



Identification of Pathogen Bacteria from Bovine Mastitis In Yozgat Province And Determination of Antimicrobial Susceptibility

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ABSTRACT

Background/Aim: The aim of this study was to isolate and identify the bacterial microorganisms causing subclinical mastitis and to determine their susceptibility and resistant to antibiotics in Yozgat province and districts.

Results and Conclusion: In isolated milk samples (174) with bacterial growth, Gram positive (63) and Gram negative (111) microorganisms were respectively isolated at 36.21% and 63.79%. A total of 238 bovine milk samples with mastitis were used and the isolated agents were identified as *Aeromonas hydrophila* 30 (17.24%), *Citrobacter diversus* 4 (2.29%), *Citrobacter freundii* 2 (1.14%), *Corynebacterium* sp. 24 (13.79%), *Escherichia coli* 18 (10.34%), *Enterobacter cloacae* 2 (1.14%), *Flavobacter* sp. 3 (1.72%), *Lactobacillus* sp. 21 (12.06%), *Pasteurella pneumonia* 2 (1.14%), *Plesiomonas shigelloides* 11 (6.32%), *Providencia* sp. 2 (1.14%), *Salmonella* sp. 2 (1.14%), *Serratia liquefacians* 2 (1.14%), *Shigella* sp. 18 (10.34%), *Vibrio cholerae* 6 (3.45%), *Yersinia enterocolitica* 1 (0.57%), coagulase-negative staphylococcus (CNS) 18 (10.34%) and *Staphylococcus aureus* 8 (4.59%). *Aeromonas hydrophila* (17.24%) was the most isolated agent from milk samples. Antibiotic resistance was evaluated in all isolated pathogens and the highest resistance was observed against erythromycin (63/174, 36.20%) followed by penicillin G (53/174, 30.45%), amoxicillin (38/174, 21.83%) and gentamicin (23/174, 13.21%).

Keyword: Antibiotic Susceptibility Test, Cow, Identification, Mastitis.

Yozgat İlinde Bulunan Sığır Mastitislerinden Patojen Bakterilerin İdentifikasyonu Ve Antibiyotiklere Duyarlılıklarının Belirlenmesi

Ö Z E T

Öz bilgi/Amaç: Bu çalışmada Yozgat ili merkez ve ilçelerinde bulunan ve mastitis enfeksiyonlarına neden olan bakteriyel etkenlerin izolasyonu ve identifikasyonu ile bu etkenlere karşı etkili ve dirençli olan antibiyotiklerin belirlenmesi amacıyla toplam 238 adet mastitisli sığır süt örneği kullanılmıştır.

Bulgular ve Sonuç: Bakteriyel üremenin meydana geldiği süt örneklerinde (174) %36.21 oranında Gram pozitif (63) ve %63.79 oranında Gram negatif (111) mikroorganizma izole edilmiştir. İzole edilen etkenler *Aeromonas hydrophila* 30 (%17.24), *Citrobacter diversus* 4 (%2.29), *Citrobacter freundii* 2 (%1.14), *Corynebacterium* sp. 24 (%13.79), *Escherichia coli* 18 (%10.34), *Enterobacter cloacae* 2 (%1.14), *Flavobacter* sp. 3 (%1.72), *Lactobacillus* sp. 21 (%12.06), *Pasteurella pneumonia* 2 (%1.14), *Plesiomonas shigelloides* 11 (%6.32), *Providencia* sp. 2 (%1.14), *Salmonella* sp. 2 (%1.14), *Serratia liquefacians* 2 (%1.14), *Shigella* sp. 18 (%10.34), *Vibrio cholerae* 6 (%3.45), *Yersinia enterocolitica* 1 (%0.57), Koagülaz Negatif Staphylococcus 18 (%10.34) ve *Staphylococcus aureus* 8 (%4.59) olarak patojenlerin antibiyotik direnç durumu değerlendirildiğinde en fazla direnç eritromisin'de (63/174, %36.20) görülmüş ve penisilin G (53/174, %30.45), amoksisilin (38/174, %21.83) ve gentamisin (23/174, %13.21) 'nin takip ettiği gözlenmiştir. **Anahtar Kelimeler:** Antibiyotik Duyarlılık Test, İdentifikasyon, İnek, Mastitis.

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Introduction

In dairy industry, quality and stability of milk production is possible with healthy udder structure of the animals. Infective, pathologic, physiologic and chemical changes on udder tissue affect the trading and nutritious value of milk negatively. Mastitis, racial difference, hormonal disorders of animals, high protein feeding, maintenance, milking, age and inflammation situation on udder tissue depending on microbiological factors all cause this negativity and account for 26% of total bovine diseases. Per year 11 million tons of milk is produced in dairy farms and it is specified that mastitis cause 10% reduction of milk production capacity in Turkey. According to 2014 data of Turkish Statistical Institute lots of mastitis events are detected and it is determined that 21.4% of these events are clinical, 78.6% of these events are subclinical. Also mastitis influences the milk composition such as increased pH, changing sodium and chlorine levels parallel to blood values. Due to mastitis the total annual economic loss of the enterprises which have milk yields between 6000-9000 kg in lactation period is as high as 58% to 68%. It also causes bitter taste and salinity which lowers the commercial value of milk. Therefore, prevention and early diagnosis of mastitis is important for both trading and business economics (Çetin and Alan, 2008; Eberhart, 1986; Kandasamy et al., 2011; Koneman et al., 1997; Özdemir and Kaymaz, 2013; Wolfova et al., 2006). Bovine mastitis is highly caused by bacterial pathogens. *Staphylococcus* sp., *Streptococcus* sp., *Mycoplasma* sp. and coliform bacteria are the common species in udder disease etiology. *S.aureus*, *S.agalactiae*, *S.dysgalactiae* and *S.uberis* are the causative agents for 95% of the mastitis and the other organisms are responsible for infections in a 5%. Essentially this situation is attributed to the ability of these groups of bacteria to form biofilms that improve their resistance mechanisms (Svensson et al., 2006; Smith, 1983). Streptococci (*S.uberis*, *S.bovis*, *S.dysgalactiae*), coliform (*E.coli*, *Klebsiella pneumoniae*, *K.oxytoca*, *Enterobacter aerogenes*) and enterococci (*Enterococcus faecium*, *E.faecalis*) bacteria species that are acquired from the environment such as soil, fertilizer, resting place, base, are the major causes of mastitis infections in cows (Neave et al., 1969). Clinical mastitis cases can occur before prenatal period 5% at the first week of lactation 30% and predominantly between 7.-30. days after birth more than 50% (Acar et al., 2012; Szweda et al., 2014). Studies on isolation and identification of mastitis agents and their resistance situation to antibiotics have been performed on cows, from different territories of Turkey, but no research is found in Yozgat province and districts. Aim of this study, to determine the prevalence of mastitis, bacteria associated with mastitis using the milk samples from the aforementioned cows and to investigate the antibiotic resistance of these bacteria.

Material and Methods

In the study, 238 milk samples were collected from different breeds of dairy cows raised in large and small family type milk producers located in Yozgat province and districts (Akdağmadeni, Boğazlıyan, Çekerek, Sorgun and Yerköy) between June and August in 2015. Fifteen milliliters of milk was collected in a sterile Falcon tube from udder after cleaning the teat orifice with 70% ethyl alcohol and discarding few streams of foremilk. Then the samples were brought to the Adnan Menderes University Faculty of Veterinary Medicine Department of Microbiology Laboratory with cold chain for the analysis. Milk samples were cultured in 5% sterile defibrinated sheep blood agar and left to incubation at 37°C for 24-48 hours. After incubation the suspected colonies were examined by Gram staining method with a light microscope and biochemical tests were performed

for identification of bacteria. The characteristic biochemical identification of bacteria was performed using catalase, coagulase, oxidase, nitrate reduction, DNA'seagar, clumping factor, hemolysis (in blood agar), arginine dihydrolase, lysine decarboxylase, urease, Methyl Red Voges Proskauer and OF tests. Also fermentation tests performed were intended for acid production from sucrose, maltose, D-mannitol, D-trehalose and raffinose. The isolates from mastitis milk samples were evaluated for their resistance and susceptibility by disk diffusion tests using amoxicillin (10 µg), gentamicin (10 µg), danofloxacin (5 µg), erythromycin (15 µg), enrofloxacin (5 µg), kanamycin (5 µg), penicillin G (10 µg) and oxytetracycline (30 µg) antibiotics. 0.1 ml from each isolate was poured to Mueller Hinton agar to obtain 10⁵ CFU/ml. Then, multi discs were left for incubation at 37°C over the course of 24 hours. Results were evaluated according to the criteria of Clinical Laboratory Standards Institute (CLSI) (Clinical Laboratory Standards Institute, 2002).

Results

Microbiological growth occurred in 174 (73.11%) of total 238 milk samples and no any growth occurred in remaining of 64 (26.89%) samples. Milk samples with bacterial growth, Gram positive (63) and Gram negative (111) bacteria were isolated at 36.21% and 63.79%, respectively. As a result of the isolation *Aeromonas hydrophila* 30 (17.24%), *Citrobacter diversus* 4 (2.29%), *Citrobacter freundii* 2 (1.14%), *Corynebacterium* sp. 24 (13.79%), *E.coli* 18 (10.34%), *Enterobacter cloacae* 2 (1.14%), *Flavobacter* sp. 3 (1.72%), *Lactobacillus* sp. 21 (12.06%), *Pasteurella pneumonia* 2 (1.14%), *Plesiomonas shigelloides* 11 (6.32%), *Providencia* sp. 2 (1.14%), *Salmonella* sp. 2 (1.14%), *Serratia liquefaciens* 2 (1.14%), *Shigella* sp. 18 (10.34%), *Vibrio cholerae* 6 (3.44%), *Yersinia enterocolitica* 1 (0.57%), coagulase-negative staphylococcus (CNS) 18 (10.34%) and *Staphylococcus aureus* 8 (4.59%) species have been identified. *A.hydrophila* is determined as the most abundant species with 17.24% (Table 1).

A.hydrophila (17/36, 47.22%) in Boğazlıyan and (8/24, 33.33%) in Akdağmadeni, *Corynebacterium* sp. (13/34, 38.23%) and *Lactobacillus* sp. (6/34, 17.64%) in Yerköy, CNS (7/35, 20%) and *P.shigelloides* (4/35, 11.42%) in Yozgat and *Shigella* sp. (9/24, 37.5%) in Sorgun were the most commonly isolated organisms in milk samples with mastitis. At the top of the list *E.cloacae* isolated in Akdağmadeni (1/24, 4.16%) and in Sorgun (1/24, 4.16%), *E.coli* isolated in Boğazlıyan (5/36, 13.88%) and in Yozgat (5/35, 14.28%), *Flavobacter* sp. isolated in Boğazlıyan (1/36, 2.77%), in Yozgat (1/35, 2.85%) and in Yerköy (1/34, 2.94%), *C.diversus* isolated in Çekerek (2/21, 9.52%), *C. freundii* isolated in Yerköy (1/34, 2.94%) in Çekerek (1/21, 4.76%), *S.aureus* isolated in Çekerek (3/21, 14.28%), *P. pneumonia* isolated in Çekerek (1/21, 4.76%) and in Yerköy (1/34, 2.94%), *Providencia* sp. isolated in Yerköy (1/34, 2.94%), *Salmonella* sp. isolated in Çekerek (1/21, 4.76%) and Yerköy (1/34 2.94%), *S.liquefaciens* isolated in Akdağmadeni (1/24, 4.16%) and in Boğazlıyan (1/36, 2.77%), *V.cholerae* isolated in Yozgat (6/35, 17.14%), *Y.enterocolitica* isolated in Boğazlıyan (1/36, 2.77%) when the microbiological growth considered all in all. Antibiotic resistances were measured in identified microorganisms using with amoxicillin, gentamicin, danofloxacin, erythromycin, enrofloxacin, kanamycin, penicillin G and oxytetracycline discs. Antibiotic resistances were determined in many strains to the antibacterial agents, respectively, were as follows: *A.hydrophila* (30%), *C.diversus* (75%) CNS (89%) and *S.aureus* (63%) for amoxicillin, *A.hydrophila* (57%) and *C.diversus* (100%) for gentamicin, *A.hydrophila* (53%), *C.diversus* (75%), *E.cloacae* (100%), *Flavoba-*

Table 1. The isolated and identified microorganism species in Yozgat province and around and distribution to counties.**Tablo 1.** Yozgat ili ve çevresinde izole ve identifiye edilen mikroorganizma türleri ve ilçelere göre dağılımı.

ORGANİZM	Total (n=174)	Akdağmadeni (n=24)	Boğazlıyan (n=36)	Çekerek (n=21)	Yozgat (n=35)	Sorgun (n=24)	Yerköy (n=34)
<i>A. hydrophila</i>	30	8	17	5	0	0	0
<i>C. diversus</i>	4	1	1	2	0	0	0
<i>C. freundii</i>	2	0	0	1	0	0	1
<i>Corynebacterium</i> sp.	24	2	3	2	2	2	13
<i>E. cloacae</i>	2	1	0	0	0	1	0
<i>E. coli</i>	18	3	5	2	5	1	2
<i>Flavobacter</i> sp.	3	0	1	0	1	0	1
CNS	18	0	2	3	7	3	3
<i>S. aureus</i>	8	0	2	3	0	2	1
<i>Lactobacillus</i> sp.	21	5	3	0	4	3	6
<i>P. pneumonia</i>	2	0	0	1	0	0	1
<i>P. shigelloides</i>	11	3	0	0	4	3	1
<i>Providencia</i> sp.	2	0	0	1	0	0	1
<i>Salmonella</i> sp.	2	0	0	1	0	0	1
<i>S. liquefaciens</i>	2	1	1	0	0	0	0
<i>Shigella</i> sp.	18	0	0	0	6	9	3
<i>V. cholerae</i>	6	0	0	0	6	0	0
<i>Y. enterocolitica</i>	1	0	1	0	0	0	0

cter sp. (100%), *P.pneumonia* (100%) and *S.aureus* (63%) for erythromycin, *C.freundii* (100%) for enrofloxacin, *Lactobacillus* sp. (72%), *C.freundii* (100%), *P.pneumonia* (100%), *P. shigelloides* (100%), *Providencia* sp. (100%), *Salmonella* sp. (100%) and *S. liquefaciens* (100%) for penicillin G (Table 2).

Discussion

Mastitis is an important inflammatory reaction of the udder tissue that causes potentially large losses in milk yield, milk quality and economy of dairy industry and in Turkey and all over the world. Mastitis infections account for 26% of total bovine diseases (Koneman et al., 1997). Many mastitis cases were determined as 21.4% clinical and 78.6% subclinical in our country. As a result of the studies on dairy cows in our country, possibility rate of subclinic mastitis was reported as 54.37% in Kırıkkale, 72.4% in Şanlıurfa, 23.0% in Konya, 15.78% in Kars, 28.21% in Hatay and 8.2% in Elazığ (Ateş et al., 1991; Ergün et al., 2004; Gülcü and Ertaş, 2004; Macun et al., 2011; Şahin et al., 1997; Tel et al., 2009). In this study, we determined mastitis as 73.11% and this rate was in close proximity of the study in Şanlıurfa, however the prevalence was higher compared to the studies carried out in other cities in Turkey. The mastitis causing microorganisms in Turkey were reported in literature. Tel et al. (2009) reported as 27.5% CNS followed by 8.9% *Streptococcus* sp., 6.2% *E.coli*, 3.1% *C.bovis*, 1.9% *E.aerogenes*, 1.5% *P.multocida*, 1.5% *C.diversus* species in Şanlıurfa and Çokal and Konuş (2012) reported as 17.1% CNS followed by 12.0% *E.aerogenes*, 9.4% *E.coli*, 8.5% *Streptococcus* sp., 5.1% *Citrobacter* sp. species in Balıkesir and Ateş et al. (1991) reported as 16.3% *C. pyogenes* and 8.2% *E. coli* species in Konya and Yeşilmen et al. (2012) reported as 8.58% *E.coli* and 3.73% *E.cloacae* species in Diyarbakır. In this study we determined the mastitis causing agents as 10.34% CNS, 10.34% *E.coli*, 13.79% *Corynebacterium* sp., 1.14% *E. cloacae*, 1.14% *P. pneumonia* and 2.29% *C. diversus*. *E.coli* and CNS findings showed approximately the same

value with the results of obtained in other subclinical mastitis studies but other isolated pathogens were determined in regional diversity. *E.coli* (10.34%) was the most encountered infection in Boğazlıyan (13.8%) and Yozgat (14.28%). Also CNS (20%) and *Corynebacterium* sp. (38.23%) were the most frequently isolated pathogen in Yozgat and Yerköy, relatively. There were not any literature scans according to the etiology of subclinical mastitis in Yozgat and the districts, but it has been thought to be parallel in studies which are done in another provinces and districts. Many factors may have a direct or indirect influence on the onset of mastitis such as collecting milk samples from neglected small type of family barns, inadequate teats dipping after milking, using obsolete milking equipment, insufficient knowledge of farm workers about milking hygiene. We isolated *Shigella* sp. (37.5%) in Sorgun and *A.hydrophila* (47.22%) in Boğazlıyan districts that we attributed these results to these factors. A wide spectrum of pathogenic mastitis agents can be resistant to one or more antibiotics that may lead to many difficulties in treatment of the disease. Macun et al. (2011) reported that the isolates from milk samples collected in Kırıkkale were resistant to penicillin (27.14%) followed by amoxicillin (16.43%), erythromycin (3.57%), gentamicin (2.14%), tetracycline (2.14%), enrofloxacin (1.43%) and danofloxacin (0.71%). In this study we determined that isolates were resistant to penicillin (25.60%) followed by amoxicillin (18.36%), enrofloxacin (2.42%), erythromycin (30.43%), gentamicin (11.59%), tetracycline (1.93%) and danofloxacin (3.86%). We have concluded that excessive using of penicillin G and amoxicillin caused resistance in the geographically close regions. Also, we observed the high resistance to gentamicin and erythromycin as a result of widespread utilization in Yozgat. Makovec and Ruegg (2003) reported that the resistance to penicillin has regressed from 6% to 1% in *Streptococcus* sp. isolates. We determined the resistance to penicillin G as 4% and this value falls within of normal antibiotic usage range in districts. Dinç et al. (2012) repor-

Table 2. The antibiotic resistance percentages of identified microorganism strains.**Tablo 2.** İdentifiye edilen mikroorganizma türlerinin antibiyotik direnç yüzdeleri.

ORGANISM	ANTIBIOTIC RESISTANCE (%)							
	AML	CN	DFX	E	ENR	K	P	OT
<i>A. hydrophila</i> (n=30)	30	57	3	53	0	13	13	0
<i>C. diversus</i> (n=4)	75	100	0	75	0	0	0	0
<i>C. freundii</i> (n=2)	0	0	100	0	100	0	100	0
<i>Corynebacterium</i> sp. (n=24)	0	10	5	10	5	10	5	5
<i>E. cloacae</i> (n=2)	0	0	0	100	0	0	0	0
<i>E. coli</i> (n=18)	0	0	0	47	0	6	29	0
<i>Flavobacter</i> sp. (n=3)	0	0	0	100	0	0	0	0
CNS (n=18)	89	0	0	39	0	6	17	6
<i>S. aureus</i> (n=8)	63	0	0	63	0	0	0	0
<i>Lactobacillus</i> sp. (n=21)	28	0	0	22	6	0	72	6
<i>P. pneumonia</i> (n=2)	0	0	0	100	0	0	100	0
<i>P. shigelloides</i> (n=11)	0	0	36	18	9	18	55	0
<i>Providencia</i> sp. (n=2)	0	0	0	0	0	0	100	0
<i>Salmonella</i> sp. (n=2)	0	0	0	0	0	0	100	0
<i>S. liquefaciens</i> (n=2)	0	0	0	0	0	0	100	0
<i>Shigella</i> sp. (n=18)	0	0	0	28	6	0	39	6
<i>V. cholerae</i> (n=6)	0	0	0	0	0	0	0	0
<i>Y. enterocolitica</i> (n=1)	0	0	0	0	0	0	0	0

AML; amoxicillin, CN; gentamicin, DFX; danofloxacin, E; erythromycin, ENR; enrofloxacin, K; kanamycin, P; penicillin G, OT; oxytetracycline.

ted that *E.coli* strains were resistant to two or more numbers of antibiotics. We also found that *E.coli* being resistant to two or more antimicrobials. In the present study, the highest resistance was determined against erythromycin (36.20%) followed by penicillin G (30.45%), amoxicillin (21.83%) and gentamicin (13.21%). In all isolates, we determined that *E.coli*, CNS, *Corynebacterium* sp., and *A.hydrophila* respectively had resistance to erythromycin (13.00%), amoxicillin (42.00%), oxytetracycline (25.00%), gentamicin (74.00%) and kanamycin (17.00%).

As a result, the microorganisms isolated from subclinical mastitis in Yozgat and districts were mostly resistant to erythromycin and other resistance rates showed difference according to agent type. It is suggested that this study will make a significant contribution to the future research on antibiotic resistance and etiology of subclinical and clinical conditions of mastitis in Yozgat and districts. Finally, we found a high incidence of mastitis in Yozgat and districts, therefore producers in dairy cow industry should be informed about the milking hygiene.

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