

Sensitivity of *Staphylococcus aureus* isolated from subclinical bovine mastitis to Ciprofloxacin in Tabriz dairy herd in 2012

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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 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 Ciprofloxacin in Tabriz dairy herd. This study is conducted in ten dairy herd of Tabriz on about one thousand 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 Ciprofloxacin, 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. intermedius*). The antimicrobial sensitivity test shown that 88.23% of *Staphylococcus aureus* isolated from bovine mastitis in Tabriz dairy herd were susceptible to Ciprofloxacin, 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 Ciprofloxacin has a great effect on *Staphylococcus aureus* isolated from subclinical bovine mastitis at in vivo.

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Keywords: Mastitis, *Staphylococcus aureus*, Ciprofloxacin.

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 maybe no microorganisms are isolated in laboratory (NCCLS, 1999a). 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; Jian-ping 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 work. 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 Ciprofloxacin, 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 disinfected previously were used for sampling. 3 to 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 (National Mastitis Council, 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 to 48 hours. After the mentioned period clones grew, so staphylococcus aureus was identified by means of common bacteriological methods (Quinn et al., 1984).

Susceptibility determining test by agar disk diffusion method:

With a sterile loop, the tops of four to five colonies of *S. Aurous* 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 McFarland standard. The entire surface of a Mueller-Hinton agar plate was inoculated using a sterile swab. Disks containing 15µg of Ciprofloxacin, 10 IU of penicillin, 25µg of Co-trimoxazole, 30 µg of Oxytetracyclin, 2 meq of lincomycin, and 10µg 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, 1999b).

Results:

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 gram bacilli and 11 gram negative (diagram 1).

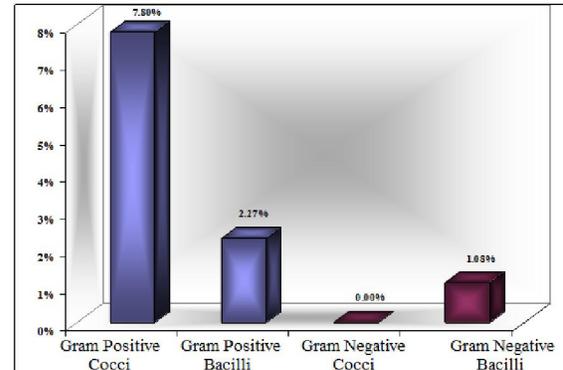


Diagram 1: the results of evaluating the percentage and the kind of bacterial infection in understudied cow pens according to gram staining and morphology

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) (diagram 2). Also staphylococcus aureus and staphylococcus intermedius, consist about 30.08 and 9.73 percent of infectious cases.

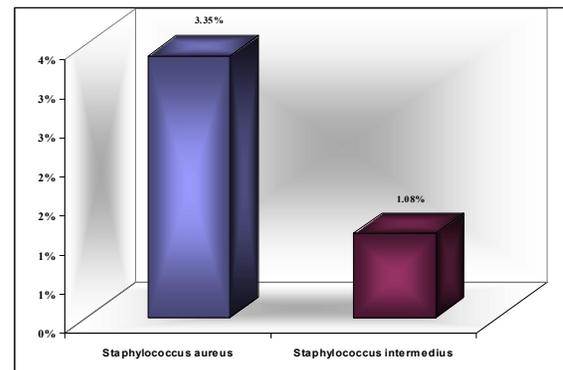


Diagram 2: the results of evaluating the infectious percent to staphylococcus aureus and staphylococcus intermedius in 10 understudied cow pens

The results of the present study demonstrated that the susceptibility rate of isolated staphylococcus aureus to Ciprofloxacin was 88.23 % as well as 85.29% of isolated staphylococcus aureus from dairy cattle mastitis of Tabriz city demonstrated susceptibility to sulfadiazine + tri methoprim (Cotrimoxazole), 14.71% to Oxytetracycline, 32.35%

to lincomycine, 94.1% to Cefquinome and 5.88% to penicillin (diagram 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,1999b).

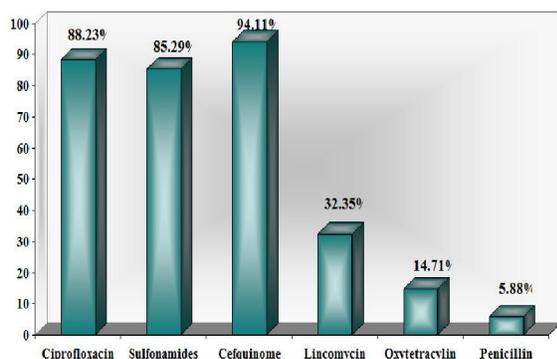


Diagram 3: comparison the susceptibility rate of isolated staphylococcus aureus from mastitis cases to administrated antibiotics in the present study and status of Ciprofloxacin

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 et al., 2011 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%).

In another study conducted by Sukuru (2005) entitled "Identification and antimicrobial susceptibility of staphylococcus aureus and staphylococcus 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 staphylococcus 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. 95% of isolated staphylococcus aureus from mastitis cases were resistant to penicillin and 60% to oxacillin (Sukuru, 2005).

In a study which was conducted in 2003 by Pengov and Ceru 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 percent, respectively.

The results of a study which was conducted in 2009 by Jian-ping et al., 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 vencomycin (0%), cephoctin (2.7%), ciprofloxacin (8%).

The results of a study which was conducted in 2006 by Tenhagen et al., 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. in 2004 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. in 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 Giannchini et al. in 2001 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%, 46.6% to ampicillin, 2% to oxacillin and 0% to cephalothin, enrofloxacin, gentamycin, amoxicillin + clavlanic acid (Co-amoxiclav), sulfamemethoxi + trimethoprim.

In a study conducted by Akbarnia et al. in 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%. 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 cephterioxone 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 (Akbarnia et al., 2008; Pitkala et al., 2004).

Antibiotic susceptibility determining test conducted in our study shows that Ciprofloxacin has a great effect on staphylococcus aureus. 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 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 Ciprofloxacin has great effect on staphylococcus aureus.

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