Dept. of Animal Hygiene and Zoonoses,
Fac. Vet. Medicine, Assiut Univ.

SURVEILLANCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN RAW MILK AND MILK HANDLERS WITH *NUC* GENE SEQUENCING OF THE ISOLATED STRAINS

(With 2 Tables and 2 Figures)

By

AMAL S.M. SAYED and ASMAA A. HUSSEIN

(Received at 4/9/2010)

مدى تواجد methicillin resistant *S. aureus* فى اللبن الخام والقائمين على حلب الحيوانات ودراسة تتابع *nuc* gene للعترات المعزولة

أمل سيد محمد سيد ، أسماء عبد الناصر حسين

فى الآونة الأخيرة تزايدت خطورة ميكروبات methicillin resistant *S. aureus* على صحة الإنسان. ولذلك أجريت هذه الدراسة لتحديد مدى تواجد MRSA فى ألبان المزارع والألبان المباعة فى الأسواق. وتم جمع ١٠٠ عينة بصورة عشوائية من اللبن الخام فى المزارع والمباع فى الأسواق ، بواقع ٥٠ عينة من كل منهم، لدراسة مدى تلوثها بهذه البكتريا. كما تم فحص مسحات من ايدى العاملين والقائمين على حلب الحيوانات بتلك المزارع لمعرفة دورهم فى نقل MRSA. وقد أسفرت النتائج عن تواجد MRSA بنسب ٢٢ ، ٨ % فى كل من لبن المزارع واللبن الخام المباع فى الأسواق على التوالى. وقد وجد ان ٥٥,٦ % من العترات المعزولة قادرة على افراز السموم وكان SE typeC هو النوع السائد بنسبة ٩٠% ، ثم SE typeB بنسبة ١٠%. وقد تم عزل MRSA من القائمين على حلب ورعاية الحيوانات الحلابة بنسبة ١٠% وكانت ٦٦,٧% من العترات المعزولة القادرة على إفراز السموم وكان SE type C هو النوع السائد. وقد اظهرت النتائج ان ٢٧,٨ % من العزلات تحمل *nuc* gene وقد تم عمل تحليل جينى لتحديد مدى تقارب العزلات ، هذا وتمت مناقشة الأهمية الصحية والوبائية لميكروبات MRSA وعزلاتها المختلفة والشروط الواجب إتباعها ، وخاصة فى مزارع الألبان من توعية للعاملين بها لمنع تلوث الألبان بهذه الميكروبات لدرء خطرها عن المستهلك.

SUMMARY

The emergence and spread of methicillin resistant *Staphylococcus aureus* (MRSA) infections are considered a global health issue. This study was designed to determine the prevalence of MRSA in milk from dairy herds and markets. The role of milk handlers as a source of MRSA infection had

been studied. Genotyping of the isolated MRSA strains was investigated. A total of 100 samples of farm milk and market milk (50 each) as well as 30 hand swabs of milk handlers were collected randomly from Assiut Governorate. Methicillin resistant *Staphylococcus aureus* was isolated and enterotoxigenic strains were investigated. Polymerase chain reaction (PCR) was performed to amplify the *nuc* gene in the isolated strains. Moreover, sequencing of the amplified PCR products and phylogenetic analysis was performed. MRSA strains were isolated from 13.85% of the examined samples (22% and 8% of the examined farm and market milk, respectively) and 55.6% of the isolated MRSA strains were enterotoxigenic. In this study, staphylococcal enterotoxin C was the most enterotoxin detected in the isolated MRSA strains with a rate of 90%. However, enterotoxin type B was detected in 10% of the isolated MRSA strains. In addition, 25% of MRSA strains isolated from market milk were enterotoxigenic with one strain belong to type C. Enterotoxigenic MRSA strains were isolated with a rate of 66.7% from milk handlers and enterotoxin type C was the type of toxin produced by these strains. *Nuc* gene was detected in 5 (27.8%) out of the 18 MRSA strains. Phylogenetic analysis of the amplified products sequences was done and the results were discussed. Public health hazard of MRSA was discussed and suggestive measures for control were explained.

Key words: *MRSA, milk, milk handler, PCR, Nuc gene, sequencing.*

INTRODUCTION

The emergence and spread of methicillin resistant *Staphylococcus aureus* (MRSA) infections are considered a global health issue (Normanno *et al.,* 2007). MRSA organisms are frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolids, chloramphinicol, tetracycline and fluoroquinolones (Mandell *et al.,* 1995). Although *S. aureus* is a serious problem in dairy production causing subclinical and clinical mastitis in dairy herds, there is a limited number of publications on the epidemiological aspects of MRSA infections in dairy herds (Devriese and Hommez, 1975; Seguin *et al.,* 1999; Lee, 2003 and Normanno *et al.,* 2007).

New evidence also suggests that domestic animals including food animals are capable of serving as reservoirs and shedders of MRSA and that transmission between host species may be possible (Loo *et al.,* 2007 and Normanno *et al.,* 2007). Human handlers, infected animals, milking equipments and the environment are implicated as possible sources of bulk

milk contamination in dairy herds (Zadoks *et al.*, 2002). Recent studies have shown genetic similarity between MRSA isolates from food animals, including dairy cows and those in humans, suggesting a mode of transmission between them (Juhasz-Kazanyitz *et al.*, 2007 and Moon *et al.*, 2007).

Food borne acquired MRSA outbreaks have been reported and most of them occurred from milk and milk products (Kluytmans *et al.*, 1995; De Buyser *et al.*, 2001; Jones *et al.*, 2002 and Lee, 2003). Food associated intoxication are commonly mediated by heat-stable staphylococcal enterotoxins (Wilson *et al.*, 1991). To date, 18 staphylococcal enterotoxins (SEs) have been described and designed SEA to SEE; SEG to SER and SEU (Dings *et al.*, 2000; Jarraud *et al.*, 2001; Leterte *et al.*, 2003).

This study was designed to determine the prevalence of MRSA in milk from dairy farms and markets. The role of milk handlers as a source of MRSA infection had been studied and genotyping of the isolated MRSA strains was investigated.

MATERIALS and METHODS

Sample collection

A total of 100 samples of farm milk and market milk (50 each) were collected randomly from Assiut Governorate in the period from March to August 2008. Moreover, 30 swabs of hands of milk handlers were obtained. Each swab was emulsified in sterile physiological saline.

Isolation of methicillin resistant *S. aureus* (MRSA):

1ml of each milk sample or 1ml of swab emulsion were enriched in tryptone soya broth (TSB) containing 70mg /ml NaCl and incubated at 37°C for 18- 20 h. A loopfull from the enriched media was plated onto selective medium for MRSA isolation (Mannitol salt agar containing oxacillin 4 mg/litre) and incubated at 37° C for 24-48h. MRSA strains are capable of fermenting mannitol within 24h. (Indicated by a color change from red to yellow) however, few strains of MRSA ferment mannitol slowly, so negative plates after 24h should be incubated for additional 24h (Compernolle *et al.*, 2007). Presumptive colonies were identified as *S. aureus* by using conventional methods including Gram stain, catalase, DNase, Voges-Proskauer and mannitol fermentation tests.

Coagulase test:

Tube coagulase test for detection of free coagulase was performed with rabbit plasma (BioMereux). The test was performed by diluting the plasma in freshly prepared normal saline (1:6). Four pure colonies were

emulsified in 1ml diluted plasma and incubated at 37° C. The tubes were inspected at 1h, 2h, 3h and 4h and incubated overnight if no clot formation was observed (Baired, 1996).

Detection of Staphylococcal enterotoxins (SEs):

The isolated *S. aureus* strains were grown in tryptone soya broth and incubated at 37° C for 24h. The cultures were centrifuged for 5 min. at 3500 rpm /15°C. The supernatant was filtered through 0.45µm low-protein binding filter and the filtrate was used for enterotoxin detection. Detection of enterotoxins (A,B,C,D,E) was done by using ELISA test kit (RIDASCREEN SET A,B,C,D,E (R- Biopharm AG, Darmstadt, Germany) according to the manufacturer's procedure.

PCR assay for detection of *nuc* gene:

DNA of the *S. aureus* colonies was extracted by using QIAamp DNA mini kit (Qiagen, GmbH) according to the manufacturer's protocol. The *nuc* gene, which encodes thermonuclease was used as a target DNA to identify *S. aureus* .The primers
F 5′-GCGATTGATGGTGATACGGTT-3′ and
R 5′AGCCAAGCCTTGACGAACTAAAGC -3′ (Brakstad *et al.,* 1992) were used to amplify *nuc* gene. The Amplification was carried out in 0.5ml tubes in a final reaction volume 50µl. The PCR mixture consisted of 1.5 mM MgCl2, 10mM Tris-HCl (pH 9.0). 50mM KCl. 0.1% Triton®X-100, 200µM (each) deoxynucleotide triphosphate, 0.2µM of each primer and 0.625U *Taq* polymerase. The PCR was conducted in a Biometra Thermal cycler (Biometra – Germany) and the amplification condition was one cycle of 95°C for 10 min, followed by 37cycles of 94°C for 1min, 50°C for 30 sec, and 72°C for 1.5 min and final extension at 72°C for 5 min. PCR products (10µl) were analyzed by electrophoreses on 2% agarose gel containing ethidium bromide and visualized under UV illumination and PCR product size is 270 bp.

Sequencing of the amplified PCR products:

Sequences of the PCR products were determined by the dideoxy chain termination method using Big Dye Terminator v 3.1 Cycle sequencing Kit according to the manufacturer's cycle sequencing protocol.

Phylogenetic analysis:

The *nuc* gene sequences were aligned with CLUSTAL W software (Thompson *et al.,* 1994) and examined using the program MEGA (Molecular Evolutionary Genetics Analysis) version 4 (Tamura *et al.,* 2007). Phylogenetic tree was constructed by the neighbor-joining method with the distance algorithms in the MEGA package. Bootstrap values were

determined with 1000 replicates of the data set and compared with *S. aureus strains* registered in the DDBJ/EMBL/GeneBank including: *S. aureus* EMRSA 16 (Accession No: ADAT01000022.1); *S. aureus* ST 398 (Accession No: AM990992.1), *S. aureus* ED 133 (Accession No: CP001996.1), *S. aureus* JKD 6008 (Accession No: CP002120.1), *S. aureus* TW 20 (Accession No: FN433596.1).

RESULTS

Table 1: Prevalence of MRSA strains in the examined samples

Source of samples	No. of samples	MRSA	%	MRSA (*nuc* gene)	%
Farm milk	50	11	22	3	27.3
Market milk	50	4	8	1	25
Milk handlers	30	3	10	1	33.3
Total	130	18	13.85	5	27.8

Table 2: Incidence of enterotoxigenic MRSA with enterotoxins typing

Source of samples	MRSA	Enterotoxigenic strains		Type of toxins				
				A	B	C	D	E
		No.	%	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)
Farm milk	11	7	63.6	-	1(14.3%)	6(85.7%)	-	-
Market milk	4	1	25	-	-	1(100%)	-	-
Milk handlers	3	2	66.7	-	-	2(100%)	-	-
Total	18	10	55.6	-	1(10%)	9(90%)	-	-

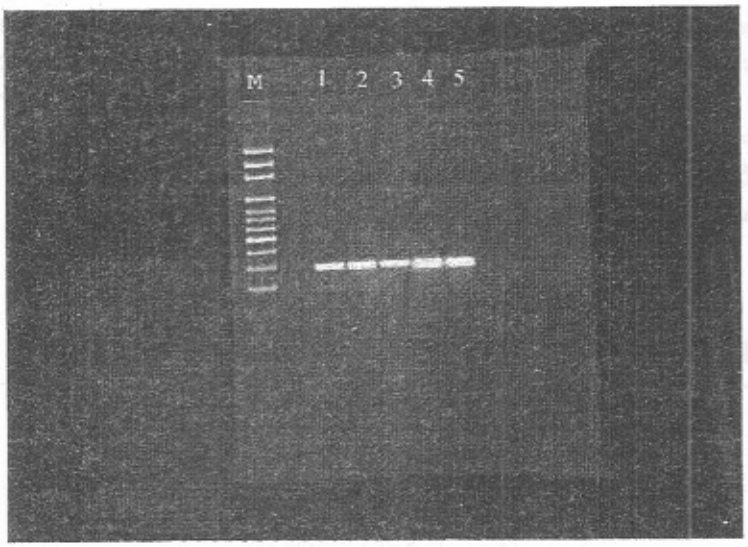

Fig. 1: PCR identification of MRSA, *nuc* gene
M: 100 bp DNA size marker. Lanes 1, 2, 3, 4, 5: MRSA strains positive for *nuc* gene (270bp).

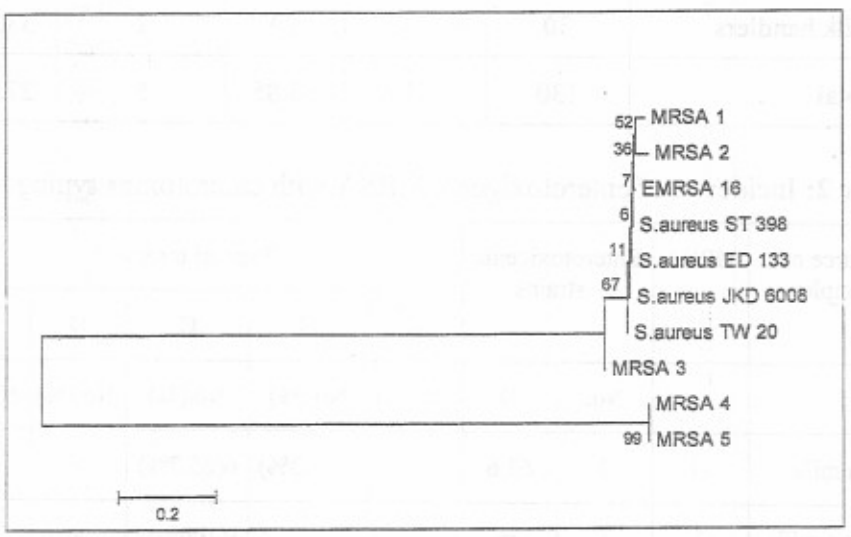

Fig. 2: Phylogenetic tree of *nuc* gene sequences
MRSA1, MRSA 4, MRSA 5 strains obtained from farm milk
MRSA 2: strain obtained from market milk
MRSA 3 strain obtained from milk handler.
S. aureus strains (EMRSA 16, *S. aureus* ST 398, *S. aureus* ED 133, *S. aureus* JKD 6008, *S. aureus* TW 20)
registered in the DDBJ/EMBL/Gene Bank.

DISCUSSION

The improper use of antibiotics in dairy herds especially in mastitis control in lactating and dry cows had lead to the emergence of bacterial resistant strains into the food chain and thus considered a public health hazard (White and McDermontt, 2001and Lee, 2003).

Coagulase positive MRSA strains were isolated from 13.85% of the examined samples (Table 1). MRSA strains were isolated from 22% and 8% of the examined farm and market milk, respectively (Table 1). It has been isolated with a lower prevalence rates (3%) and (2.8%) in other studies (Lee, 2006 and Moon *et al.*, 2007), respectively. Higher prevalence rate (42.9%) was reported in another study (Lee, 2003).

In this study 55.6% of the isolated MRSA strains (Table 2) were enterotoxigenic. A similar result was reported previously by Jorgensen *et al.*, 2005. However, variable rates of enterotoxigenic MRSA strains were reported 42.9% and 74% by Adesiyun *et al.*, 1998 and Valle *et al.*, 1990, respectively. Staphyloccol enterotoxin C was the most enterotoxin detected in the isolated MRSA strains with a rate of 90% however, enterotoxin type B was detected in 10% of the isolated MRSA strains (Table 2). Our result is in concurrent with Wilson *et al.*, 1991 who reported that Staph enterotoxin type C was the enterotoxin most commonly associated with dairy products. 63.6% of the isolated MRSA strains from farm milk were enterotoxigenic where, 6 (85.7%) strains belong to type C and one strain belong to type B (14.3%) (Table 2). However, 25% of MRSA strains isolated from market milk were enterotoxigenic with one strain belong to type C (Table 2). Normanno *et al.*, 2007 reported that the most frequently detected (SE) was SED alone or in association with either SEA and SEC.

Enterotoxigenic MRSA strains were isolated from 10% of the examined milk handlers (Table 1) and 66.7% from the strains produced enterotoxin type C (Table 2). However, *S. aureus* strains were isolated from hand swabs of milkers belonged to SEC, SEB and SEA with a rate of 32.4%, 24.3% and 13.5%, respectively (Adesiyun *et al.*, 1998).

Corrente *et al.* (2005) found that SEA was the prevalent SE synthesized by MRSA strains isolated from humans. The presence of enterotoxigenic strains in milk handlers emphasizes the role of human as an important reservoir for MRSA and their potential role of contamination of milk during handling.

Multiple studies have characterized the genotypic diversity of *S. aureus* from cases of bovine mastitis (Sabour *et al.*, 2004) but few have investigated *S. aureus* from bulk milk (Casciano *et al.*, 2003 and Scherrer *et al.*, 2004). Knowledge about the genotypic variation among *S. aureus*

isolates from bulk milk could aid in the implementation of strategies to decrease *S. aureus* level in bulk milk and could be useful in future investigation of staph food poisoning (SFP) from raw milk products in the presence of certain genotypes in the product might point to a possible source of contamination (Jorgensen *et al.*, 2005). Moreover, DNA sequencing is a useful tool in epidemiological investigation to observe the genotypic variation and to determine the relation between the isolates.

Although the isolated MRSA strains was identified by conventional methods, only 5 (27.8%) out of the 18 MRSA strains were positive for *nuc* gene by PCR (Table 1 & Figure 1). This discrepancy could be explained by polymorphism of primer annealing site or partial deletion of the *nuc* gene in the MRSA negative strains (Klaassen *et al.*, 2003). Similar results were reported by Loo *et al.* (2007) and Kateete *et al.* (2010). The possibility of mutation needs to be considered in the design of probes and primers to avoid false negative or inaccurate quantitative PCR. The use of single species specific gene as target for molecular based MRSA screening may lead to misidentification. It is better to incorporate an additional internal species specific gene detection to increase strain coverage and identification (Van Leeuwen *et al.*, 2008).

The phylogenetic analysis (Figure 2) classified the MRSA 4 and MRSA 5 into a separate clade from MRSA 1, MRSA 2 and MRSA 3. MRSA 4 and MRSA 5 are closely related to each other however, both of them had relatively low level of homology with sequences from MRSA 1, MRSA 2 and MRSA 3. Interestingly, MRSA 4 and MRSA 5 were isolated from the same farm and phylogenetic analysis revealed that they are epidemiologically related. On the other hand, MRSA 1 and MRSA2 are epidemiologically related, although MRSA 1 was isolated from farm milk and MRSA 2 were isolated from market milk and this result points out to the same source of MRSA from the farm. The genetic variation of *nuc* gene sequence between MRSA 1, MRSA 2 and MRSA 4 & MARSA 5 may be attributed to geographic variation. MRSA 3 was isolated from milk handler and had relatively low level of homology with MRSA 1, MRSA 2, MRSA 4 and MRSA 5. This result reveals the genetic variation between the strains isolated from milk and that isolated from human. The sequence of MRSA strains obtained in this study was compared with 5 strains registered in the DDBJ/EMBL/Gene Bank and phylogenetic analysis revealed that MRSA 1 and MRSA 2 are closely related to *S. aureus* strains (EMRSA 16, *S. aureus* ST 398, *S. aureus* ED 133, *S. aureus* JKD 6008, *S. aureus* TW 20) registered in the DDBJ/EMBL/Gene Bank. However, MRSA 3, MRSA 4 and MRSA 5 had relatively low homology with them (Figure 2).

Presence of MRSA strains in milk may constitute a lethal risk for consumers, especially for immunocompromised individuals where their immune system are not able to act as barriers to prevent colonization of the gastrointestinal tract (Kluytmans *et al.*, 1995). It is recommended that infected cows with *S. aureus* should be rapidly culled or treated early with antimicrobial dry cow therapy to prevent the transmission to healthy cows and to control the somatic cell count in bulk tank milk. Efficient chilling of bulk milk until pasteurization, followed by efforts to prevent recontamination minimizes the risk of SFP. Better sanitary education of milk handlers on sanitary practices focusing on their potential role as reservoirs and spreaders of food borne pathogens is recommended.

REFERENCES

Adesiyun, A.A.; Webb, L.A. and Romain, H.T. (1998): Prevalence and characteristics of *Staphylococcus aureus* strains isolated from bulk and composite milk and cattle handlers. J. Food Protec. 61(5): 629-632.

Baired, D. (1996): Staphylococcus: cluster forming gram positive cocci. In Mackie & Mac Cartney Practical Medical Microbiology. 14 th edition. Edited by: Collee J.G., Fraser, A.G.; Marmion, B.P; Simmons, A. New York; Churchill Livingstone. pp 245-261.

Brakstad, O.G.; Asbakk, K. and Macland, J.A. (1992): Detection of *Stapylococcus aureus* by polymerase chain reaction amplification of *nuc* gene. J. Clinic. Microbiol. 30: 1654-1660.

Casciano, R.; Alberghini, L.; Peccio, A; Serraino, A. and Rosmni, R. (2003): Typing of *Stapylococcus aureus* isolates from raw milk. Vet. Res. Commun. 27 (1): 289-291.

Compernolle, V.; Verschraegen, G. and Claeys, G. (2007): Combined use of Pastorex Staph- plus and either of two new chromogenic agars, MRSA ID and CHROM agar MRSA, for detection of methicillin-resistant *Stapylococcus aureus.* J. Clinic. Microbiol. 45(1): 154- 158.

Corrente, M.; Monno, R; Totano, M.; Marella, V.; Buonavoglia, D.; Rizzo, C.; Ricci, D.; Rizzo, G. and Buonavoglia, C. (2005): Characterization of methicillin- resistant *Stapylococcus aureus* (MRSA) isolated at the Policlinico Hospital of Bari (Italy). The New Microbiolgica 28: 57-65.

De Buyser, M.L.; Dufour, B.; Maire, M. and Lafarge, V. (2001): Implication of milk and milk products in food borne diseases in France and in different industrialized countries. Int. J. Food Microbiol. 67: 1-17

Dinges, M.M.; Orwin, P.M. and Schlievert, P.M. (2000): Exotoxins of *Staphylococcus aureus*. Clinic. Microbiol. Rev. 13:16-34.

Diverse, L.A. and Hommez, J. (1975): Epidemiology of methicillin resistant *Staphylococcus aureus* in dairy herds. Res. Vet. Sci. 19: 23-27.

Jarraud, S.; Peyrat, A.M.; Lim, A.; Tristan, A.; Bes, M.; Maugel, C.; Etienne, J.; Vandenesch, F.; Bonneville, M. and Lina, G. (2001): *ecg*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigen in *Staphylococcus aureus*. J. Immunol.166: 669-677.

Jones, T.F.; Kellum, M.E.; Porter, S.S.; Bell, M. and Schaffiner, W. (2002): An outbreak of community–acquired food borne illness caused by methicillin resistant *Staphylococcus aureus*. Emerging Infect. Dis. 8: 82-84.

Jorgensen, J.H.; Mork, T.; Caught, A.D.; Kearn, A. and Rorvilk, M.L. (2005): Genetic variation among *Staphylococcus aureus* strains from Norwegian bulk milk. Appl. Environ. Microbiol. 71(12): 8352-8361.

Juhasz- Kaszanyitzky, E.; Janosi, S.; Somogyi, P.; Dan, A; VanderGraaf-Van Bloois, L.; Van Duijkeron, E. and Wagenaar, J.A. (2007): MRSA transmission between cows and humans. Emerg. Infect. Dis. 13: 630-632.

Kateete, D.P.; Kimani, C.N.; Katabazi, F.A.; Okeng, A.; Okee, M.S.; Nanteza, A.; Joloba, M.L. and Najjuka, F.C. (2010): Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of Clinical Microbiology and Antimicrobials. 9: 23-29.

Klassen, C.H.; de Valk, H.A. and Horrevorts, A.M. (2003): Clinical *Staphylococcus aureus* isolate negative for the Sa 442 fragment. J. Clinic. Microbiol. 41: 4493

Kluytman, J.; Van leeuwen, W.; Goessens, W.; Hollis, R.; Messer, S.; Herwarld, L.; Bruining, H.; Heck, M.; Rost, J.; Van Leeuven, N.; Van Belkum, A. and Verburg, H. (1995): Food initiated outbreak of methicillin resistant *Staphylococcus aureus* analyzed by phenol- and genotyping. J. Clinic. Microbiol. 33: 1121-1128.

Lee, J.H. (2006): Occurrence of methicillin -resistant *Staphylococcus aureus* strains from cattle and chicken, and analyses of their *mecA*, *mecR1* and *mecI* genes. Vet. Microbiol. 114: 155-159.

Lee, J.H. (2003): Methicillin (oxacillin) resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol. 69: 6489-6494.

Leterte, C.; Perelle, S.; Dilasser, F. and Fech, P. (2003): Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*. J. Appl. Microbiol. 95: 38-43.

Loo, I.; Huijsdens, X.; Tiermersma, F.; Neeling, A.; Van de sande-Brnisma, D.; Beanjean, D.; Voss, A. and Klutmans, J. (2007): Emergence of methicillin- resistant *Stapylococcus aureus* animal orign in humans. Emerg. Infect. Dis. 13: 1834-1839.

Mandell, G.; Douglas, J. and Benett, R. (1995): Principles and practices of infectious diseases,4th ed. Churchill Livingstone, Ltd, Edinburg, United Kingdom.

Moon, J.S.; Lee, A.R.; Kang, H.M.; Lee, E.S.; Kim, M.N.; Paik, Y.H.; Park, Y.H.; Joo, Y.S. and Koo, H.C. (2007): Phenotypic and genetic antibiogram of methicillin-resistant *Staphylococci* isolated from bovine mastitis in Korea. J. Dairy Sci. 90: 1176-1185.

Normanno, G.; Corrente, M.; Salandra, La, G.; Dambrosio, A.; Quaglia, N.C.; Parisi, A.; Gerco, G.; Bellacicco, L.A.; Virgillo, S. and Celano, V.G. (2007): Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. Int. J. Food Microbiol. 117: 219-222.

Sabour, P.M.; Gill, J.J.; Lepp, D.; Pecan, J.C.; Ahmed, R.; Dingwell, R. and Lesile, K. (2004): Molecular typing and distribution of *Stapylococcus aureus* isolates in Eastern Canadian dairy herds. J. Clinic. Microbiol. 42: 3449-3455.

Scherrer, D.; Corti, S.; Muehlherr, E.; Zweifel, C. and Stephan, R. (2004): Phenotyping and genotypic characterstics of *Staphylococcus aureus* from raw bulk tank milk samples of goats and sheep. Vet. Microbiol. 101: 101-107.

Seguin, J.C.; Walker, R.D.; Caron, J.P.; Kloos, W.E.; George, C.G.; Hollis, R.J.; Johnes, R.N. and Pfaller, M.A. (1999): Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital; potential human to animal transmission. J. Clinic. Microbiol. 37: 1459-1463.

Tamura, K.; Dudley, J.; Nei, M. and Kumar, S. (2007): Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Thompson, J.D.; Higgins, D.G. and Jibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680.

Valle, J.; Gomez-Lucia, E.; Priz, S.; Goyuche, J.; Orden, J.A. and Vadillo, S. (1990): Enterotoxin production by *Staphylococcus aureus* isolated from healthy goats. Appl. Environ. Microbiol. 56: 1323-1326.

Van Leeuwen, W.; Roorda, L.; Hendrik, W.; Francois, P. and Schrenzel, J. (2008): A *nuc* deficient methicillin- resistant *Staphylococcus aureus* strain. FEMS Immunol. Med. Microbiol.54: 157.

White, D.G. and McDermott, P.F. (2001): Emergence and transfer of antibacterial resistance. J. Dairy Sci. 84(E Suppl.): 151-155.

Wilson, I.G.; Cooper, J.E. and Gilmour, A. (1991): Detection of enterotoxigenic *Staphylococcus aureus* in dried skimmed Milk: Use of the polymerase chain reaction for amplification and detection of Staphylococcal enterotoxin genes *ent B* and *ent C*, and the thermonuclease Gene *nuc*. Appl. Environ. Microbiol. 57(6): 1793-1798.

Zadoks, R.V.; Van Leeuwen, W.B.; Kreft, D.; Fox, L.K.; Barkema, H.W. Schukken, Y.H. and Van Belkum, A. (2002): Comparison of *Staphylococcus aureus* isolates from bovine and human skin, milking equipment and bovine milk by phage typing pulsed field gel electrophoreses and binary typing. J. Clinic. Microbiol. 40: 3894-3902.