (typical coloration for *Macrococcus* spp.) to pink-purple (typical coloration for *S. aureus*) on the MRSA agar were submitted to Matrix-Assisted Laser-Desorption/Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS) (Microflex LT, Bruker, Daltonics, Billerica, Massachusset, United States), using the Biotyper software with Bruker MBT 7854 MSP Library and own MSPs for the *Macrococcus* type strains *M. bohemicus* CCM7100^T, *M. bovicus* CCUG38365^T, *M. brunensis* CCUG47200^T, *M. canis* KM45013^T, *M. carouselicus* CCUG38360^T, *M. epidermidis* CCM7099^T, *M. goetzii* CCM4927^T, *M. hajekii* CCUG47201^T, *M. llamae* CCUG47199^T. MALDI-TOF MS samples were prepared by direct transfer protocol according to manufacturer's instructions. For initial identification of possible *Macrococcus* spp. from MRSA selective agar a score value of > 1,7 was set. *S. aureus* strains isolated from MRSA selective agar were used to determine the prevalence of MRSA in food producing animals within the national surveillance monitoring program[19] while *Macrococcus* spp. were analyzed in this study.

After the initial identification, the strains were routinely grown in pure culture on TSA-SB plates (BD BBL™ Trypticase^{MT} Soy Agar with 5 % Sheep Blood, Becton, Dickinson and Company) aerobically at 37°C overnight. For reliable species identification a score of > 2,0 was used as recommended by the manufacturer. Identification of *M. caseolyticus* was additionally confirmed by *nuc*-PCR.[10] Other *Macrococcus* species were identified by 16S rRNA and *hsp*60 gene sequence analysis. A 1415-bp fragment of the 16S rRNA gene fragment was amplified with the primers 16SUNI-L (5'-AGAGTTTGATCATGGCTCAG) and 16SUNI-R (5'-GTGTGACGGGCGGTGTGTAC), using the following conditions: 35 cycles at 94°C for 30 s, 54°C for 30 s, 72°C for 30 s and a final extension step of 7 min at 72°C.[20] A 1210-bp fragment of the *hsp*60 gene sequence was amplified with the primers *hsp*60-mc-F (5'-AAGATGCAAGACGCTCAATGCT) and *hsp*60-mc-R (5'-CCTGCAACGATACCTTCTTC), (35 cycles of 94°C for 30 s, 53°C for 70 s and 72°C for 60 s). Unless specified otherwise, all PCRs were performed using the 5x HOT FIREPol® Blend Master Mix RTL (Solis BioDyne, Tartu, Estonia). The amplicons were sequenced using the same primers (Microsynth, Balgach, Switzerland). The obtained sequences were compared with the 16S rRNA and the *hsp*60 gene of the *Macrococcus* spp. type strains deposited in the GenBank using the Basic Local Alignment Search Tool (BLAST). DNA was extracted from each strain using the DNeasy® UltraClean® Microbial Kit of QIAGEN (Hilden, Germany), stored at -20°C and used for PCR and Microarrays.

Antimicrobial susceptibility testing and resistance gene detection

Minimal inhibitory concentration (MIC) of 19 antibiotics was determined by broth microdilution in cation adjusted Mueller-Hinton Broth (CAMHB) using ThermoScientific™ SENSITITRE™ EUST European Veterinary Resistance Plate for *Staphylococcus aureus* and following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[21] The plates were incubated under aerobic conditions at 37°C for 18–20h.

The clinical resistance breakpoints (R) set for *Staphylococcus* spp. were taken from the CLSI guidelines and from European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org) and used for *Macrococcus* spp. as suggested by Cotting *et al.*[10]: clindamycin (R ≥ 4 mg/L), tetracycline (R > 2 mg/L), rifampin (R ≥ 4 mg/L), streptomycin (R > 8 mg/L), fusidic acid (R > 1 mg/L), penicillin (R ≥ 0,25mg/L), chloramphenicol (R > 8 mg/L), kanamycin (R > 8 mg/L), quinupristin/dalfopristin (R ≥ 4 mg/L), vancomycin (R ≥ 16 mg/L), gentamicin (R > 1 mg/L), trimethoprim (R > 4 mg/L), erythromycin (R > 2 mg/L), ciprofloxacin (R > 1 mg/L), cefoxitin (R ≥ 8 mg/L), linezolid (R ≥ 8 mg/L), sulfamethoxazole (R ≥ 512 mg/L) (breakpoint values indicated by «≥» are form CLSI and by «>» are from EUCAST). No breakpoints were available for tiamulin and mupirocin. Inducible clindamycin-resistance was identified through a double disk diffusion test following the CLSI guidelines.[22]

The MIC of oxacillin for *Macrococcus* sp. strains 19Msa0295 and 19Msa0966 was determined in 96-well plate containing 2-fold dilution ranging from 0,5 to 256 mg/L under aerobic incubation at 37°C for 18–20h. Oxacillin-susceptible and *mec*-negative *M. caseolyticus* strains CCUG 15606^T and KM1352[14] were used as control. We tentatively used the resistance breakpoint set for coagulase-negative staphylococci by the CLSI guidelines (R ≥ 1 mg/L).[23]

The antimicrobial resistance genes were detected by microarray using AMR+ve-5,1 tubes (Alere Technologies GmbH, Jena, Germany) capable of detecting up to 117 antibiotic resistance genes.[24] The erythromycin resist-

ance genes *erm*(45) and *msr*(G) which are not present on the microarray were detected by PCR using the primer pair *erm*(45)-F (5'-CATAATTTATGAGGTTGGAACTGG) and *erm*(45)-R (5'-GAATACTCTTTATTAACCCACTTTG),[25] and the primer pair *msr*(G)-F (5'-TGAACAGCAGAGTCAAGCAC) and *msr*(G)-R (5'-ATATATCTGTGCCGACAAACAAC) (35 cycles with 94°C for 30 s, 55°C for 60 s and 72°C for 60 s).[26] Mutations in the 30S ribosomal protein S12 (encoded by the *rpsL* gene) were identified by amino acid comparison with S12 sequence of *M. caseolyticus* strains deposited in GenBank (e.g. type strain DSM 20597 [GenBank acc. no. PPRM00000000.1], strain IMD0819 [CP021058.1] and strain JCSC5402 [AP009484.1]) using Clustal Omega and deduced amino acid sequence from Whole Genome Sequencing (WGS).

Genotyping

The sequence types (ST) of *M. caseolyticus* were determined by Multilocus Sequence Typing (MLST) using the *M. caseolyticus* scheme from pubMLST homepage (https://pubmlst.org/). No MLST-schemes are available at the moment for *M. bohemicus*.

The type of *Macrococcus* resistance island *mecD* (McRI$_{mecD}$) was determined using the multiplex PCR protocol developed by Schwendener *et al.*[8]. The protocol was adapted with an universal primer for macrococcal 30S ribosomal protein S9 (*rspI*) genes (rpsI-MC-F: 5'-TTAGACTTAAACCAACCATTCGA) that replaced the *M. caseolyticus* specific *rpsI* primer. PCRs were performed in 30 µl volume with 1,5 U of HOT FIREPol® DNA polymerase (Solis BioDyne, Tartu, Estonia), 200 µM deoxynucleoside triphosphates and 0,2 µM primers as described. [8]

Whole Genome Sequencing (WGS)

The strains whose McRI$_{mecD}$ remained untypable, as well as the strains carrying *mecB* were submitted to WGS. The DNA was extracted using the MasterPure™ Complete DNA and RNA Purification Kit of Lucigen® Corporation, LCG Biosearch Technologies (Hoddeson, United Kingdom). DNA sequencing was performed using a NEBNext Ultra II directional DNA library with TruSeq adapters on an Illumina NovaSeq 6000 system (2 × 150-bp paired-end reads) in-house and at Eurofins Genomics (Germany) and in-house using MinION system (Oxford Nanopore Technologies [ONT] (Oxford, United Kingdom); R9.4.1 SpotON flow cell, MinION MK1b device) as described previously.[27] The Illumina sequences were quality filtered and paired using trimmomatic v0.36 with adapter clipping to remove Nextera Transposase Sequence (5'-CTGTCTCTTATA) (ILLUMINACLIP) and the following parameters: phred33, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, MINLEN:36. The ONT reads were base called and demultiplexed using Guppy software v4.4.1 and end trimmed and optionally size fitted using Cutadapt v2.5. The genomes were *de novo* assembled and circularized using Unicycler v0.4.4 run in bold mode with paired-end Illumina reads and ONT reads. Genomes that could not be closed with this method were first *de novo* assembled with Canu v2.1.1 using ONT reads only. The Canu assembly was subsequently used as a reference for Unicycler v0.4.8 hybrid assembly. All calculation were performed on the high-performance computing (HPC) cluster UBELIX at the University of Bern (http://www.id.unibe.ch/hpc). The genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline service.[28] The genome sequences of the *M. caseolyticus* strains 19Msa0287, 19Msa0499, 19Msa1047, 19Msa0687, the *M. bohemicus* strains 19Msa0936, 19Msa0383 and the *Macrococcus* sp. 19Msa1099 have been deposited in GenBank and are available under NCBI BioProject PRJNA744395.

The genomic regions containing *mecB* and *mecD* was localized by blast search, and the elements were analyzed by genetic comparison with closely related elements from the GenBank. Analysis and illustration were performed using EasyFig 2.1 Software (Beatson Lab, University of Queensland, Australia).

Results

Prevalence, identification and genotyping of methicillin-resistant Macrococcus strains from calves, pigs, beef and pork meat

Forty-nine methicillin-resistant *Macrococcus* spp. were isolated from nasal cavities of 299 calves and 303 pigs raised in 10 Swiss cantons and slaughtered at different slaughterhouses and from 311 fresh retail pork and 309 beef meat samples in 2019. The prevalence of methicillin-resistant *Macrococcus* spp. was of 13,38 % (95 % CI, 9,98 % – 17,70 %) in calves and 1,32 % (95 % CI, 0,36 % – 3,35 %) in pigs (Figure 1). This prevalence in calves is mainly to be associated with the predominant isolation of *M. caseolyticus* [12,71 % (95 % CI, 9,40 % – 16,96 %)], while in pigs the prevalence seems to be associated with the isolation of a few *M. bohemicus* [0,99 % (95 % CI, 0,20 % – 2,87 %)].

Out of a total of 49 strains (44 from nasal swabs, five from retail meat), 42 were identified as *M. caseolyticus* (37 in calves, one in pigs and four in beef meat), four as *M. bohemicus* (one in cattle and three in pigs) and three (two from cattle and one from pigs) could not be identified to the species level neither by MALDI-TOF MS nor by *hsp60* and 16S rRNA gene sequence analysis (Tables 1 and 2). Nevertheless, 16S rRNA gene and *hsp60* comparative sequence analysis confirmed that they be-

Table 1: Antimicrobial resistance phenotypes and genotypes, canton of origin, sequence types (ST) and genetic elements containing *mecD* or *mecB* of *Macrococcus* spp. isolated from the nasal cavities of calves at slaughter and from Swiss retail beef meat

Nr.	Sample Name	Origin	Phenotype[a] and Genotype[b]	ST	*mec*-element
			Nasal cavities		
			Macrococcus caseolyticus		
1	19Msa0117	SG	CLI [*erm*(B)], TET [*tet*(L)], PEN [*mecD*], KAN [*aac(6')-Ie – aph(2'')-Ia, aph(2')-Ic, ant(4')-Ia*], GEN [*aac(6')-Ie – aph(2'')-Ia*], ERY [*erm*(B)], FOX [*mecD*]	21	McRI$_{mecD}$-3
2	19Msa0198	LU	PEN [*mecD*], FOX [*mecD*]	26	McRI$_{mecD}$-3
3	19Msa0290	GR	PEN [*mecD*], FOX [*mecD*]	9	McRI$_{mecD}$-1
4	19Msa0287	SZ	CLI [*erm*(B)], TET [*tet*(L)], STR [*rspL* (P22A; K56R)], PEN [*mecD*], KAN [*ant(4')-Ia*], ERY [*erm*(B), *msr*(G)], FOX [*mecD*]	47	McRI$_{mecD}$-3
5	19Msa0421	TG	TET [*tet*(L)], FUS [*fusC*], PEN [*mecD*], KAN (8mg/L) [*ant(4')-Ia, aph(2')-Ib*], FOX [*mecD*]	26	McRI$_{mecD}$-3
6	19Msa0367	BE	PEN [*mecD*], FOX (4mg/L) [*mecD*]	21	McRI$_{mecD}$-3
7	19Msa0477	BE	PEN [*mecD*], FOX [*mecD*]	21	McRI$_{mecD}$-3
8	19Msa0586	UR	PEN [*mecD*], FOX [*mecD*]	21	McRI$_{mecD}$-3
9	19Msa0592	SG	PEN [*mecD*], FOX [*mecD*], [*bleO*] [b]	6	McRI$_{mecD}$-2
10	19Msa0618	LU	CLI [*erm*(B)], PEN [*mecD*], ERY [*erm*(B)], FOX [*mecD*]	25	McRI$_{mecD}$-3
11	19Msa0624	AG	CLI [*erm*(B)], PEN [*mecD*], ERY [*erm*(B)], FOX (4mg/L) [*mecD*]	25	McRI$_{mecD}$-3
12	19Msa0613	ZH	TET [*tet*(L)], PEN [*mecD*], FOX [*mecD*]	25	McRI$_{mecD}$-3
13	19Msa0670	UR	TET [*tet*(L)], STR [*str*], FUS [*fusC*], PEN [*mecD*], KAN [*ant(4')-Ia*], FOX [*mecD*], [*fosB*]	26	McRI$_{mecD}$-3
14	19Msa0726	LU	TET [*tet*(L)], PEN [*mecD*], FOX [*mecD*]	64	McRI$_{mecD}$-3
15	19Msa0725	LU	CLI [*erm*(B)], TET [*tet*(L)], STR [*str*], FUS (<=0,5 mg/l) [*fusC*], PEN [*mecD*], CHL [*cat*$_{pC221}$], KAN [*ant(4')-Ia, aph(2')-Ic*], ERY [*erm*(B)], FOX [*mecD*]	6	McRI$_{mecD}$-2
16	19Msa0730	LU	STR [*str*], PEN [*mecD*], FOX [*mecD*]	40	McRI$_{mecD}$-3
17	19Msa0752	ZH	TET [*tet*(L)], PEN [*mecD*], FOX [*mecD*]	25	McRI$_{mecD}$-3
18	19Msa0793	TG	TET [*tet*(L)], STR [*str*], FUS [*fusC*], PEN [*mecD*], KAN [*ant(4')-Ia*], FOX [*mecD*], [*fosB*]	26	McRI$_{mecD}$-3
19	19Msa0834	BE	PEN [*mecD*], FOX (4 mg/l) [*mecD*]	21	McRI$_{mecD}$-3
20	19Msa0835	BE	TET [*tet*(M)], STR [*str*], PEN [*mecD*], FOX [*mecD*]	23	McRI$_{mecD}$-1
21	19Msa0837	BE	TET [*tet*(K), *tet*(L)], STR [*str*], PEN [*mecD*], FOX [*mecD*]	21	McRI$_{mecD}$-3
22	19Msa0853	LU	PEN [*mecD*], FOX (4 mg/l) [*mecD*]	21	McRI$_{mecD}$-3
23	19Msa0878	AG	PEN [*mecD*], FOX [*mecD*]	22	McRI$_{mecD}$-1
24	19Msa0879	AG	PEN [*mecD*], FOX [*mecD*]	22	McRI$_{mecD}$-1
25	19Msa0880	AG	PEN [*mecD*], FOX [*mecD*]	22	McRI$_{mecD}$-1
26	19Msa1005	SG	PEN [*mecD*], FOX [*mecD*]	26	McRI$_{mecD}$-3
27	19Msa0996	LU	STR [*str*], PEN [*mecD*], FOX [*mecD*]	40	McRI$_{mecD}$-3
28	19Msa0960	ZH	PEN [*mecD*], FOX [*mecD*]	66	McRI$_{mecD}$-3 (rt)
29	19Msa0962	SG	PEN [*mecD*], FOX [*mecD*]	66	McRI$_{mecD}$-3 (rt)
30	19Msa0963	FR	PEN [*mecD*], FOX [*mecD*]	66	McRI$_{mecD}$-3 (rt)
31	19Msa1101	AG	TET [*tet*(L)], PEN [*mecD*], KAN [ant(4')-Ia], FOX [*mecD*]	65	McRI$_{mecD}$-1
32	19Msa0611	ZH	CLI [*erm*(B)], PEN [*mecD*], ERY [*erm*(B)], FOX [*mecD*]	9	McRI$_{mecD}$-1
33	19Msa0631	FR	TET [*tet*(L)], STR [*str*], PEN [*mecD*], KAN (8mg/l) [*ant*(4')-*Ia*], FOX [*mecD*]	21	McRI$_{mecD}$-1
34	19Msa0723	SZ	CLI [*erm*(B)], TET [*tet*(L)1], STR [*str*], FUS (<=0,5 mg/l) [*fusC*], PEN [*mecD*], CHL [*cat*$_{pC221}$], KAN (8 mg/l) [*ant*(4')-Ia], ERY [*erm*(B)], FOX [*mec*D], [*bleO*] [b]	6	McRI$_{mecD}$-3 (rt)
35	19Msa0802	SG	PEN [*mecD*], FOX [*mecD*]	21	McRI$_{mecD}$-1
36	19Msa0850	AG	TET [*tet*(K)], PEN [*mecD*], KAN [*ant(4')-Ia, aph(2')-Ic*], FOX (4 mg/l) [*mecD*]	21	McRI$_{mecD}$-3
37	19Msa0848	SZ	TET [*tet*(K)], PEN [*mecD*], FOX (4 mg/l) [*mecD*]	21	McRI$_{mecD}$-3
			Macrococcus bohemicus		
38	19Msa0422	TG	PEN [*mecD*], FOX [*mecD*]	n.d.[c]	SCC*mecD*
			***Macrococcus* sp.**		
39	19Msa0966	SG	STR [*ant(6)-Ia*], FUS [*fusC*], PEN (<=0,12) [*mecD*], FOX (0,5 mg/l) [*mecD*]	n.d.[c]	McRI$_{mecD}$-1
40	19Msa0295	LU	CLI [*erm*(45)], STR [*ant(6)-Ia*], FUS (1mg/l) [*fusC*], PEN [*mecD*], ERY [*erm*(45)], FOX (1 mg/l) [*mecD*]	n.d.[c]	McRI$_{mecD}$-1
			Meat samples (Origin: CH)		
			Macrococcus caseolyticus		
41	19Msa0499	CH	PEN [*blaZm, mecB*], FOX [*mecB*]	61	plasmid
42	19Msa1047	CH	PEN [*blaZm, mecB*], FOX [*mecB*]	67	plasmid
43	19Msa0679	CH	PEN [*mecD*], FOX [*mecD*]	63	McRI$_{mecD}$-3
44	19Msa0687	CH	PEN [*blaZm, mecB*], FOX [*mecB*]	60	plasmid

[a] The resistance phenotype is indicated by antibiotics for which MIC was measured above the defined resistance breakpoint. For those strains harboring a resistance gene but exhibiting a MIC below the resistance breakpoint, MIC values of the respective antibiotics were indicated in brackets.
[b] No MICs were determined for strains containing the bleomycin (*bleO*) and fosfomycin (*fosB*) genes, and therefore only the genes are listed.
[c] n.d. = not determined (no MLST scheme available for *M. bohemicus* and *Macrococcus* sp.).
Cantons: SG, Sankt Gallen; LU, Luzern; GR, Graubünden; SZ, Schwyz; TG, Thurgau; BE, Bern; UR, Uri; AG, Aargau; ZH, Zurich; FR, Fribourg
Country: CH, Switzerland
Antibiotics: CLI, clindamycin; TET, tetracycline; PEN, penicillin; KAN, kanamycin; GEN, gentamicin, ERY, erythromycin; FOX, cefoxitin; STR, streptomycin; FUS, fusidic acid, CHL, chloramphenicol
Antimicrobial resistance genes and function: *erm*(B), *erm*(45), macrolide, lincosamide and streptogramin B 23S-rRNA methylase gene; *tet*(L) and *tet*(K), tetracyclin efflux genes; *tet*(M), ribosome protection tetracycline resistance gene; *mecB* and *mecD*, transpeptidase coding gene [PBP2a (penicillin binding protein)] for resistance to all β-lactam antibiotics; *blaZm*, β-lactamase gene; *aac(6')-Ie – aph(2'')-Ia*, gentamicin and kanamycin acetyltransferase and phosphotransferase tandem genes; *ant(4')-Ia*, aminoglycoside nucleotidyltransferase gene; *aph(2')-Ib, aph(2')-Ic*, aminoglycoside phosphotransferase gene; *msr*(G), ABC-F protein gene; *fusC*, gene for cytoplasmic protein that protects EF-G from binding fusidic acid; *ant(6)-Ia* and *str*, streptomycin nucleotidyltransferase gene; *cat*$_{pC221}$, chloramphenicol acetyltransferase gene; *rpsL*, gene encoding for ribosomal protein S12; *bleO*, bleomycin resistance gene; *fosB*, Mn^{2+}-dependent fosfomycin-inactivating enzyme gene

longed to the genus *Macrococcus* with the strains 19Msa0295 and 19Msa0966 exhibiting 99,74 % identities to 16S rRNA gene sequence of the closest relative *M. canis* and the strain 19Msa1099 exhibiting 99,81 % with 16S rRNA of the closest relative *M. caseolyticus*. MALDI-TOF MS identifications for *M. caseolyticus* were confirmed through *nuc*-PCR, which allowed the identification of 42 strains as *M. caseolyticus*, even though ten of them displayed a MALDI-TOF MS score between 1,92 – 1,99. *Macrococcus* sp. 19Msa1099 also displayed a MALDI-TOF MS score of 1,99 for *M. caseolyticus*, but was negative in the PCRs. Four strains were identified as *M. bohemicus* by MALDI-TOF MS with scores between 1,81 and 2,16, and were confirmed as such by *hsp*60 sequence analysis.

Antimicrobial Resistance Phenotype and Genotype

All the *Macrococcus* species grew on the selective plates BD BBL™ CHROMagar™ MRSA II and contained a methicillin resistance gene with *mecD* found in 45 strains (39 *M. caseolyticus*, four *M. bohemicus* and two *Macrococcus* spp.) and *mecB* in four strains (three *M. caseolyticus* and one *Macrococcus* sp.). They exhibited an MIC above the cefoxitin breakpoint of 8 mg/L, except for one *M. caseolyticus* (19Msa0848) that exhibited an MIC of 4 mg/L and two *mecD*-carrying *Macrococcus* spp. (19Msa0295 and 19Msa0966), that exhibited an MIC of 0,5 mg/L for cefoxitin (Table 1 and 2). The two *mecD*-carrying *Macrococcus* spp. were also tested for oxacillin resistance and they both exhibited an MIC of 16 mg/L, which is above the MIC of oxacillin for *mec*-negative *M. caseolyticus* strains CCUG 15606^T (≤0,25 mg/L) and KM1352 (0,5 mg/L)[14] and the CLSI oxacillin resistance breakpoint of *Staphylococcus* other than *S. aureus* of ≥ 1 mg/L.

Thirty-two strains also showed reduced susceptibility to additional antibiotics including tetracycline, clindamycin, streptomycin, erythromycin, kanamycin, fusidic acid and gentamicin. Tetracycline resistance was associated with the presence of the tetracycline efflux genes *tet*(L) (n=13) and *tet*(K) (n=4) and the ribosome protection tetracycline resistance gene *tet*(M) (n=1). One strain contained both *tet*(L) and *tet*(K). Strains exhibiting resistance to both erythromycin and clindamycin (n=9) contained the 23 rRNA methylase genes *erm*(B) (n=7) and *erm*(45) (n=2). In the *erm*(45) containing strains, clindamycin resistance was inducible. One of the strains had an additional macrolide ABC-F ribosomal protection gene *msr*(G). Streptomycin resistance was associated with the streptomycin nucleotidyltransferase genes *str* (n=10) and *ant(6)-Ia* (n=2) and one strain exhibited two mutations in the *rpsL* gene resulting in a Proline to Alanine substitution at position 22 (P22A) and a Lysine to Arginine substitution at position 56 (K56R) in the 30S ribosomal protein S12. One strain was resistant to gentamicin and carried the tandem genes *aac(6')-Ie – aph(2'')-Ia* encoding for gentamicin and kanamycin acetyltransferase and phosphotransferase (Tables 1 and 2).

Table 2: Antimicrobial resistance phenotypes and genotypes, canton of origin, sequence types (ST) and genetic elements containing *mecD* or *mecB* of *Macrococcus* spp. from nasal cavities of pigs at slaughter and from Swiss retail pork meat

Nr.	Sample Name	Origin	Phenotype[a] and Genotype[b]	ST	*mec*-element
Nasal cavities					
Macrococcus caseolyticus					
1	19Msa0847	LU	TET [*tet*(K)], PEN [*mecD*], FOX [*mecD*]	21	McRI$_{mecD}$-3
Macrococcus bohemicus					
2	19Msa0936	TG	STR [*str*], PEN [*mecD*], FOX [*mecD*]	n.d.[c]	SCC*mecD*
3	19Msa1083	BE	CLI [*erm*(45)], FUS [*fusC*], PEN [*mecD*], ERY [*erm*(45)], FOX [*mecD*]	n.d.[c]	McRI$_{mecD}$-1
4	19Msa0383	TG	PEN [*mecD*], FUS [*fusC*], FOX [*mecD*]	n.d.[c]	SCC*mecD*
Meat samples (Origin: CH)					
***Macrococcus* sp.**					
5	19Msa1099	CH	PEN [*blaZm*, *mecB*], FOX [*mecB*]	n.d.[c]	plasmid

[a] The resistance phenotype is indicated by antibiotics for which MIC was measured above the defined resistance breakpoint. For those strains harboring a resistance gene but exhibiting a MIC below the resistance breakpoint, MIC values of the respective antibiotics were indicated in brackets.
[b] No MICs were determined for strains containing the bleomycin (*bleO*) and fosfomycin (*fosB*) genes, and therefore only the genes are listed.
[c] n.d. = not determined (no MLST scheme available for *M. bohemicus* and *Macrococcus* sp.).
Cantons: LU, Luzern; TG, Thurgau; BE, Bern; ZH, Zurich.
Country: CH, Switzerland
Antibiotics: CLI, clindamycin; TET, tetracycline; PEN, penicillin, ERY, erythromycin; FOX, cefoxitin; STR, streptomycin; FUS, fusidic acid.
Antibiotic resistance genes and function: *erm*(45), macrolide, lincosamide and streptogramin B 23S-rRNA methylase gene; *tet*(K), tetracyclin efflux genes; *mecB* and *mecD*, transpeptidase coding gene [PBP2a (penicillin binding protein)] for resistance to all β-lactam antibiotics; *blaZm*, β-lactamase gene; *fusC*, gene for cytoplasmic protein that protects EF-G from binding fusidic acid; *str*, streptomycin nucleotidyltransferase gene.

Characterization of the genetic elements containing *mecD* and *mecB*

The genetic elements containing *mecD* were characterized by multiplex PCR developed for molecular typing of McRI$_{mecD}$. WGS was performed for three *mecD*-containing *M. bohemicus* strains, whose genetic element remained untypable and for all *mecB*-containing strains. Twenty-eight *M. caseolyticus* strains had a McRI$_{mecD}$-3, two *M. caseolyticus* strains carried a McRI$_{mecD}$-2, and McRI$_{mecD}$-1 was present in nine *M. caseolyticus* but also in two *Macrococcus* spp. and in one *M. bohemicus* (Table 1 and Table 2). Four of the McRI$_{mecD}$-3-containing *M. caseolyticus* strains carried the reverse transcriptase gene (*rt*), that are also present on McRI$_{mecD}$-2. The *M. bohemicus* strains with untypable elements (19Msa0422, 19Msa0383 and 19Msa1083) contained the *mecD* gene on novel SCC*mecD* elements (Figure 2). In a previous report, we described the element of 19Msa0422

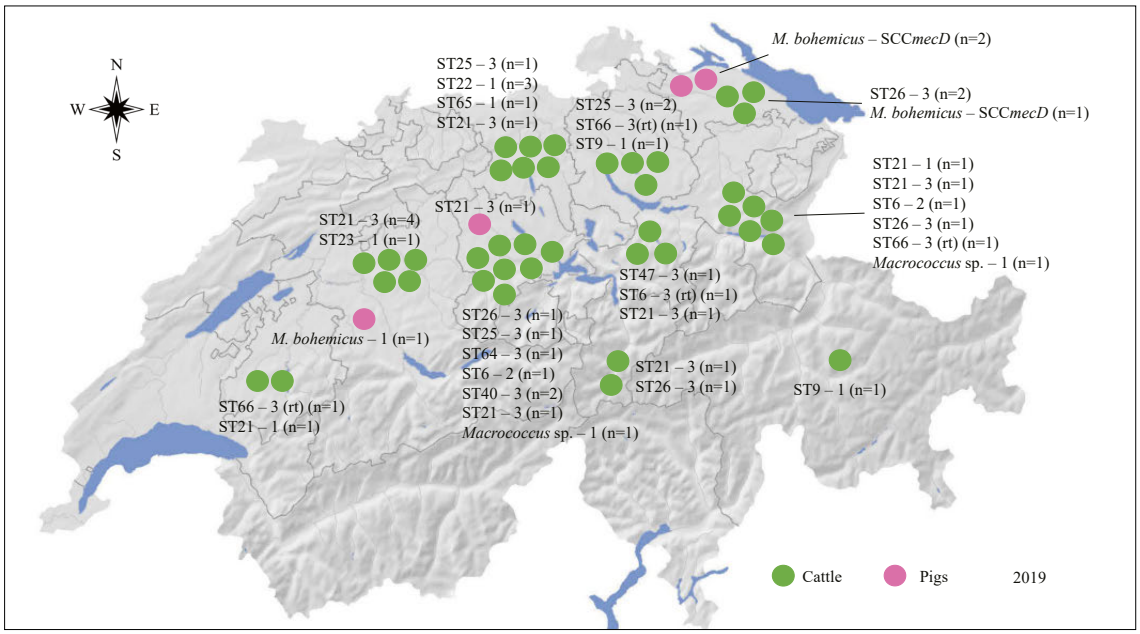

Figure 1: Distribution of methicillin-resistant *Macrococcus* spp. isolated from nasal swabs of slaughtered pigs and calves in Switzerland in 2019. ST, sequence type; the numbers following the STs (-1, -2, -3, -3(rt)) indicate the type of McRI$_{mec}$ containing *mecD*. Strains containing SCC*mecD* are indicated.

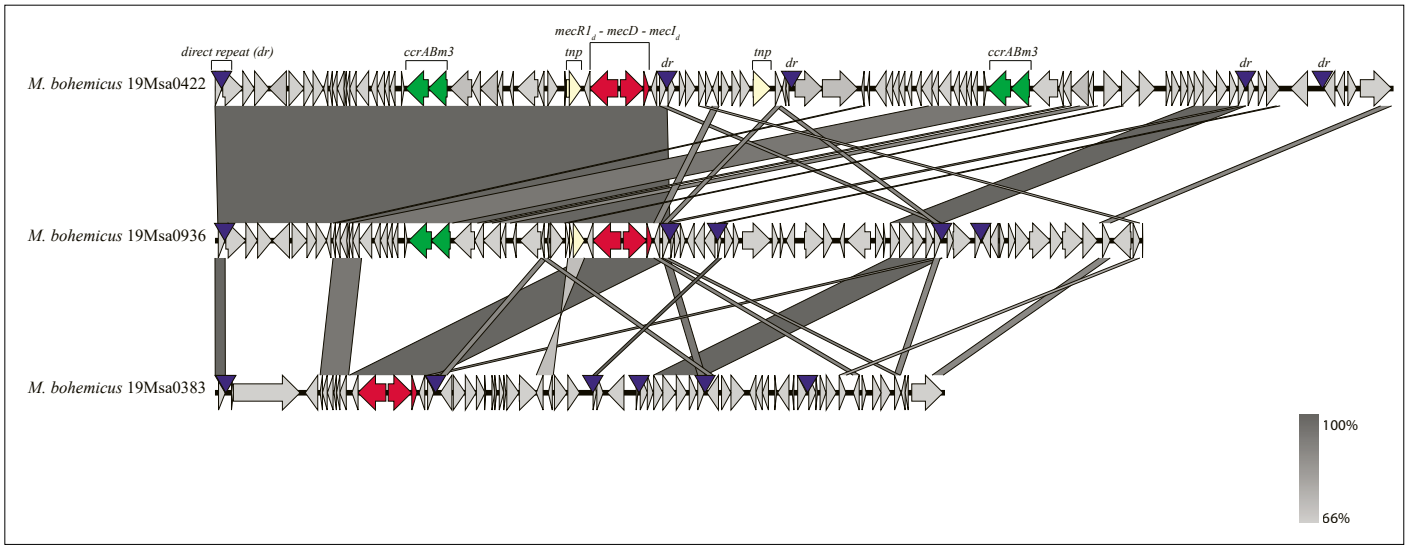

Figure 2: Comparison between SCC*mecD* of strains 19Msa0422 and 19Msa0936 and the SCC*mec* of 19Msa0383. The direct repeats that flank the subunits of the complex SCC*mec* elements are marked as blue pointer. The direct repeats were identified using the following consensus sequence for the integration site sequence (ISS) for SCC: N-[ATG]-[AG]-[GAT]-N-N-T-A-[TC]-C-A-[CT]-A-A-[AG]-T-[AG]-[AG], N indicating any base, positions with more than one base allowed are in square brackets with possible bases given. The arrows represent the open reading frames (*orf*). The *ccrABm3* genes are highlighted in green, the *mecD* gene complex composed of the *mec*D gene and the regulatory genes *mecR1*$_d$, *mecI*$_d$ in red.

since it was the first detection of *mecD* on a SCC*mec*.[17] *M. bohemicus* 19Msa0936 carried an almost identical SCC*mecD* element (99,99 % DNA identity) with a cassette chromosome recombinase *ccrABm3* genes as 19Msa0422. The third *M. bohemicus* strain, 19Msa0383, carried the same *mecD* operon downstream of the *rlmH* gene, but in a novel ΨSCC*mecD* element of 15'115 bp, which lacks the *ccrABm3* genes and most of SCC*mecD*$_{19Msa0422}$ (Figure 2). In this ΨSCC*mecD* the *rlmH* gene was followed by an open reading frame (*orf*) of 4'836 bp coding for an helicase of the DEAD-like family. All three strains carried additional unique ΨSCC (segment without *ccr* genes) and SCC (segment with *ccr* genes) in the *rlmH* region and formed (Ψ)SCC*mecD* composite islands. These complex elements were segmented by direct repeats (dr) that contain an imperfect copy of the integration site sequence (ISS) for SCC (Figure 2).[29]

The *mecB* gene associated with the *mec* transposon Tn*6045* was present on a plasmid in three *M. caseolyticus* strains as well as in one *Macrococcus* sp. strain isolated from Swiss meat (Figure 3, Tables 1 and 2). The plasmids were similar to each other and to the already described plasmids of *M. caseolyticus* JCSC5402[15] and *M. canis* KM0218[16]. However, they were smaller as they did not carry other resistance genes (Figure 3).

Genotyping

Genotypic analysis of the *M. caseolyticus* strains revealed 16 different STs with ST21 being the most predominant (n=12), followed by ST26 (n=6), ST25 (n=4), ST6, ST22, ST66 (n=3 each) and ST9, ST40 (n=2 each) (Table 1 and Table 2). Other STs were unique. *M. caseolyticus* ST21 was found in both pigs and calves (Figure 1). Strains of ST21 harbored either McRI$_{mecD}$-1 or -3 (Figure 1). This diverse population of *M. caseolyticus* was disseminated in different geographical regions of Switzerland (Figure 1). The four *M. caseolyticus* strains isolated from beef meat belonged to four different STs, which were not detected among the isolates from animals (Table 1).

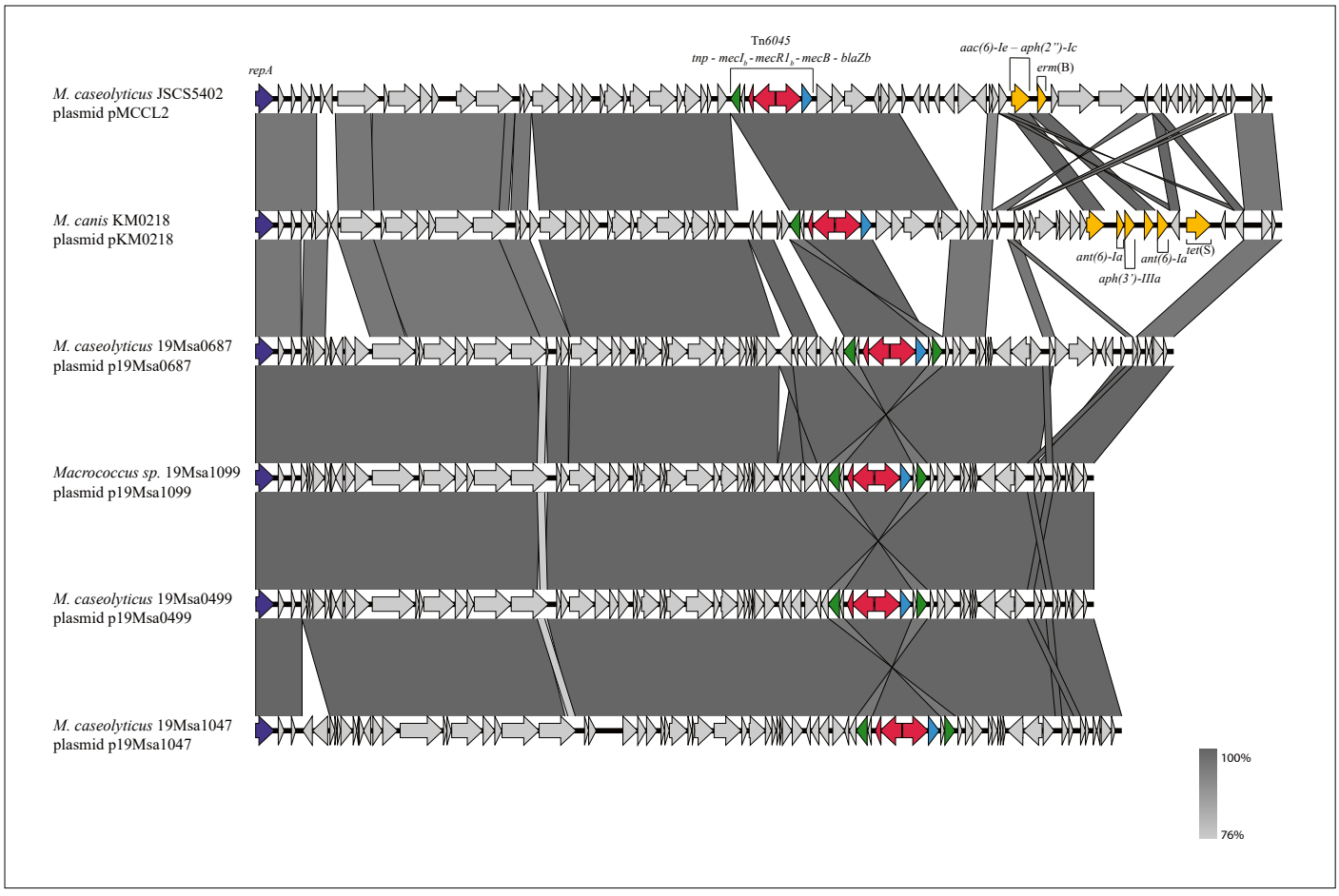

Figure 3: Comparison between the *mecB*-carrying plasmids of *M. caseolyticus* JCSC5402, *M. canis* KM0218, *M. caseolyticus* 19Msa0687, 19Msa0499, 19Msa1047 and *Macrococcus* sp. 19Msa1099. The plasmid replication initiation protein gene (*repA*) is highlighted in navy. The four strains of this study carried the *mec* transposon Tn*6045*.[15] The Tn*6045* transposase (*tnp*) is highlighted in green, the *mecB* gene complex composed of the *mecB* gene and the regulatory gees *mecR1$_b$*, *mecI$_b$* in red and the *blaZb* genes are indicated in blue. Various resistance genes in light orange.

Discussion

The use of chromogenic plates developed for the isolation of MRSA also permits the selection of methicillin-resistant macrococci. On this selective medium, *M. caseolyticus* colonies exhibit light-pink coloration, while those of *S. aureus* display pink to dark purple coloration. These criteria were therefore applied for the selection of the *S. aureus* destined to the national monitoring of MRSA[19] as well as for the identification of the putative *Macrococcus* colonies. It happened that *M. bohemicus* as well as some other *Macrococcus* spp. could also be isolated using this approach.

Identification of *Macrococcus* spp. at the species level could be achieved by MALDI-TOF MS for a majority of the isolates. However, for some of them, the score was below the cut off value of 2,0 set for reliable identification and further identification with molecular techniques were needed. For instance, *nuc*-PCR was applied to clearly differentiate *M. caseolyticus* from *M. canis*,[10] and *hsp60* gene sequence analysis was used to distinguish between the closely related *M. bohemicus*, *M. epidermidis* and *M. goetzii* species. The use of *hsp60* has been shown to be a reliable choice since *M. goetzii*, *M. epidermidis* and *M. bohemicus* are closely related species that cannot be distinguished based on 16S rRNA gene sequence.[5] Sequencing of the 16S rRNA gene was however used to further identify three strains which remained unidentified by MALDI-TOF MS, *nuc*-PCR and *hsp60* gene analysis. The 16S rRNA gene sequence revealed that these strains represent putative novel species of the *Macrococcus* genus. This approach consisting of using both MALDI-TOF MS and molecular tests allowed to identify the strains at the species level and also showed that animals represent a source of novel species of *Macrococcus*.

The prevalence of methicillin-resistant *M. caseolyticus* in nasal cavities of calves [12,71 % (95 % CI, 9,40 % – 16,96 %)] was higher than the prevalence of MRSA calculated within the monitoring program [3,68 % (95 % CI, 2,07–6,47 %)] with the same samples.[19] On the other hand, the prevalence of methicillin-resistant *Macrococcus* sp. in nasal cavities of pigs was 2,65 % (95 % CI, 1,35–5,14 %) and much lower than that of MRSA [52,81 % (95 % CI, 47,18–58,36 %)]. This is likely due to the fact that *M. caseolyticus* is rather a bacteria associated with milk and dairy animals than with pigs, and nasal carriage may be transient and associated with milk feeding. Feeding calves with raw milk is a common practice in Switzerland,[30] and may be one of the source of *M. caseolyticus* in the nasal cavities of calves. Methicillin-resistant *M. caseolyticus* have already been isolated from mastitis milk of dairy cows in Switzerland[8,14] and in tank milk in UK and Wales.[31] However, the driving force selecting for methicillin-resistance in *Macrococcus* from pigs and calves is not known so far. The presence of the same *mec*-containing elements in genetically diverse *Macrococcus* in different geographical locations and animals suggests an independent acquisition of the genetic elements, which may be driven by the use of β-lactam antibiotics in farming environment. The presence of methicillin-resistant *Macrococcus* spp. in meat is likely to be associated with contamination during processing. However, the macrococci from meat rather harbored the methicillin resistance gene *mecB* on plasmids, whereas the macrococci from animals contained *mecD*. This genetic difference suggests that the source of contamination may not be the same. Besides the methicillin-resistance gene *mecB* and *mecD* which seems to be specific to macrococci, other resistance genes found in macrococci were however the same as those also occurring in staphylococci from animals of both cattle and pig origin.[32,33] The presence of identical resistance determinants in both genera indicates either an exchange of genes or a common source of genes. For instance, the *erm*(45) gene, an MLS_B resistance gene first described in a *Mammaliicoccus fleurettii* strain from milk in Switzerland,[25] was also found during this study for the first time in macrococci from calves and pigs.

Methicillin-resistance genes were found in strains which exhibited a MIC below the resistance breakpoint of cefoxitin (R ≥ 8 mg/L) used for the detection of methicillin resistance in *S. aureus*. Three of the *mecD*-carrying strains exhibited a MIC for cefoxitin ranging from 0,5 mg/L to 4 mg/L, even though they all grew well on the MRSA selective agar. We observed that the two strains 19Msa0295 and 19Msa0966 exhibiting a low cefoxitin MIC (0,5 mg/L) showed an MIC of oxacillin above the MIC of methicillin-susceptible *M. caseolyticus* strains and the CLSI breakpoint (R ≥ 1 mg/L) used to predict methicillin-resistance in staphylococci other than *S. aureus*. A similar result was already described by MacFadyen et *al.* who observed that antimicrobial susceptibility testing using the Vitek2 system (Biomérieux) presented a discrepancy between resistance to cefoxitin and oxacillin.[31] In that study, all strains containing eighter the *mecB* or *mecD* resistance genes exhibited resistance to oxacillin using the Vitek2 system, but only one strain showed resistance to cefoxitin. A larger analysis of different isolates of *Macrococcus* spp. should be conducted to determine whether oxacillin or cefoxitin is most suitable to predict methicillin resistance in *Macrococcus* spp. as it has been done for example for *S. pseudintermedius* and *S. schleiferi*.[34,35] However, our results and those of MacFadyen et al.[31] suggest that oxacillin should be preferred as a predictor of methicillin-resistance in macrococci as it is used for *Staphylococcus* other than *S. aureus*.[23] We also observed that the MIC of cefoxitin for some *mecD* containing macrococci may decrease to 1 mg/L after several

passages of the strains on blood plates. We therefore recommend to perform MIC measurement from colonies which were not passaged more than two times on blood plates after their selection from the selective MRSA agar.

One streptomycin resistant strain harbored mutation of the *rpsL* encoded 30S ribosomal protein S12. While the role of P22A mutation is not known, the K56R mutation has been well characterized and shown to be associated with streptomycin resistance in other bacteria like e.g. *Lactobacillus* and *Escherichia coli*.[36,37]

Overall, this study gives for the first time an indication of the prevalence of methicillin-resistant *Macrococcus* species in food producing animals and in meat in Switzerland. It also highlights livestock as a possible source for new *Macrococcus* species and further demonstrates that *Macrococcus* plays a role as reservoir for antimicrobial resistance genes. Those results suggest that further attention should be paid to this genus due to its role as turntable for the acquisition and dissemination of antimicrobial resistance genes and the potential of some of its members to cause infections.

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Prévalence et caractérisation des *Macrococcus* spp. résistants à la méthicilline chez les animaux de rente et dans la viande en Suisse en 2019

La prévalence des macrococques résistants à la méthicilline chez les veaux et les porcs à l'abattoir et dans la viande de bœuf et de porc au détail a été déterminée à partir d'échantillons prélevés en 2019 dans le cadre du monitoring national des *Staphylococcus aureus* résistants à la méthicilline chez les animaux de rente en Suisse. Les isolats ont été soumis à des tests de sensibilité à 19 antibiotiques et à des techniques moléculaires (par exemple PCR, microarray, WGS) pour l'identification des gènes de résistance, des éléments contenant les gènes *mec* responsible de la résistance à la méthicilline, ainsi que du type de séquence (ST).

Des macocoques résistants à la méthicilline (*M. caseolyticus* (n=38), *M. bohemicus* (n=4) et *Macrococcus* spp. (n=2)) ont été isolés dans 40 des 299 écouvillons nasaux de veaux, ce qui représente une prévalence de 13,38 % (IC 95 %, 9,98 % – 17,70 %), et dans quatre des 303 écouvillons nasaux de porcs [1,32 % (IC 95 %, 0,36 % – 3,35 %)]. Un des 311 échantillons de viande de porc suisse contenait un *Macrococcus* sp. [0,32 % (IC 95 %, 0,01 % – 1,78 %)], et quatre des 309 échantillons de viande de bœuf (260 domestiques et 49 importés) contenaient *M. caseolyticus* [1,29 % (IC 95 %, 0,35 % – 3,28 %)].

Les souches de *M. caseolyticus* appartenaient à divers ST, le ST21 étant le plus fréquent chez les porcs et les veaux. Le gène *mecD* a été localisé sur des éléments du

Prevalenza e caratterizzazione di *Macrococcus* spp. meticillino-resistente negli animali da reddito e nella carne in vendita al dettaglio in Svizzera nel 2019

La prevalenza di *Macrococcus* spp. meticillino-resistente nei vitelli e nei suini destinati al macello e nella carne bovina e suina in vendita al dettaglio è stata determinata utilizzando campioni prelevati nel 2019 nel quadro del monitoraggio nazionale dello *Staphylococcus aureus* meticillino-resistente negli animali da reddito in Svizzera. Gli isolati sono stati sottoposti a test di suscettibilità antimicrobica per 19 antibiotici ed a diverse tecniche molecolari (ad es. PCR, microarray, WGS) per l'identificazione dei geni di resistenza, degli elementi contenenti i geni di resistenza alla meticillina *mec* e della variante di sequence type (ST).

I *Macrococcus* spp. meticillino-resistenti (*M. caseolyticus* (n=38), *M. bohemicus* (n=4) e *Macrococcus* spp. (n=2)) sono stati isolati in 40 dei 299 tamponi nasali dei vitelli con una prevalenza del 13,38 % (95 % CI, 9,98 % – 17,70 %), e in quattro dei 303 tamponi nasali dei maiali [1,32 % (95 % CI, 0,36 % – 3,35 %)]. Uno dei 311 campioni di carne suina svizzera conteneva un *Macrococcus* sp. [0,32 % (95 % CI, 0,01 % – 1,78 %)], e quattro dei 309 campioni di carne bovina (260 nazionali e 49 importati) contenevano *M. caseolyticus* [1,29 % (95 % CI, 0,35 % – 3,28 %)].

I ceppi di *M. caseolyticus* si sono rivelati appartenere a diversi sequence types, il più comune dei quali è risultato essere ST21 sia nei maiali che nei vitelli. Il gene *mecD* è stato riscontrato a livello cromosomico in 42

chromosome McRI$_{mecD}$ dans 42 souches et SCC*mecD* dans trois souches, tandis que le gène *mecB* se trouvait sur des plasmides dans quatre souches. En plus de la résistance aux β-lactamines, les souches étaient également résistantes à la tétracycline (n=17 ; *tet*(L), *tet*(K), *tet*(M)), à la streptomycine (n=13 ; *str*, *ant(6)-Ia*, mutation *rpsL* [K56R dans la protéine ribosomale S12]), à la kanamycine (n=10 ; *aac(6')-Ie–aph(2'')-Ia*, *aph(2')-Ib*, *aph(2')-Ic*, *ant(4')-Ia*), clindamycine (n=9 ; *erm*(B), *erm*(45)), érythromycine (n=9 ; *erm*(B), *msr*(G), *erm*(45)), acide fusidique (n=9 ; *fusC*) et gentamicine (n=1 ; *aac(6')-Ie–aph(2'')-Ia*).
Cette étude est la première à déterminer prévalence des *Macrococcus* spp. résistants à la méthicilline chez les porcs, les veaux, la viande de porc et de bœuf en Suisse et a révélé un réservoir de souches génétiquement diverses et portant plusieurs traits de résistance.

Mots clés: Résistance aux antibiotiques; *Macrococcus* spp.; McRI$_{mecD}$; monitoring; SCC*mec*; WGS

ceppi sull'isola di resistenza *Macrococcus mecD* (McRI-$_{mecD}$) e in tre ceppi sull'elemento mobile *Staphylococcal cassette chromosome mec* (SCC*mecD*), mentre *mecB* è stato trovato sui plasmidi in quattro ceppi. Oltre alla resistenza ai β-lattamici, i ceppi hanno mostrato anche resistenza alla tetraciclina (n=17*; tet*(L), *tet*(K), *tet*(M)), streptomicina (n=13; *str*, *ant(6)-Ia*, mutazione *rpsL* [K56R nella proteina ribosomiale S12]), kanamicina (n=10; *aac(6')-Ie–aph(2'')-Ia*, *aph(2')-Ib*, *aph(2')-Ic*, *ant(4')-Ia*), clindamicina (n=9; *erm*(B), *erm*(45)), eritromicina (n=9; *erm*(B), *msr*(G), *erm*(45)), acido fusidico (n=9; *fusC*) e gentamicina (n=1; *aac(6')-Ie–aph(2'')-Ia*).
Questo studio rappresenta il primo studio di prevalenza nazionale di *Macrococcus* spp. resistente alla meticillina in suini, vitelli, carne di maiale e di manzo in Svizzera e ha rivelato un serbatoio di ceppi geneticamente diversi che portano diversi tratti di resistenza.

Parole chiave: resistenza agli antibiotici, *Macrococcus* spp., McRI$_{mecD}$, monitoring, SCC*mec*, WGS

Literaturnachweis

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Korrespondenzadresse

Prof.Dr. Vincent Perreten
Institute of Veterinary Bacteriology, University of Bern
Länggassstrasse 122
CH-3012 Bern
Telefon: +41 31 684 24 30
E-mail: vincent.perreten@vetsuisse.unibe.ch