

Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among Swiss veterinary health care providers: Detection of livestock- and healthcare-associated clones

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Summary

We screened a total of 340 veterinarians (including general practitioners, small animal practitioners, large animal practitioners, veterinarians working in different veterinary services or industry), and 29 veterinary assistants for nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) at the 2012 Swiss veterinary annual meeting. MRSA isolates (n = 14) were detected in 3.8% (95% CI 2.1–6.3%) of the participants whereas MRSP was not detected. Large animal practitioners were carriers of livestock-associated MRSA (LA-MRSA) ST398-t011-V (n = 2), ST398-t011-IV (n = 4), and ST398-t034-V (n = 1). On the other hand, participants working with small animals harbored human healthcare-associated MRSA (HCA-MRSA) which belonged to epidemic lineages ST225-t003-II (n = 2), ST225-t014-II (n = 1), ST5-t002-II (n = 2), ST5-t283-IV (n = 1), and ST88-t186-IV (n = 1). HCA-MRSA harbored virulence factors such as enterotoxins, β -hemolysin converting phage and leukocidins. None of the MRSA isolates carried Panton-Valentine leukocidin (PVL). In addition to the methicillin resistance gene *mecA*, LA-MRSA ST398 isolates generally contained additional antibiotic resistance genes conferring resistance to tetracycline [*tet*(M) and *tet*(K)], trimethoprim [*df*rK, *df*rG], and the aminoglycosides gentamicin and kanamycin [*aac*(6')-Ie-aph(2')-Ia]. On the other hand, HCA-MRSA ST5 and ST225 mainly contained genes conferring resistance to the macrolide, lincosamide and streptogramin B antibiotics [*erm*(A)], to spectinomycin [*ant*(9)-Ia], amikacin and tobramycin [*ant*(4')-Ia], and to fluoroquinolones [amino acid substitutions in GrlA (S84L) and GyrA (S80F and S81P)]. MRSA carriage may represent an occupational risk and veterinarians should be aware of possible MRSA colonization and potential for developing infection or for transmitting these strains. Professional exposure to animals should be reported

Methicillin-resistente *Staphylococcus aureus* (MRSA) im Nasenabstrich von Beschäftigten im Schweizer Veterinärsektor: Nachweis von Nutztier-assoziierten und Spital-assoziierten Klonen

Insgesamt 340 Tierärzte/Tierärztinnen und 29 Tiermedizinische Praxisassistentinnen wurden am Tierärzte-Kongress 2012 mittels Nasentupferproben auf das Vorkommen von Methicillin-resistenten *Staphylococcus aureus* (MRSA) und Methicillin-resistenten *Staphylococcus pseudintermedius* (MRSP) untersucht. Unter den beprobten Tierärzten und Tierärztinnen befanden sich Allgemeinpraktiker, Kleintierspezialisten, Grosstierärzte und Tierärzte, welche in der Industrie oder in einem Amt arbeiten und demzufolge keinen direkten Kontakt zum Tier aufweisen. Insgesamt konnten 14 MRSA (3.8%, 95% CI 2.1–6.3%) isoliert werden, MRSP wurden keine gefunden. Grosstierärzte waren Träger von «Grosstier-assoziierten» MRSA (livestock-associated MRSA, LA-MRSA) ST398-t011-V (n = 2), ST398-t011-IV (n = 4) und ST398-t034-V (n = 1). Personen, die mit Kleintieren arbeiten, sind hingegen Träger von humanen «Spital-assoziierten» MRSA (healthcare-associated MRSA, HCA-MRSA), wie ST225-t003-II (n = 2), ST225-t014-II (n = 1), ST5-t002-II (n = 2), ST5-t283-IV (n = 1) und ST88-t186-IV (n = 1). HCA-MRSA tragen Virulenzfaktoren wie Enterotoxine, phagenkodierte Virulenzfaktoren und Leukocidine. Bei keinem MRSA-Isolat konnte das Panton-Valentin-Leukozidin (PVL) nachgewiesen werden. LA-MRSA vom Typ ST398 wiesen neben dem Methicillin-Resistenzgen *mecA* zusätzliche Resistenzen gegenüber Tetracyclin [*tet*(M) und *tet*(K)], Trimethoprim [*df*rK, *df*rG] und den Aminoglykosiden Gentamicin und Kanamycin [*aac*(6')-Ie-aph(2')-Ia] auf. Bei HCA-MRSA ST5 und ST225 konnten Resistenzgene gegen Makrolide, Linkosamide und Streptogramin B [*erm*(A)], sowie gegen Spectinomycin [*ant*(9)-Ia], Amikacin und Tobramycin [*ant*(4')-Ia] und gegen Fluoroquinolone [Aminosäuresubstitution in GrlA

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upon hospitalization and before medical intervention to allow for preventive measures. Infection prevention measures are also indicated in veterinary medicine to avoid MRSA transmission between humans and animals, and to limit the spread of MRSA both in the community, and to animal and human hospitals.

Keywords: human, nose, prevalence, genotyping, antibiotic resistance, *mecA*, *Staphylococcus aureus*, *S. pseudintermedius*

(S84L) und GyrA (S80F and S81P)] gefunden werden. MRSA-Trägertum ist ein Berufsrisiko und Tierärzte sollten sich einer möglichen MRSA-Kolonisation, bzw. des Risikos einer Infektion und einer möglichen Übertragung bewusst sein. Bei einer Hospitalisierung oder vor einem operativen Eingriff sollte das Berufsrisiko für MRSA-Trägertum erwähnt werden, damit präventive Massnahmen ergriffen werden können. Darüber hinaus sollte in der Veterinärmedizin die Infektionsprävention konsequent durchgeführt werden. So kann die mögliche Übertragung zwischen Tier und Mensch verhindert werden und die Verbreitung von MRSA in der Gesellschaft, im Spital und in der Tierklinik begrenzt werden.

Schlüsselwörter: Mensch, Nase, Prävalenz, Genotypisierung, Antibiotika-Resistenz, *mecA*, *Staphylococcus aureus*, *S. pseudintermedius*

Introduction

Staphylococcus (S.) aureus can colonize different parts of the human body, but the anterior nares are the main ecological niche (Wertheim et al., 2005). Longitudinal studies reported different percentage ranges of nasal carriage pattern, with an average of approximately 20 % of healthy human individuals being persistent carriers of one particular strain, about 30 % being intermittent carriers with colonization status and type of strains varying overtime, and about 50 % being non-carriers as they never carry *S. aureus* (Wertheim et al., 2005). The prevalence of *S. aureus* nasal carriage may depend on population, health status, occupation and age (Wertheim et al., 2005). Like *S. aureus* in humans, *S. pseudintermedius* belongs to the normal flora of dogs and also primarily colonizes the nasopharynx (Bannoehr and Guardabassi, 2012). Although *S. pseudintermedius* is not part of the normal human flora, transmission between dogs and humans has been reported (Weese and van Duijkeren, 2010; van Duijkeren et al., 2011; Bannoehr and Guardabassi, 2012).

Methicillin-resistant *S. aureus* (MRSA) and *S. pseudintermedius* (MRSP) both have acquired staphylococcal chromosome cassette *mec* (SCC*mec*) elements that contain the *mecA* gene which confers resistance to all β -lactam antibiotics, with the exception of ceftaroline, a new broad-spectrum cephalosporin (Chambers, 1997; Steed and Rybak, 2010). MRSA and MRSP often contain additional resistance mechanisms leading to multidrug resistance and limiting therapeutical options (de Lencastre et al., 2007; Frank and Loeffler, 2012).

Until the 1990s, MRSA mainly led to problems in the hospital environment, in particular as the cause of

postoperative infections. This type of MRSA was called hospital-acquired or healthcare-associated MRSA (HA-MRSA and HCA-MRSA, respectively). Since the mid-1990s, a new form of MRSA infections has emerged in many countries, independent of the healthcare setting, as a cause of invasive skin and soft tissue infections: the community-acquired MRSA (CA-MRSA) (David and Daum, 2010). Since the beginning of the 2000s, livestock-associated MRSA (LA-MRSA) has emerged in domestic animal species like pigs, horses and cattle, and has also the potential to colonize and cause infections in humans (Graveland et al., 2011a; Fluit, 2012; Verkade and Kluytmans, 2013). LA-MRSA mainly belongs to the clonal complex (CC) 398 (Verkade et al., 2013). Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), on the other hand, has been reported since 2005 in numerous countries, predominantly causing infection in dogs and cats, and occasionally in humans (van Duijkeren et al., 2011; Bannoehr and Guardabassi, 2012).

Transmission of MRSA and MRSP between veterinarians, farmers and animal owners has been well documented (Davis et al., 2012; Bannoehr and Guardabassi, 2012; Fluit, 2012). Contact to animals is a risk factor of MRSA carriage in humans and the likelihood of carriage correlates with the intensity of animal exposure (Graveland et al., 2011b; Feingold et al., 2012; Garcia-Graells et al., 2012). Higher prevalence of MRSA and MRSP nasal carriage in veterinarians has been observed in several countries around the world (van Duijkeren et al., 2011; Paterson et al., 2013). In Switzerland, a study conducted in 2009 revealed that 3 % of the tested veterinarians were carriers of MRSA, which belonged to the CC398

and CC8 lineages (Huber et al., 2010). In the present study, veterinary health care providers who participated in a screening study provided nasal samples to be tested for the presence of both MRSA and MRSP, and gave information on their occupation. The molecular characterization of the methicillin-resistant staphylococci should reveal if new emerging clones are present among veterinary health workers in Switzerland and determine if a link exists between occupation and nasal carriage of specific clonal lineages of methicillin-resistant staphylococci.

Material and Methods

Sampling, isolation and identification of methicillin-resistant *Staphylococcus*

A total of 369 veterinary health care providers were screened at the annual national meeting in June 2012 in Switzerland. All the 369 screened persons participated voluntarily in the study and completed and signed a questionnaire asking the field of profession (small animal practice, horse practice, rural practice, general practice, veterinary assistant, or other) and locality. Both anterior nares were tested using a single sterile swab that was pre-moistened with physiological saline solution (0.85% NaCl). Samples were taken by veterinarians themselves under supervision and stored in transport medium at 6 °C until cultivation (Oxoid Ltd, Basingstoke, England). All swabs were processed within one week. Methicillin-resistant staphylococci were cultured in a two-step enrichment procedure (Overesch et al., 2011) and selected on chromogenic selective MRSA agar plates (Brilliance MRSA 2 agar (Oxoid)). Colonies were purified onto trypton soy agar plates containing 5% sheep blood (TSA-S) (Becton, Dickinson and Company) and identified to the species level by Matrix-Assisted-Laser-Desorption/Ionization-Time-Of-Flight-Mass-Spectrometry (MALDI-TOF MS) analysis (Microflex LT, Bruker Daltonics GmbH, Bremen). The presence of the methicillin-resistance gene *mecA* was confirmed by PCR (Overesch et al., 2011) and its expression was determined by latex agglutination (Oxoid). The prevalence and its 95% confidence interval were calculated using the online Confidence Interval Calculator (www.measuringusability.com).

Determination of the antibiotic resistance profile and genotyping

The minimal inhibitory concentration (MIC) of 19 antibiotics [cefotixin, chloramphenicol, clindamycin, ciprofloxacin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, penicillin, quinupristin/dalfopristin, rifampicin, tetracycline, streptomycin, sulfamethoxazole, trimethoprim, tiamulin (a pleuromutilin

antibiotic), and vancomycin] was determined by broth microdilution in Mueller-Hinton using the Sensititre susceptibility plate EUST (Trek Diagnostic Systems, East Grinstead, England; MCS Diagnostics BV, Swalmen, The Netherlands), except for spectinomycin and amikacin, which were tested using homemade microbroth dilution plates. The MICs were interpreted using breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines <http://www.eucast.org>, except for streptomycin and kanamycin for which breakpoints came from the French Society for Microbiology (www.sfm-microbiologie.org), and sulfamethoxazole, for which they came from CLSI (Clinical and Laboratory Standards Institute, 2013). No breakpoint was available for tiamulin and spectinomycin and resistance for these drugs was attributed after the detection of a resistance gene and elevated MIC of > 4 mg/L and > 256 mg/L, respectively. The antibiotic resistance genes were detected using a custom-made microarray (AMR + ve-4 array tubes, Alere technologies GmbH, Jena, Germany) (Perreten et al., 2005). Mutations in the chromosomal isoleucyl-tRNA synthetase gene of mupirocin-resistant MRSA were identified by PCR and sequence analysis (Fujimura et al., 2003). Mutations in the quinolone resistance-determining regions of GyrA, GyrB, GrlA and GrlB were determined by amino acid sequence analysis derived from PCR products (Schmitz et al., 1988). The toxin genes were detected using *S. aureus* Genotyping Kit 1.0 (Alere technologies). Genotyping was performed by multilocus sequence typing (MLST), *spa* typing, and SCC*mec* typing (Sieber et al., 2011).

Results

Fourteen of the 369 participants were tested positive for MRSA nasal carriage (3.8%) (95% CI 2.1–6.3%) and none were tested positive for MRSP (Tab. 1). MRSA were detected among general practitioners (4.5%) (95% CI 1.5–10.2%), small animal practitioners (2.7%) (95% CI 0.8–6.9%), large animal practitioners (6.5%) (95% CI 0.8–21.4%), in one veterinary assistant (3.5%) (95% CI 0.09–17.8%), in one veterinarian belonging to those professionals who do not directly work with animals (2.27%) (95% CI 0.06–12.0%), and in one whose specific field of work remained unknown (Tab. 1).

Eight different MRSA genotypes were found, including ST398-t011-IV (n = 4), ST398-t011-V (n = 2), ST398-t034-V (n = 1), ST225-t003-II (n = 2), ST225-t014-II (n = 1), ST5-t002-II (n = 2), ST5-t283-IV (n = 1), and ST88-t186-IV (n = 1) (Tab. 1 and Tab. 2). MRSA belonging to the livestock-associated ST398 lineages were exclusively found in general and large animal practitioners, whereas MRSA of the healthcare associated lineages ST5, ST225 and ST88 were detected in small animal practitioners and in one small animal vet-

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Table 1: Distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *S. pseudintermedius* (MRSP) among veterinary health care providers in Switzerland.

Field of occupation	total	MRSP-positive		MRSA-positive		MRSA genotypes (MLST- <i>spa</i> -SCC <i>mec</i>)											
		N	%	N	%	ST398-ton-IV	ST398-ton-V	ST398-to11-IV	ST398-to11-V	ST398-t034-V	ST225-to03-II	ST225-to14-II	ST5-to02-II	ST5-t283-IV	ST88-t186-IV		
						N	%	N	%	N	%	N	%	N	%	N	%
Small animal	146	0	2.73	4	2.73												
Large animal	31	0	6.45	2	6.45		2										
General	111	0	4.50	5	4.50	4	3.60	1	0.90								
Horses	7	0	0	0	0												
Zoo	2	0	0	0	0												
Veterinary assistants	29	0	3.44	1	3.44					1	3.44						
Non-animal related	44		2.27	1	2.27					1	2.27						
- Laboratory	7	0	0	0	0												
- Administration	16	0	0	0	0												
- Industry	9	0	NA	1	NA					1	NA						
- Other	10	0	0	0	0												
Unknown	1	0	NA	1	NA									1	NA		
Total	369	0	3.79	14	3.79	4	1.08	2	0.54	1	0.27	2	0.54	1	0.27	2	0.54

NA, not applicable due to low number.

erinary assistant, as well as in one person working in the industry (Tab. 1). MRSA belonging to a specific genetic lineage also exhibited the same antibiotic resistance profile emphasizing the spread of specific clones (Tab. 2). In addition to *mecA*, MRSA ST398-t011-IV contained additional genes conferring resistance to tetracycline [*tet*(M)], gentamicin and kanamycin [*aac*(6')-*Ie*-*aph*(2')-*Ia*], and to trimethoprim [*dfrK*]. MRSA ST398-t011-IV contained both the tetracycline resistance genes *tet*(M) and *tet*(K). MRSA ST398-t034-IV also contained both the tetracycline resistance genes *tet*(M) and *tet*(K), the trimethoprim resistance gene *dfrG*, the macrolide, lincosamide and streptogramin B resistance gene (A), the spectinomycin resistance gene [*ant*(9)-*Ia*], the streptomycin resistance gene *str* and the pleuromutilin, lincosamide and streptogramin A resistance gene *vga*(E). Entirely different resistance profiles were observed in MRSA ST5, ST225 and ST88. MRSA ST5 and ST225 contained genes conferring resistance to macrolides, lincosamides and streptogramins B [*erm*(A)], to spectinomycin [*ant*(9)-*Ia*], to fluoroquinolones (GrlA and GyrA mutations), and to chloramphenicol [*cat*_{Pc221}]. They contained the amikacin resistance gene *ant*(4')-*Ia*, but displayed MIC for amikacin of 4 to 8 µg/ml which are situated below the resistance breakpoint of > 16 µg/ml. They also contained the bleomycin resistance gene *ble*. MRSA ST88 only exhibited an additional resistance to trimethoprim encoded by *dfrA* (Tab. 2). Of note, the two MRSA ST5-t002-II exhibited decreased susceptibility to mupirocin (MIC 256 mg/L) and contained a valine-to-phenylalanine substitution at position 588 (V588F) in the chromosomal isoleucyl-tRNA synthetase gene.

The only toxin genes detected in all MRSA ST398 were associated to the γ-hemolysin [*hlgA*, *hlgB* (*lukF*), *hlgC* (*lukS*)], with the exception of ST398-t034-V which lacked *hlgA*. In contrast, MRSA ST5 and ST225 contained the γ-hemolysin genes [*hlgA*, *hlgB*, *hlgC*], leukocidin genes (*lukD*, *lukE*), as well as genes associated with β-hemolysin (*hly*) converting phages (*sak*, *chp*, *scn*), and enterotoxin genes (*sed*, *seg*, *sei*, *sej*, *selm*, *sels*, *selo*, *ser*, *selu*). MRSA ST225-t003-II, MRSA ST225-t014-II, as well as ST5-t283-IV had an additional enterotoxin A gene (*sea-N315* and *sea*, respectively). MRSA ST88 contained the γ-hemolysin genes (*hlgA*, *hlgB*, *hlgC*), leukocidin genes (*lukD*, *lukE*), as well as β-hemolysin converting phage genes (*sak*, *chp*, *scn*). All MRSA isolates were negative for the PVL gene (Tab. 2).

Discussion

The MRSA nasal colonization prevalence determined among Swiss veterinarians did not increase substantially between 2009 (3%) (Huber et al., 2010) and 2012 (3.8%). However, this is still higher than the prevalence in the general population in Switzerland where 1.5% were reported to be nasal MRSA carriers (Sakwinska et al., 2009), and where 2.4% of patients are MRSA-positive at hospital admission (Pasricha et al., 2013). In other European countries, carriage rates in the general population were also low, ranging from below 0.1% to 2.1% (den Heijer et al., 2013). However, other surveys undertaken at veterinary conferences in different countries also found higher prevalences of MRSA carriage among veterinarians (1.6% to 17.3%) (Paterson et al., 2013). Of note, none of the tested Swiss veterinarians harbored MRSP. Low MRSP prevalence among veterinary personnel was also observed in Hong Kong (0.6%) (Boost et al., 2009), but reached 3% in The Netherlands (van Duijkeren et al., 2011), 3.9% in Italy (Paul et al., 2011), 5.3% in the USA (Morris et al., 2010), and 7.9% in Japan (Ishihara et al., 2010).

Our study revealed the presence of epidemic clones of ST5-MRSA-II, ST5-MRSA-IV, ST225-MRSA-II, and ST88-MRSA-IV in healthy veterinary health care providers in Switzerland. ST5-MRSA-II is a pandemic clone which has been reported in many human hospitals worldwide, including Switzerland (Grundmann et al., 2010; Monecke et al., 2011; Senn et al., 2013). ST225-MRSA-II represents a single locus variant of ST5-MRSA-II which has rapidly disseminated in Germany and is now one of the most frequent MRSA lineages causing hospital-acquired infections there (Deurenberg et al., 2009; Schaumburg et al., 2012). The ST225-t003-II clone has also been detected in hospital patients in the Czech republic, Poland, Belgium, The Netherlands and Denmark (Bartels et al., 2007; Deurenberg et al., 2009; Grundmann et al., 2010). ST5-MRSA-II has already been reported in veterinarians from the USA and Germany (Hanselman et al., 2006). In our study, the epidemic clonal lineages of HA-MRSA ST5 and ST225 were exclusively detected in people working with small animals, whereas veterinarians working with large animals harbored LA-MRSA ST398. Three different MRSA ST398 lineages could be identified: ST398-t011-IV, ST398-t011-V, and ST398-t034-V. Each ST398 lineage exhibited a specific antibiotic resistance profile which corresponded to profiles that have already been detected among animals in Switzerland. ST398-t034-V which contained multiple resistance genes [*mecA*, *blaZ*, *tet(M)*, *dfrG*, *ant(9)-Ia*, *erm(A)*, *str*, *vga(E)*] is the most frequent MRSA clonal lineage detected in pigs in Switzerland (Overesch et al., 2011; Schwendener and Perreten, 2011). MRSA ST398-t011-V which contained *mecA*, *blaZ*, *tet(M)* and *tet(K)* has also been detected in pigs in Switzerland (Overesch et al., 2011). Furthermore, ST398-t011-IV containing *mecA*, *blaZ*, *tet(M)*, *dfrK*, and *aac(6')-Ie-aph(2')-Ia* is the most frequent lineage causing infections in horses and

found to colonize the nares of horse clinic personnel in Switzerland (Sieber et al., 2011). It is very likely that Swiss veterinarians colonized with either pig- or horse-associated MRSA ST398 have been working with these animal-species, since contact with livestock and horses is a risk factor for acquiring MRSA ST398 (Sieber et al., 2011; Garcia-Graells et al., 2012; Oppliger et al., 2012). However, to our knowledge, human infection caused by MRSA ST398 has not yet been reported in Switzerland (van Cleef et al., 2011). One participant was a carrier of MRSA ST88-t186-IV, a MRSA lineage which is widespread in Africa (Breurec et al., 2011). In Switzerland, this MRSA lineage has so far only been detected in African children who underwent surgery in Switzerland (Blanc et al., 2007).

Virulence genes associated to enterotoxins, β -hemolysin converting phages or leukocidins D and E were exclusively detected in MRSA ST5, ST225 and ST88 as already described in epidemic strains of these lineages (Monecke et al., 2011), whereas livestock-associated MRSA ST398 only contained the γ -hemolysin as virulence factor. Indeed, the majority of LA-MRSA strains characterized to date have been found to be negative for enterotoxins and to toxins associated with β -hemolysin converting phages (Monecke et al., 2011).

Conclusion and recommendations

This study demonstrates that Swiss veterinarians and veterinary health care workers act as a reservoir for MRSA that are not only related to livestock clones, but also to HA-MRSA clones that have disseminated in hospitals throughout Europe. It is therefore important that veterinarians as well as other people working with or owning animals notify their doctors upon hospitalization or prior to surgical intervention, because MRSA carriers have a higher risk of developing subsequent infection (Wertheim et al., 2005; Safdar and Bradley, 2008). Screening for MRSA carriage should be given consideration in such patients. However, systematic decolonization using mupirocin in conjunction with chlorhexidine skin disinfection as used in hospital patients (Marschall and Mühlemann, 2006) is not routinely recommended for healthy veterinary practitioners given that recolonization may reappear quickly while working with animals (Sieber et al., 2011). On the other hand, standardized infection prevention measures as they exist in current human health care should be introduced into veterinary medicine to avoid pathogen transmission between humans and animals. Such measures are essential in limiting the spread of MRSA through the community, and into animal and human hospitals.

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Table 2: Genetic characteristics of MRSA isolated from the nares of healthy veterinarians and veterinary assistants in Switzerland

Strain	Genotype	Field of activity	Virulence genes	FOX	PEN	TMP
	MLST- <i>spa</i> -SCC <i>mec</i>			> 4	> 0.12	> 4
IMD418/12	ST398-t011-V	Large animals	<i>hlgA hlgB hlgC</i>	<i>mecA</i>	<i>blaZ</i>	
IMD439/12	ST398-t011-V	Large animals	<i>hlgA hlgB hlgC</i>	<i>mecA</i>	<i>blaZ</i>	
IMD423/12	ST398-t011-IV	General	<i>hlgA hlgB hlgC</i>	<i>mecA</i>	<i>blaZ</i>	<i>dfrK</i>
IMD443/12	ST398-t011-IV	General	<i>hlgA hlgB hlgC</i>	<i>mecA</i>	<i>blaZ</i>	<i>dfrK</i>
IMD444/12	ST398-t011-IV	General	<i>hlgA hlgB hlgC</i>	<i>mecA</i>	<i>blaZ</i>	<i>dfrK</i>
IMD569/12	ST398-t011-IV	General	<i>hlgA hlgB hlgC</i>	<i>mecA</i>	<i>blaZ</i>	<i>dfrK</i>
IMD416/12	ST398-t034-V	General	<i>hlgB hlgC</i>	<i>mecA</i>	<i>blaZ</i>	<i>dfrG</i>
IMD427/12	ST225-t003-II	Industry	<i>hlgA hlgB hlgC</i> <i>sea-N315 sed seg sei sej selm seln selo ser selu</i> <i>sak chp scn</i> <i>lukD lukE</i>	<i>mecA</i>	<i>blaZ</i>	
IMD428/12	ST225-t003-II	Small animals (veterinary assistant)	<i>hlgA hlgB hlgC</i> <i>sea-N315 sed seg sei sej selm seln selo ser selu</i> <i>sak chp scn</i> <i>lukD lukE</i>	<i>mecA</i>	<i>blaZ</i>	
IMD429/12	ST225-t014-II	Small animals	<i>hlgA hlgB hlgC</i> <i>sea-N315 sed seg sei sej selm seln selo ser selu</i> <i>sak chp scn</i> <i>lukD lukE</i>	<i>mecA</i>	<i>blaZ</i>	
IMD421/12	ST5-t002-II	Small animals	<i>hlgA hlgB hlgC</i> <i>sed seg sei sej selm seln selo ser selu</i> <i>sak chp scn</i> <i>lukD lukE</i>	<i>mecA</i>	<i>blaZ</i>	
IMD447/12	ST5-t002-II	Small animals	<i>hlgA hlgB hlgC</i> <i>sed seg sei sej selm seln selo ser selu</i> <i>sak chp scn</i> <i>lukD lukE</i>	<i>mecA</i>	<i>blaZ</i>	
IMD430/12	ST5-t283-IV	Unknown	<i>hlgA hlgB hlgC</i> <i>sea sed seg sei sej selm seln selo ser selu</i> <i>sak chp scn</i> <i>lukD lukE</i>	<i>mecA</i>	<i>blaZ</i>	
IMD417/12	ST88-t186-IV	Small animals	<i>hlgA hlgB hlgC</i> <i>sak chp scn</i> <i>lukD lukE</i>	<i>mecA</i>	<i>blaZ</i>	<i>dfrA</i>

BLE, bleomycin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; PEN, penicillin; SPC, spectinomycin; STR, streptomycin; TET, tetracycline; TMP, trimethoprim. Antibiotic resistance genes and their functions: *mecA*, methicillin-resistance gene encoding PBP2a for resistance to all β -lactam-antibiotics; *blaZ*, β -lactamase gene; *dfrA*, *dfrG*, and *dfrK*, trimethoprim resistance dihydrofolate reductase gene; *tet(K)*, tetracycline efflux gene; *tet(M)*, ribosome protection tetracycline resistance gene; *aac(6')-Ie-aph(2')-Ia*, aminoglycoside acetyltransferase and phosphotransferase tandem genes; *erm(A)*, macrolide, lincosamide and streptogramin B 23S rRNA methylase gene; *ant(9)-Ia*, spectinomycin nucleotidyltransferase gene; *ant(4')-Ia*, amikacin and tobramycin nucleotidyltransferase gene; *str*, streptomycin adenylyltransferase gene; *vga(E)*, pleuromutilin, lincosamide and streptogramin A ATP binding transporter gene; *cat_{Pc221}*, chloramphenicol acetyltransferase gene; *ble*, bleomycin resistance gene; GrlA, topoisomerase IV; GyrA, topoisomerase II.

Virulence genes and their functions: *hlgA hlgB hlgC*, γ -hemolysin genes; *sea sed seg sei sej selm seln selo ser selu*, enterotoxin genes; *sak chp scn*, β -hemolysin converting phage genes; *lukD lukE*, leukocidin genes.

a) The MIC breakpoints that determine resistance were recommended from EUCAST for staphylococci (www.eucast.org). Resistance breakpoints for streptomycin and kanamycin were those recommended from the French Society for Microbiology (www.sfm-microbiologie.org), and the resistance breakpoint for sulfamethoxazole was from the CLSI (CLSI, 2013). The minimal inhibitory concentrations (MICs) for the strains containing a resistance gene or a mutation in the topoisomerase genes *gyrA* and *grlA* were situated above the corresponding antibiotic resistance breakpoints; otherwise the isolates were susceptible with MICs situated below the resistance breakpoint.

NA, no resistance breakpoint available for tiamulin and spectinomycin [resistance to tiamulin and spectinomycin was attributed in the presence of the *vga(E)* gene (MIC > 4 mg/L) and *ant(9)-Ia* (MIC > 256 mg/L), respectively]; ND, not defined, susceptibility bleomycin was not measured, only the gene was detected; blank spaces indicate either no resistance or no mutations. b) The MIC of amikacin was situated below the resistance breakpoint despite the presence of the *ant(4')-Ia* gene.

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