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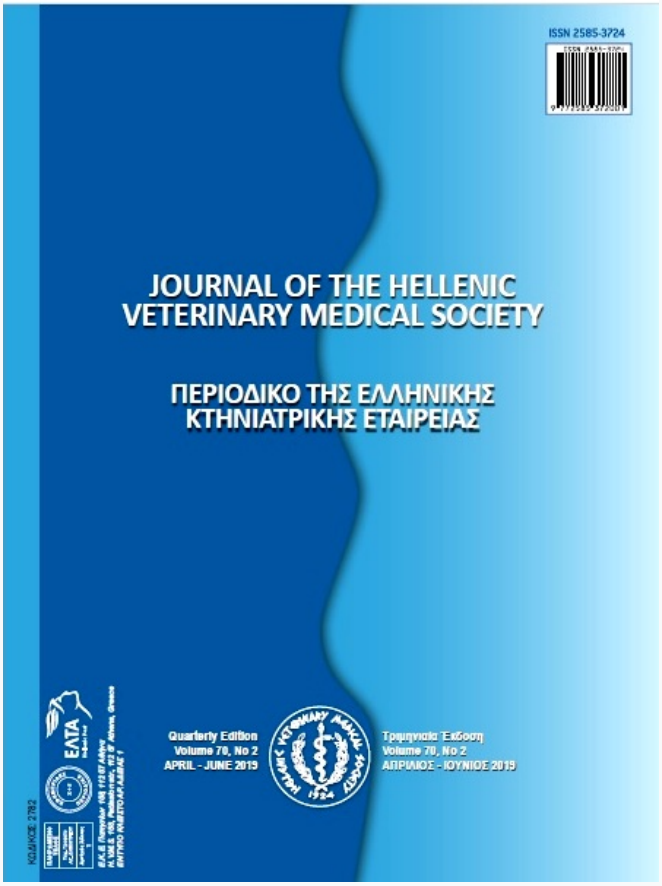

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Research article
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Staphylococcus aureus and bovine mastitis: molecular typing of methicillin-resistance and clinical description of infected quarters

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ABSTRACT. This study targeted *Staphylococcus aureus (S. aureus)*-mastitis of bovine as a possible source of livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA), to describe clinical signs of mastitis associated with MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA). The study area was the Gharbia and Kafrelsheikh governorates, in the central and northern regions of the Egyptian Delta. Clinical examination of animals was performed to detect clinical mastitis (CM) and clinically normal cases were tested by the California mastitis test (CMT) to identify subclinical mastitis (SCM). Accordingly, 38 mastitis cases (68 infected quarters) were detected. Milk samples were collected aseptically and were cultured on Baird Parker agar. Fifty nine Gram-positive cocci-shaped isolates were selected and preserved. In addition, 33 *Staphylococcus* spp. isolates originated from bovine mastitis at the same study area were obtained from Animal Health Research Institute (AHRI). The cocci-shaped Gram-positive bacteria and AHRI *Staphylococcus* spp. isolates were used for molecular identification of *S. aureus* and MRSA. Molecular screening had yielded 17 *S. aureus* isolates, from which five isolates (29.41%) were MRSA and 12 isolates (70.59%) were MSSA. The five MRSA isolates were *mecA* positive, but *mecC* negative. Multilocus sequence typing (MLST) of the five MRSA isolates indicated that all were sequence type 1 (ST1). *S. aureus*-associated cases showed different clinical forms of mastitis, including subclinical, acute, chronic, and gangrenous. However, subclinical mastitis was the only detected form associated with MRSA, which may represent a potential hidden risk for humans. Phenotypic antimicrobial-resistance pattern of MRSA isolates showed resistance to all of the tested β-lactam antimicrobials, with marked

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resistance to tetracycline and gentamycin. Based on our knowledge, this is the first report to identify MRSA ST1 in Egypt. Bovine mastitis could be a source for the dissemination of MRSA to humans and other animals. Additionally, while methicillin-resistance may have no effect on the clinical severity of mastitis, it does affect therapeutic success, particularly when β-lactam antimicrobials are used.

Keywords**:** *MRSA, Bovine, Mastitis*, *Staphylococcus aureus*

INTRODUCTION

Staphylococci, particularly *S. aureus*, are common pathogens of mastitis in bovines (Haveri et al., 2007), and the *Staphylococcus* spp. associated mastitis is responsible for considerable economic losses (Lammers et al., 2000). *S. aureus* accounts for 25–30% of all intra-mammary infections (IMI) in cows (Poutrel, 1985). Importantly, *S. aureus* IMI results in a 10–25% milk reduction in infected animals (Anderson, 1983). *S. aureus* can induce either CM or SCM, but the subclinical form is more predominant (Anderson, 1983; Lammers et al., 2000; Akineden et al., 2001). While CM has detectable clinical symptoms, SCM has no detectable symptoms and necessitates screening using the CMT (Kasikci et al., 2012). The success in staphylococcal mastitis therapy is dependent on the individual animal, treatment, and pathogen factors (Barkema et al., 2006), and the resistance to antimicrobials is a major factor affecting the cure rates of staphylococcal mastitis (Barkema et al., 2006).

Staphylococci, notably *S. aureus*, have shown resistance to various antimicrobials (Wang et al., 2015), and MRSA strains have gained worldwide attention. MRSA is classified into three categories according to its origin: livestock-associated (LA-MRSA), healthcare-associated (HA-MRSA), and community-associated (CA-MRSA) (Stefani et al., 2012). There is an increasing global interest in LA-MRSA because of its animal and human associated health implications (Graveland et al., 2011). Many sequence types (ST1, ST9, ST97, ST130, ST398, and ST425) of MRSA had been recorded in both cattle and humans (García-Álvarez et al., 2011; Paterson et al., 2012; Spoor et al., 2013; Alba et al., 2015; Cuny et al., 2015). In humans, LA-MRSA strains can colonize tissues, resulting in pneumonia, endocarditis, and other life threatening conditions (Ekkelenkamp et al., 2006; Witte et al., 2007). Molecular epidemiology studies on MRSA in southern Mediterranean countries are limited (Borg et al., 2007). In Egypt, there are very few studies on MRSA strains originating from bovine mastitis (Elhaig and Selim, 2015). Additionally, studies describing the clinical aspects of MRSA- and non-MRSA-associated bovine mastitis are limited. Therefore, this study was intended to screen for MRSA and its sequence types associated with bovine mastitis in Egypt, and to describe the clinical aspects of MRSA- and MSSA-associated bovine mastitis.

MATERIAL AND METHODS

Animals, detection of mastitis and sampling

Study animals were reared in the Gharbia and Kafrelsheikh governorates, in the central and northern regions of the Egyptian Delta. In these areas, the dairy animals are reared in small groups rather than organized farms. Mastitis cases were detected by clinical examination of the animals with special attention to the udder according to Houe et al. (2002), clinically normal cases were tested for SCM by CMT according to Kasikci et al. (2012).

Milk samples were aseptically collected according to Quinn et al. (1994): teats were wiped efficiently with 70% ethyl alcohol, first strip of milk was discarded and a suitable amount of milk (about 5 ml of milk) was collected in a sterile screw-capped Falcon tube. Samples were sent to the laboratory on ice quickly after collection.

Bacteriological examination

Milk samples were centrifuged (1000 *g*/5 min), the supernatant was discarded and sediment was streaked on Baird Parker agar. A 24 h incubation at 37 °C was done according to Silva et al. (2000). Gram-stained Smears of the colonies were examined. The putative *Staphylococcus* species isolates were preserved in glycerol stock at −20 °C.

Animal Health Research Institute (AHRI) *Staphylococcus* species Isolates

In addition to the above mentioned putative isolates, another 33 *Staphylococcus* spp. isolates were obtained from AHRI. Such isolates originated from bovine mastitis cases at the same study area, but clinical data of these isolates were not recorded.

Both of the isolated *Staphylococcus* spp. isolates and the obtained AHRI isolates were used in molecular procedures.

Molecular characterization and typing

For extraction of DNA, Luria-Bertani agar plates were streaked by the isolates and incubated for 24 h at 37 °C. The extraction of DNA was performed by InstaGene matrix (Bio-Rad Laboratories Inc.). The extracted DNA was preserved at −20 °C for use in PCR assays. Primer sequences of *S. aureus*, MRSA and MLST are shown in Table 1.

A PCR assay targeting a 359-bp region of the *S. aureus* thermonuclease (*nuc*) gene was used to detect *S. aureus* as described by Sasaki et al. (2010) with a few modifications. Briefly, a 25-µl reaction was prepared containing 5 µL of DNA, 0.2 mM dNTPs, 1× buffer, 0.5 U of AmpliTaq Gold (Applied Biosystems), and primers (each of 20 pmol). The thermal cycler conditions consisted of 95 °C /10 min, 35 cycles (95 °C/30 s, 56 °C/35 s, and 72 °C/1 min), followed by 72 °C/10 min.

MRSA was identified by PCR targeting 147- and 138-bp regions of *mecA* and *mecC* ($mecA_{LGA251}$) as described by Zhang et al. (2005) and Stegger et al. (2012), respectively, with a few modifications. The 25 µL reaction consisted of 5 µL of DNA, 0.2 mM dNTPs, 1× buffer, 0.5 U of AmpliTaq Gold (Applied Biosystems), and primers (20 pmol of each). Mixtures of *mecA* and *mecC* were initially heated at 94 °C for 4 min/15 min, followed by 35/30 cycles of 94 °C/30 s, 52 /59 °C for 30 s/1 min, and 72 °C for 45 s/1 min, respectively. A final extension was conducted at 72 °C/7 min.

Multi-locus sequence typing of MRSA isolates was performed using seven housekeeping genes according to Enright et al. (2000) and allelic profiles were obtained from MLST web site (http://saureus.beta.mlst.net/).

Table 1. Primers of *S. Aureus*, MRSA and MLST.

Primer	Gene	Sequence (5'-3')	Size (bp)	References
au-F3	*Nuc*	TCGCTTGCTATGATT GTGG	359	Sasaki et al., 2010
au-nucR		GCCAATGTTCTACCA TAGC		
***MecA*147-F**	*MecA*	GTG AAG ATA TAC CAA GTG ATT	147	Zhang et al., 2005
***MecA*147-R**		ATG CGC TAT AGA TTG AAA GGA T		
***mecA*$_{LGA251}$MultiFP**	*mecA*$_{LGA251}$	GAAAAAAAGGCTTAGAACGCCTC	138	Stegger et al., 2011
***mecA*$_{LGA251}$MultiRP**		GAAGATCTTTTCCGTTTTCAGC		
***arcC*-Up**	*Arc*	TTGATTCACCAGCGCGTATTGTC	456	Enright et al., 2000
***arcC*-Dn**		AGGTATCTGCTTCAATCAGCG		
***aroE*-Up**	*aroE*	ATCGGAAATCCTATTTCACATTC	456	
***aroE*-Dn**		GGTGTTGTATTAATAACGATATC		
***glpF*-Up**	*GlpF*	CTAGGAACTGCAATCTTAATCC	465	
***glpF*-Dn**		TGGTAAAATCGCATGTCCAATTC		
***gmk*-Up**	*Gmk*	ATCGTTTTATCGGGACCATC	429	
***gmk*-Dn**		TCATTAACTACAACGTAATCGTA		
***pta*-Up**	*Pta*	GTTAAAATCGTATTACCTGAAGG	474	
***pta*-Dn**		GACCCTTTTGTTGAAAAGCTTAA		
***tpi*-Up**	*Tpi*	TCGTTCATTCTGAACGTCGTGAA	402	
***tpi*-Dn**		TTTGCACCTTCTAACAATTGTAC		
***yqiL*-Up**	*YqiL*	CAGCATACAGGACACCTATTGGC	516	
***yqi*L-Dn**		CGTTGAGGAATCGATACTGGAAC		

Antimicrobial susceptibility testing

All *S. aureus* isolates were examined for their susceptibility to ampicillin, tetracycline, ciprofloxacin, gentamicin, sulfamethoxazole and trimethoprim, teicoplanin, ceftriaxone, amoxicillin and clavulanic acid, oxacillin, and cefoxitin. The antibiotic disc diffusion guidelines of The Clinical and Laboratory Standards Institute (2005) were followed.

RESULTS

Clinical and descriptive aspects of mastitis cases

A total of 38 mastitis cases (68 affected quarters) were detected. Twenty five cases had single diseased quarter, while 13 cases had multiple affected quarters. Different clinical forms of mastitis were noticed as shown in Figure 1.

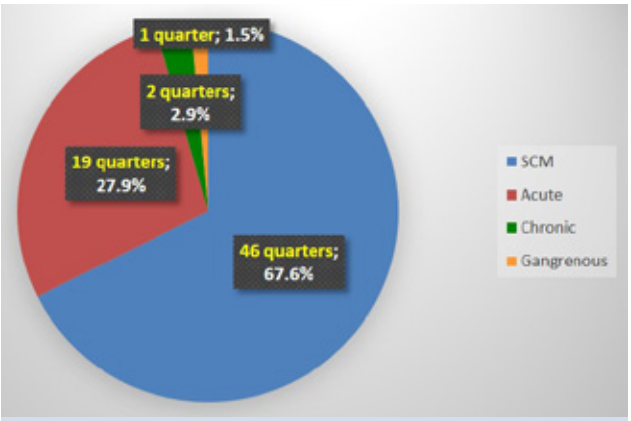

Figure 1.

Subclinical mastitis presented no obvious symptoms except of reduced amount of milk from the affected quarters, the physical characters of milk were normal. On the other hand, acute mastitis showed hotness, pain and enlargement of the affected quarter. Systemic reaction appeared as fever, congested mucous membranes, reduced appetite, increased heart and respiratory rates were recorded in a few cases of acute mastitis. The physical characters of milk were changed; yellowish semi-transparent offensive odor milk were the most common detected changes. Chronic affected quarters were fibrous discharging scanty amount of watery milk, while gangrenous quarter was of blackish discoloration, cold temperature and discharging bloody milk.

Identification of *S. aureus*

Gram-positive cocci-shaped bacteria were isolated from 36/38 mastitis cases (94.7%) and 49/68 infected quarters (72.1%). Based on Baird Parker agar growth and Gram-stained smears, 59 gram-positive cocci-shaped isolates were selected. Molecular screening for *S. aureus* yielded seven isolates from seven individual cases (four subclinical cases, one acute, one chronic and one gangrenous case). In addition, molecular screening of the AHRI isolates resulted in an additional 10 isolates of *S. aureus*. Consequently, 17 *S. aureus* isolates were used in further phenotypic and genotypic investigations.

Antimicrobial susceptibility of *S. aureus* isolated strains

A marked resistance of *S. aureus* to teicoplanin and ampicillin was observed. In addition, oxacillin-resistance was evident in five *S. aureus* isolates. *S. aureus* phenotypic and genotypic profiles are listed in Table 2.

Molecular screening/typing of MRSA

Out of the 17 *S. aureus* strains, 12 isolates (70.59%) were MSSA and five (two originated from the clinical cases and three AHRI isolates) strains contained *mecA* (29.41%) and were identified as MRSA ST1. However, *mecC* was absent in all of the *S. aureus* isolates.

Clinical nature of MRSA-associated bovine mastitis

The two cases (AHRI isolates had no clinical data) which were infected by MRSA ST1 had showed SCM, which may indicate that MRSA ST1 may be unable to induce severe mastitis cases.

DISCUSSION

LA-MRSA strains are reported to induce endocarditis, pneumonia, soft tissue and skin conditions in humans (Ekkelenkamp et al., 2006; Witte et al., 2007). Interestingly, the current study identified a high percentage of MRSA ST1 (29.41%) amongst the *S. aureus* strains isolated from mastitis in bovines. However, larger scale investigations are important to assess LA-MRSA of bovine mastitis origin and its potential risk for humans in Egypt. MRSA ST1 is a wide spread LA-MRSA lineage with a broad host range including humans (Alba et al., 2015). Although it is commonly isolated from pigs, recent studies had recorded MRSA ST1 associated with cattle and dairy farming in some countries such as Italy and Hungary (Juhász-Kaszanyitzky et al., 2007; Alba et al., 2015). Moreover, studies had showed high genetic similarity (90-100%) between human and cattle associated MRSA ST1 and confirmed complete ability of the later to colonize and infect humans (Juhász-Kaszanyitzky et al., 2007; Alba et al., 2015).

Table 2. Phenotypic and genotypic profiles of *S. Aureus* isolates.

Isolates	Phenotypic resistance		Genotypic
	β-lactams	Other antimicrobials	resistance
Sa28	AMP, FOX	TEC	
Sa69	AMP, AMC, FOX, CRO, OXA	TET, TEC	*mecA*
Sa70	AMP, AMC, FOX, CRO	TET, GEN, TEC	*mecA*
Sa101	AMP, AMC, FOX, CRO, OXA	TET, GEN, TEC	*mecA*
Sa104	FOX	TEC	
Sa107	AMP	TET, GEN,TEC	
Sa119	AMP	GEN, TEC	
Sa120	AMP	-	
Sa131	AMP, AMC, FOX, CRO, OXA	TET, GEN, TEC	*mecA*
Sa135	AMP, FOX	TET, TEC	
Sa136	-	TET, TEC	
Sa137	AMP, AMC, FOX, CRO, OXA	GEN, TEC	*mecA*
Sa140	AMP	-	
Sa144	AMP, AMC, FOX, CRO, OXA	TET, GEN, TEC	
Sa146	AMP, FOX	-	
Sa158	AMP, AMC	-	
Sa164	AMP	-	

TET, tetracycline; CIP, ciprofloxacin; SXT, sulfamethoxazole and trimethoprim; GEN, gentamicin; AMP, ampicillin; AMC, amoxicillin and clavulanic acid; FOX, cefoxitin; TEC, teicoplanin; CRO, ceftriaxone; OXA, oxacillin.

The affection of a single quarter, in spite of lack of hygienic milking procedures, in most of the detected mastitis cases indicated the low contagiousness of the involved pathogens. This was confirmed by PCR which showed that most of Staphylococcus isolates were not *S. aureus*. In addition, SCM was more prevalent than CM, which agrees with a recent study in Egypt (Elhaig & Selim, 2015), and highlights the importance and widespread nature of SCM.

Staphylococcus aureus was associated with different clinical forms of mastitis. The resulting clinical form of *S. aureus* mastitis is a multifactorial process which is influenced by host's immunity and virulence determinants of *S. aureus* isolates (Haveri et al., 2007). On the other hand, the current study showed that MRSA ST1 was associated only with SCM. This is because most LA-MRSA isolates lack many mastitis-associated virulence factors such as toxic shock syndrome toxin 1, hemolysins, and enterotoxins (Monecke et al., 2007; Walther et al., 2009). Despite this, the involvement of LA-MRSA (ST398) in a few CM cases has been reported previously (Vanderhaeghen et al., 2010).

Antimicrobial susceptibility of methicillin resistant and sensitive *Staphylococcus aureus* isolates revealed prominent differences. The MRSA isolates showed resistance to most of the tested antimicrobials (Table 2). The resistance of MRSA to oxacillin and other β-lactam antimicrobials can be attributed to the existence of *mecA*, which codes for a penicillin binding protein with low affinity for all β-lactams (Hartman & Tomasz, 1984).

In conclusion, SCM is more prevalent than CM, *S. aureus* associated bovine mastitis was of variable clinical nature (subclinical, acute, chronic and gangrenous), bovine mastitis is a source of LA-MRSA ST1 which is a health risk for humans and source of infection of animals. Additionally, MRSA may be unable to induce severe mastitis, but it will affect therapeutic success, particularly when β-lactams are used for treatment.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Akineden O, Annemuller C, Hassan AA, Lammler C, Wolter W, Zschock M (2001) Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. Clininical and Diagnostic Laboratory Immunology 8: 959-964.

Alba P, Feltrin F, Cordaro G, Porrero MC, Kraushaar B, Argudín MA, Nykäsenoja S, Monaco M, Stegger M, Aarestrup FM, Butaye P, Franco A, Battisti A (2015) Livestock-associated methicillin resistant and methicillin susceptible *Staphylococcus aureus* sequence type (CC)1 in European farmed animals: high genetic relatedness of isolates from Italian cattle herds and humans. PLoS ONE 10: e0137143.

Anderson JC (1983) Veterinary aspects of staphylococci, In: Easmon, C.S.F., Adlam, C. (Eds), Staphylococci and staphylococcal infections, vol 1: Clinical and epidemiological aspects. London, Academic Press. PP. 193-241.

Barkema HW, Schukken YH, Zadoks RN (2006) Invited Review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. Journal of Dairy Science 89: 1877-1895.

Borg MA, Kraker M, Scicluna E, Sande-Bruinsma NV, Tiemersma E, Jos Monen J et al. (2007) Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in invasive isolates from southern and eastern Mediterranean countries. The Journal of Antimicrobial Chemotherapy 60: 1310-1315.

Clinical and Laboratory Standards Institute (2005) Performance standards for antimicrobial susceptibility testing; 19th informational supplement M100-S15. CLSI, Wayne, PA. USA.

Cuny C, Wieler LH, Witte W (2015) Livestock-associated MRSA: The impact on humans. Antibiotics 4: 521-543.

Ekkelenkamp MB, Sekkat M, Carpaij N, Troelstra A, Bonten MJ (2006) Endocarditis due to methicillin-resistant *Staphylococcus aureus* originating from pigs. Nederlands Tijdschrift voor Geneeskunde 150: 2442-2447.

Elhaig MM, Selim A (2015) Molecular and bacteriological investigation of subclinical mastitis caused by *Staphylococcus aureus* and *Streptococcus agalactiae* in domestic bovids from Ismailia, Egypt. Tropical Animal Health and Production 47: 271-276.

Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus Sequence Typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. Journal of Clinical Microbiology 38: 1008-1015.

García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA (2011) Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. The Lancet Infectious Diseases 11(8): 595-603.

Graveland H, Duim B, van Duijkeren E, Heederika D, Wagenaar JA (2011) Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. International Journal of Medical Microbiology 301: 630-634.

Hartman BJ, Tomasz A (1984) Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aureus*. Journal of Bacteriology 158: 513-516.

Haveri M, Roslof A, Rantala L, Pyorala S (2007) Virulence genes of bovine *Staphylococcus aureus* from persistent and nonpersistent intramammary infections with different clinical characteristics. Journal of Applied Microbiology 103: 993-1000.

Houe H, Vaarst M, Enevoldsen C (2002) Clinical parameters for assessment of udder health in Danish dairy herds. Acta Veterinaria Scandinavica 43: 173-184.

Juhász-Kaszanyitzky É, Jánosi S, Somogyi P, Dán Á, Bloois LV, Duijkeren EV, Wagenaar JA (2007) MRSA transmission between cows and humans. Emerging Infectious Diseases 13: 630-632.

Kasikci G, Cetin O, Bingol EB, Gunduz MC (2012) Relations between electrical conductivity, somatic cell count, California mastitis test and some quality parameters in the diagnosis of subclinical mastitis in dairy cows. Turkish Journal of Veterinary and Animal Sciences 36: 49-55.

Lammers A, Kruijt E, van de Kuijt C, Nuijten PJM, Smith HE (2000) Identification of *Staphylococcus aureus* genes expressed during growth in milk: a useful model for selection of genes important in bovine mastitis?. Microbiology 146: 981-987.

Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehricht R (2007) Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. Veterinary Microbiology 125: 128-140.

Paterson GK, Larsen AR, Robb A, Edwards GE, Pennycott TW, Foster G, Mot D, Hermans K, Baert K, Peacock SJ, Parkhill J, Zadoks RN, Holmes MA (2012) The newly described mecA homologue, mecALGA251, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. The Journal of Antimicrobial Chemotherapy 67: 2809–2813.

Poutrel B (1985) Generalites sur les mammites de la vache laitiere. Processus infectieux, epidemiologie, diagnostics, methodes de controle. Revue de Médecine Vétérinaire 161: 497-511.

Quinn SG, Carter HE, Markey BK, Carter GR (1994) Clinical veterinary microbiology. 1st Ed. Mosby. Year Book Europe Limited.

Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu k (2010) Multiplex-PCR method for species identification of coagulase-positive Staphylococci. Journal of Clinical Microbiology 48: 765-769.

Silva WP, Destro MT, Landgraf M, Franco BDGM (2000) Biochemical characteristics of typical and atypical *Staphylococcus aureus* in mastitic milk and environmental samples of Brazalian dairy farms. Brazilian Journal of Microbiology 31: 103-106.

Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, Aarestrup FM, Kearns AM, Larsen AR, Skov RL, Fitzgerald JR (2013) Livestock origin for a human pandemicclone of community-associated methicillin-resistant *Staphylococcus aureus*. MBio 4: doi:10.1128/mBio.00356-13.

Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, MacKenzie FM (2012) Methicillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. International Journal of Antimicrobial Agents 39: 273-282.

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR (2012) Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue $mecA_{LGA251}$. Clinical Microbiology and Infection 18: 395-400.

Vanderhaeghen W, Cerpentier T, Adriaensen C, Vicca J, Hermans K, Butaye P (2010) Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. Veterinary Microbiology 144: 166-171.

Walther B, Monecke S, Ruscher C, Friedrich AW, Ehricht R, Slickers P, Soba A, Wleklinski CG, Wieler LH, Lubke-Becker A (2009) Comparative molecular analysis substantiates zoonotic potential of equine methicillin-resistant *Staphylococcus aureus*. Journal of Clinical Microbiology 47: 704-710.

Wang D, Wang Z, Yan Z, Wu J, Ali T, Li J, Lv Y, Han B (2015) Bovine mastitis *Staphylococcus aureus*: Antibiotic susceptibility profile, resistance genes and molecular typing of methicillin-resistant and methicillin-sensitive strains in China. Infection Genetics and Evolution 31: 9-16.

Witte W, Strommenger B, Stanek C, Cuny C (2007) Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerging Infectious Diseases 13: 255-258.

Zhang K, McClure J, Elsayed S, Louie T, Conly J (2005) Novel Multiplex PCR assay for Characterization and concomitant subtyping of Staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. Journal of Clinical Microbiology 43: 5026-5033.