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

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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 presence of large number of leucocytes. There is a swelling, heat, pain and edema in mammary gland in many clinical cases. 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, oxitetracyclin, 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 oxitetracyclin 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 (Dobkins, 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; Phuetes 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 coxamoxiclav, oxytetracycline, penicillin, lincomycin, 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 disinsected 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 disinsected with 70% alcohol about 5-6 times and dried; after complete drying of involved unit the milk sample milked in into 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, 10 IU 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](image1)

![Fig. 2: The results of evaluating the infectious percent to *Staphylococcus aureus* and *intermedius* in 10 understudied cow pens](image2)
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 (Kirk 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, cephalaxine 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 Zhejian province in China entitled prevalence, genetic diversity and antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated from bovine mastitis in Zhejian 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 vencymycin (0%), cepheoxitin (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 Brandenburg city in Germany, antibiotic susceptibility determining test demonstrated that *staphylococcus aureus* isolated from bovine mastitis had the maximum susceptibility to cefquinome and clavamic 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%, oxytetracycline 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%, oxytetracycline 96.7 and 100% to enrofloxacin, sulfamethoxasole + trimethoprim, gentamycin, ceftazolin and oxacillin.

In a study conducted by Gianneechnini 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, enrofloxacolin, gentamycin, amoxicillin + clavulanic acid (Co-amoxiclav), sulfinemethoxi + 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 enrofloxacain and cotrimoxazole, 83.33% to tetracycline, 27.77% to lincomycine and 27.77% to cephtheraxone 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 in Iran (Li et al., 2009; Myllys et al., 1998; Kalmus et al., 2011; Kirk et al., 2005; Akbarnia et al., 2008; Pritkala 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 (Kirk 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 Tabriz region. Perhaps the reason of this issue is high propagation of bacteria generating beta-lactamase 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**


