

## Sensitivity of *Staphylococcus aureus* Isolated from Subclinical Bovine Mastitis to Co-Amoxiclav in Tabriz Dairy Herd in 2010

<sup>1</sup>S. Mosaferi, <sup>2,3</sup>T. Jalili, <sup>4</sup>Z. Ostadi, <sup>1</sup>M. Khakpour and <sup>2</sup>H. Bodaghi

<sup>1</sup>Department of Clinical Science, Faculty of Veterinary Medicine,

<sup>2</sup>Faculty of Veterinary Medicine, <sup>3</sup>Young Researchers Club,  
Tabriz Branch, Islamic Azad University, Tabriz, Iran

<sup>4</sup>Department of Anesthesiology, Faculty of Medicine,  
Tabriz University of Medical Science, Tabriz, Iran

**Abstract:** Mastitis is an inflammation of the parenchyma of mammary gland regardless of the causes. Mastitis is characterized by a range of physical and chemical changes in the glandular tissue. The most important changes in milk include discoloration, the present clots and the present of large number of leucocytes. There is a swelling, heat, pain and edema in mammary gland in many clinical causes. Coagulase positive *S. aureus* is a major pathogen of the bovine mammary gland and a common cause of contagious mastitis in cattle. The aim of this study was the evaluation of outbreaks of *Staphylococcus aureus* mastitis and its sensitivity to Co-amoxiclav in Tabriz dairy herd. This study is conducted in ten dairy herd of Tabriz on about 1,000 cows. After doing CMT and identifying of infected cows, the milk samples obtained from infected quarters and transport to microbiological laboratories. After microbial culture of milk samples and isolated of *S. aureus*, antimicrobial sensitivity test with disk diffusion method perform by co-amoxiclav, penicillin, oxytetracyclin, co-trimoxazole, lincomycin and cefquinome. This study defined that the outbreak of subclinical coagulase positive staphylococcus mastitis in Tabriz dairy herd was 4.43% (3.35% *S. aureus* and 1.08% *S. intermedicus*). The antimicrobial sensitivity test shown that 41.17% of *Staphylococcus aureus* isolated from bovine mastitis in Tabriz dairy herd were susceptible to co-amoxiclav, 94.1% to cefquinome, 85.29 to sulfonamides (Co-trimoxazole), 32.35 lincomycin, 14.7% to oxytetracyclin and 5.88% to penicillin. Results of this study defined that Co-amoxiclav has a moderate effect on *Staphylococcus aureus* isolated from subclinical bovine mastitis in Tabriz dairy herd. It seems that cefquinome and sulfonamides has a great effect on *Staphylococcus aureus* isolated from subclinical bovine mastitis at *in vivo*.

**Key words:** Mastitis, *staphylococcus aureus*, co-amoxiclav, bovine, penicillin

---

### INTRODUCTION

Mastitis is the inflammation of mammary gland that some changes occur in appearance and structure of milk (Beaudeau *et al.*, 1995). The inflammation of mammary gland is resulted of various microorganisms, although may be no microorganisms are isolated in laboratory (Barry *et al.*, 1999). Mastitis is the most important disease of dairy cattle which imposes significant economic losses and allocates considerable treatment costs to its own. The common form of mastitis are subclinical cases which cause to gradual and significant decrease of milk production in cattle pens and impose much economic losses to cattlemen and cow-keeping industry (Dobbins, 1997; King, 1972; Losinger, 2005). *Staphylococcus aureus* is one the most important factors creating subclinical mastitis in dairy cattle's breasts all over the world

(Lipman *et al.*, 1996; Phuektes *et al.*, 2001; Li *et al.*, 2009) as well as one of the important pathogens in human being (Easmon and Adlam, 1983). The bacterium shows resistance against antibiotics due to some reasons like having beta lactamase enzyme, fast changing to abscess in to the breast, entering intra cell and changing to L form. The bacterium is so resistant against most of antibiotics across the cow-pens that most of treatments protocols used against this pathogen fail to research. Various antibiotics in treatment of mastitis caused by *Staphylococcus aureus* have been evaluated up to now. With regard to the fact that the probability of resistant strains occurrence and their incidence against common antibiotics, increased resistance of *Staphylococcus aureus* isolated from dairy cattle with mastitis against antibiotics has been reported (Myllys *et al.*, 1998; Gentilini *et al.*, 1995; Aarestrup and Jensen, 1998).

Therefore in the present study, the incidence rate of mastitis resulted of *Staphylococcus aureus* and the rate of isolated bacterium allergy to antibiotics like coamoxiclav, oxytetracycline, penicillin, lincomycine, cefquinome, sulfadiazine + trimetoprim (cotrimoxazole) have been evaluated.

### MATERIALS AND METHODS

The present study was conducted across 10 industrial cow pens of Tabriz suburbs which was consisted of 1012 Holstein dairy cattle in 8 stages. Sampling method was such that following identification of involved unit by California Mastitis Test (CMT) then rinsed thoroughly with clean water for removing any external body. The most important issue in sampling was preventing of secondary infection occurrence (Harmon *et al.*, 1990; Jasper and Jain, 1966; Lam *et al.*, 1995). Plastic capped tubes which were disinfected previously were used for sampling. And 3-4 first milking of involved units were discarded to remove the microbes existed in teat. Then, the teat was cleaned with sterile cotton and dried and finally disinfected with 70% alcohol about 5-6 times and dried; after complete drying of involved unit the milk sample milked in to the sterile tube which was inclined to teat. Finally, the mentioned plastic tubes were placed in to the flasks contained dry ice for transferring the samples to the microbiological laboratory of veterinary faculty of Tabriz Islamic Azad University to microbial culture as soon as possible. The milk samples were obtained according to NMC (1999) and transferred to the laboratory.

The milk sample of each cow was cultured in blood agar media and was placed in 37°C incubator for 24-48 h. After the mentioned period clones grew so *Staphylococcus aureus* was identified by means of common bacteriological methods (Quinn *et al.*, 1994).

### Susceptibility determining test by agar disk diffusion method:

With a sterile loop, the tops of 4-5 colonies of *S. aureus* from pure culture were picked up. The colonies were suspended in 5 mL of sterile physiologic saline. The inoculum turbidity was standardized to equivalent of a 0.5 Mc Farland standard. The entire surface of a Mueller-Hinton agar plate was inoculated using a sterile swab. Disks containing 30 µg of co-amoxiclav, 10IU of penicillin, 25 µg of co-trimoxazole, 30 µg of oxytetracyclin, 2 meq of lincomycin and 10 µg of cefquinome were placed using a sterile forceps onto the agar surface and gently pressed down to ensure contact. Plates were incubated at 35°C for 20 h. Subsequently, the

diameter of the zone of inhibition around each disk was measured. This procedure is conforming to the National Committee for Clinical Laboratory Standards (NCCLS) documents M31-A2 and M2-A7 (NCCLS, 1999).

### RESULTS

About 126 milk samples were transferred to the laboratory of which 113 samples were positive. Gram positive cocci were isolated from 79 samples of which 45 samples were positive coagulase staphylococcus, 34 cases were *Staphylococcus aureus*, 11 cases were *Staphylococcus intermedius*, 23 cases were positive g bacilli and 11 g negative (Fig. 1).

Generally, 12.45% of 1012 dairy cattle was CMT positive and in these herds 4.43% statistical society has the infection of positive coagulase staphylococcus (3.35%), *Staphylococcus aureus* and 1.08% *Staphylococcus intermedius* (Fig. 2). Also *Staphylococcus aureus* and *intermedius*, consist about

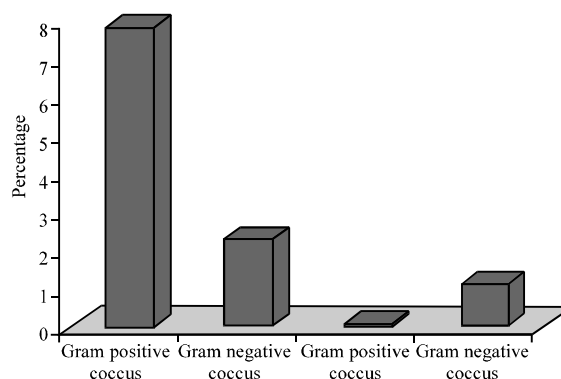


Fig. 1: The results of evaluating the percentage and the kind of bacterial infection in understudied cow pens according to gram staining and morphology

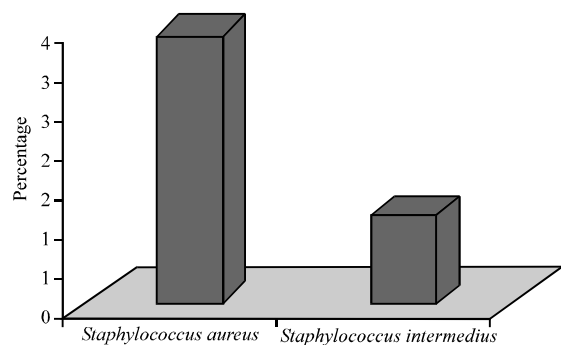


Fig. 2: The results of evaluating the infectious percent to *Staphylococcus aureus* and *intermedius* in 10 understudied cow pens

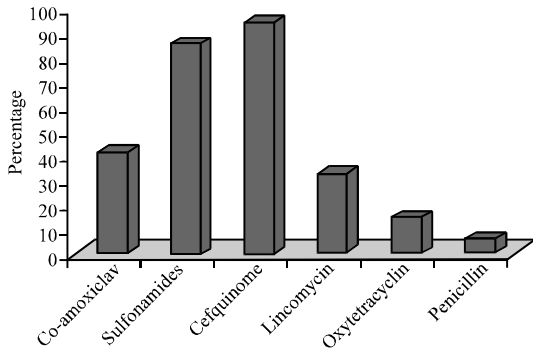


Fig. 3: Comparison the susceptibility rate of isolated *Staphylococcus aureus* from mastitis cases to administered antibiotics in the present study and status of co-amoxiclav

30.08 and 9.73% of infectious cases. The results of the present study demonstrated that the susceptibility rate of isolated *Staphylococcus aureus* to co-amoxiclav was 41.17% as well as 85.29% of isolated *Staphylococcus aureus* from dairy cattle mastitis of Tabriz city demonstrated susceptibility to sulfadiazine + trimethoprim (cotrimoxazole), 14.71% to oxytetracycline, 32.35% to lincomycin, 94.1% to cefquinome and 5.88% to penicillin (Fig. 3).

The criteria for susceptibility and resistance to antibiotics was based on the non-growth corona thickness of bacteria and its comparison with standard tables (NCCLS, 1999).

### DISCUSSION

Up to now various studies about the susceptibility rate of isolated *Staphylococcus aureus* from mastitis cases to different antibiotics have been conducted in laboratory. In a study which was conducted in 464 farms by Piret Kalmus in 2007-2009 entitled upper pathogens and their resistance to antimicrobial agents in dairy cows in stonia it was demonstrated that the bacteria which cause mainly subclinical mastitis are *Staphylococcus aureus* (20%) and staphylococcus Coagulase Negative (CNS) (15.4%). In their study, the antibiotic susceptibility determining test shows that the susceptibility of *Staphylococcus aureus* to ampicillin (40.5%), penicillin (38.6%), cephalothin (96.2%), clindamycin (81.9%), erythromycin (95.2%), tetracycline (95.9%), sulfadiazine + trimethoprim (96.6%) and gentamycin (93.2%) (Kalmus *et al.*, 2011).

In another study conducted by Sukuru in 2003 entitled identification and antimicrobial susceptibility of *Staphylococcus aureus* and *coagulase* negative

staphylococci from bovine mastitis in the Aydin region of Turkey 300 milk samples which were milked from cows' affected by mastitis as identified positively by CMT test, the following results were concluded after microbial culturing and bacteria identification.

*Staphylococcus aureus* and *coagulase* negative were isolated from 85 (28.33%) and 60 (20%) samples, respectively. Antibiotic susceptibility determining test by Disc Propagation Method cleared that 100% of isolated *Staphylococcus aureus* were sensitive to ciprofloxacin, 85% to kanamycin, 85% to cefquinome, 84% to co-amoxiclav compound and 100% to neomycin-basiteracin-tetracycline. About 95% of isolated *Staphylococcus aureus* from mastitis cases were resistant to penicillin and 60% to oxacillin (Kirkan *et al.*, 2005).

In a study which was conducted by Pengov and Ceru (2003) entitled antimicrobial drug susceptibility of *Staphylococcus aureus* strains isolated from bovine and ovine mammary gland it was demonstrated that the susceptibility of *Staphylococcus aureus* isolated from bovine milk to penicillin, oxacillin, kanamycin, cephalexine was 59.2, 61.8, 100, 92.1 and 96.1%, respectively.

The results of a study which was conducted by Li *et al.* (2009) on 864 dairy cattle in Zhejiang province in China entitled prevalence, genetic diversity and antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated from bovine mastitis in Zhejiang province in China demonstrated that the prevalence of *staphylococcus aureus* was 12% and maximum resistant of *staphylococcus aureus* was to penicillin and ampicillin (77.3%) and the minimum rate was to cephalothin and vencomycin (0%), cephoxetin (2.7%), ciprofloxacin (8%).

The results of a study which was conducted by Tenhagen *et al.* (2006) on 2560 dairy cattle of 80 farms in Bradenburge city in Germany, antibiotic susceptibility determining test demonstrated that *staphylococcus aureus* isolated from bovine mastitis had the maximum susceptibility to cefquinom and clavlanic acid-amoxicillin (co-amoxiclav) compound, respectively.

A study conducted by Moroni *et al.* (2006) in Italy demonstrated that resistance of *staphylococcus aureus* to penicillin was 69.1%, ampicillin 98.5%, amoxicillin 100%, co-amoxiclav 20.6%, oxytetracyclin 58.8% and daxicillin 47.1%. Nunes *et al.* (2007) evaluated 7 farms in Portugal demonstrated that antibiotic susceptibility of *Staphylococcus aureus* to penicillin was 33.3%, oxytetracyclin 96.7 and 100% to enrofloxacin, sulfamethoxazole + trimethoprim, gentamycin, cefazolin and oxacillin.

In a study conducted by Gianneechini *et al.* (2002) entitled antimicrobial susceptibility of upper pathogens

isolated from dairy herds in the west littoral region of Uruguay, it was demonstrated that the resistance of *Staphylococcus aureus* to penicillin was 46.1 and 46.6% to ampicillin, 2% to oxacillin and 0% to cephalothin, enrofloxacin, gentamycin, amoxicillin + clavulanic acid (Co-amoxiclav), sulfamemethoxi + trimethoprim.

In a study conducted by Akbarnia *et al.* (2008) in Tabriz entitled evaluating the rate of bacterial susceptibility of *Staphylococcus aureus* isolated from Tabriz dairy cattle mastitis to cefquinome, it was demonstrated that there was 6.25% infection to positive coagulase staphylococcus which consisted of 3.04% *Staphylococcus aureus* and 3.2% *Staphylococcus intermedius*. The antibiotic susceptibility determining test by disk propagation method demonstrates that the rate of isolated *Staphylococcus aureus* susceptibility to cefquinome is 94.44 and 88.88% of isolated *Staphylococcus aureus* from dairy cattle's mastitis in Tabriz have susceptibility to enrofloxacin and cotrimoxazole, 83.33% to tetracycline, 77.77% to lincomycine and 27.77% to cephterixaxone but no susceptibility to penicillin, amoxicillin and ampicillin was observed.

The present study shows that *Staphylococcus aureus* is one of the most important factors of mastitis such that the issue conforms to most of conducted studies in the world as well as Iran (Li *et al.*, 2009; Myllys *et al.*, 1998; Kalmus *et al.*, 2011; Kirkan *et al.*, 2005; Akbarnia *et al.*, 2008; Pittkala *et al.*, 2004).

Antibiotic susceptibility determining test conducted in the study shows that co-amoxiclav has a moderate effect on *Staphylococcus aureus* but this result has no conformation to conducted studies (Kirkan *et al.*, 2005; Pengov and Ceru, 2003; Tenhagen *et al.*, 2006; Moroni *et al.*, 2006). This issue suggests that resistance to this drug is high in Tariz region. Perhaps the reason of this issue is high propagation of bacteria generating betalactamase enzyme in nature. Also, there is probability of transferring resistant strains from animals to each other as well as to human being (Nazer and Sarmadi, 2005).

## CONCLUSION

Generally, the results of the present study show that *Staphylococcus aureus* is one of the important agents causing dairy cattle mastitis in Tabriz region in Iran. Also with regard to the findings of the present study, co-amoxiclav has moderate effect on *Staphylococcus aureus* and cefquinome and cotrimoxazole are suitable drugs against isolated *Staphylococcus aureus* from dairy cattle's mastitis.

## REFERENCES

- Aarestrup, F.M. and N.E. Jensen, 1998. Development of penicillin resistance among *Staphylococcus aureus* isolated from bovine mastitis in Denmark and other countries. *Microb. Drug Resist.*, 4: 247-256.
- Akbarnia, S., S. Mosaferi and M. Khahpour, 2008. Effect of cefquinome on *Staphylococcus aureus* isolated from subclinical mastitis in Tabriz dairy herd of Iran veterinary. Ph.D. Thesis, Veterinary Faculty of Tabriz Islamic Azad University
- Barry, A.L., W.A. Creig, H.Nadler, L.B. Reller and C.C. Sanders *et al.*, 1999. Methods for determining bactericidal activity of antimicrobial agents. Approved Guidelines, Document M26-A. National Committee for Clinical Laboratory Standards, Wayne, PA., Vol. 19. <http://www.clsi.org/source/orders/free/m26-A.pdf>.
- Beaudeau, F., V. Ducrocq, C. Fourichon and H. Seegers, 1995. Effect of disease on length of productive life of French Holstein dairy cows assessed by survival analysis. *J. Dairy Sci.*, 78: 103-117.
- Dobbins, C.N., 1997. Mastitis losses. *J. Am. Vet. Med. Assoc.*, 170: 1129-1132.
- Easmon, C.S.F. and C. Adlam, 1983. *Staphylococci and Staphylococcal Diseases*. Academic Press, London, UK.
- Gentilini, E., G. Denamiel, L. Tirante, C. Chavez and M.S. Godaly, 1995. Bovine mastitis:  $\beta$ -lactamase production. *Staphylococcus aureus* antibiotic resistance evolution. Proceedings of the 3rd International Mastitis Seminar, Session 2, May 28-June 1, 1995, Tel Aviv, Israel, pp: 84-85.
- Gianneechini, R.E., C. Concha and A. Franklin, 2002. Antimicrobial susceptibility of udder pathogens isolated from dairy herds in the west littoral region of Uruguay. *Acta Vet. Scand.*, 43: 31-41.
- Harmon, R.J., R.J. Eberhart, D.E. Jasper, B.E. Langlois and R.A. Wilson, 1990. Microbiological Procedures for the Diagnosis of Bovine Udder Infection. Natl. Mastitis Counc. Inc., Arlington VA., USA.
- Jasper, D.E. and N.C. Jain, 1966. Hemolytic behaviour of *Staphylococci* isolated from cow's milk. *Can. J. Comp. Vet. Med. Sci.*, 30: 63-70.
- Kalmus, P., B. Aasmae, A. Karsin, T. Orra and K. Kask, 2011. Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. *Acta Vet. Scand.*, Vol. 53.
- King, J.O.L., 1972. Mastitis as a production disease. *J. Vet. Rec.*, 91: 325-330.

- Kirkan, S., E.O. Goksoy and O. Kaya, 2005. Identification and antimicrobial susceptibility of *Staphylococcus aureus* and coagulase negative *Staphylococci* from bovine mastitis in the Aydin Region of Turkey. Turk. J. Vet. Anim. Sci., 29: 791-796.
- Lam, T.J., A. Pengov, Y.H. Schukken, J.A. Smit and A. Brand, 1995. The differentiation of *Staphylococcus aureus* from other *Micrococcaceae* isolated from bovine mammary glands. J. Applied Microbiol., 79: 69-72.
- Li, J.P., H.J. Zhou, L. Yuan, T. He and S.H. Hu, 2009. Prevalence, genetic diversity and antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated from bovine mastitis in Zhejiang Province, China. J. Zhejiang University Sci. B., 10: 753-760.
- Lipman, L.J., A. de Nijs, T.J. Lam, J.A. Rost, L. van Dijk, Y.H. Schukken and W. Gastra, 1996. Genotyping by PCR, of *Staphylococcus aureus* strains, isolated from mammary glands of cows. Vet. Microbiol., 48: 51-55.
- Losinger, W.C., 2005. Economic impacts of reduced milk production associated with an increase in bulk-tank somatic cell count on US dairies. J. Am. Vet. Med. Assoc., 226: 1652-1658.
- Moroni, P., G. Pisoni, M. Antonini, R. Villa, P. Boettcher and S. Carli, 2006. Antimicrobial drug susceptibility of *Staphylococcus aureus* from subclinical bovine mastitis in Italy. J. Dairy Sci., 89: 2973-2976.
- Myllys, V., K. Asplund, E. Brofeldt, V. Hirvela-Koski and T. Honkanen-Buzalski *et al.*, 1998. Bovine mastitis in Finland in 1988 and 1995. Changes in prevalence and antimicrobial resistance. Acta Vet. Scand., 39: 119-126.
- NCCLS, 1999. Performance standard for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Approved standard. NCCLS Document M31-A2. National Committee for Clinical Laboratory Standards, Wayne, PA.
- NMC., 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council Inc., Madison WI., USA.
- Nazer, A.H.K. and A. Sarmadi, 2005. Prevalence of clinical and subclinical mastitis, antibiotic resistance and determination of Minimum Inhibitory Concentration (MIC) in *Staphylococcus aureus* and *Echerichia coli* isolated from cases of bovine mastitis. J. Vet. Res., 60: 247-252.
- Nunes, S.F., R. Bexiga, L.M. Cavaco and C.L. Vilela, 2007. Technical note: Antimicrobial susceptibility of portuguese isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* in subclinical bovine mastitis. J. Dairy Sci., 90: 3242-3246.
- Pengov, A. and S. Ceru, 2003. Antimicrobial drug susceptibility of *Staphylococcus aureus* strains isolated from bovine and ovine mammary glands. J. Dairy Sci., 86: 3157-3163.
- Phuektes, P., P.D. Mansell and G.F. Browning, 2001. Multiplex polymerase chain reaction assay for simultaneous detection of *Staphylococcus aureus* and *Streptococcal causes* of bovine mastitis. J. Dairy Sci., 84: 1140-1148.
- Pittkala, A., M. Haveris, S. Pyorala, V. Myllys and T.H. Buzalski, 2004. Bovine mastitis in finland 2001-prevalence, distribution of bacteria and antimicrobial resistance. J. Dairy Sci., 87: 2433-2442.
- Quinn, R.J., M.E. Carter, B.K. Markey and G.R. Carter, 1994. Clinical Veterinary Microbiology. Mosby-Wolfe Publishing Co., Spain, pp: 118-137 and 320-345.
- Tenhagen, B.A., G. Koster, J. Wallmann and W. Heuwieser, 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg. Germany J. Dairy Sci., 89: 2542-2551.