Research Article

Isolation of Microorganisms from Goats with Subclinical Mastitis and Detection of Antibiotics Susceptibility

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ABSTRACT

In this study, microorganisms were isolated and identified to 102 (67.1%) samples from 152 hair goat’s milk. There was not obtained any bacterial isolation from the remaining 50 (32.9%) samples. Our of identified 102 samples were identified respectively; S. aureus from 71 (69.6%) samples, S. epidermidis from 8 (7.8%) samples, S. intermedius from 5 (4.9%) samples, S. hyicus from 6 (5.9%) samples, Corynebacterium sp. from 3 (2.9%) samples, Klebsiella pneumoniae from 4 (3.9%) samples, Pseudomonas sp. from 2 (2.0%) samples, E. coli from 2 (2.0%) samples and Mannheimia haemolytica from 1 (1.0%) sample. As a result of antibiotic susceptibility tests, S. aureus isolates were found susceptible to Amoxycillin-Clavulanic Acid in the ratio of 100%, susceptible to Penicilin in the ratio of 100%, resistant to Kanamycin and Oxacillin ratio of 90%. The other Staphylocci isolates were found susceptible to Amoxycillin-Clavulanic Acid in the ratio of 100%, resistant to Penicilin ratio of 100%. Other identified isolates Corynebacterium sp., Klebsiella pneumoniae, Pseudomonas sp., E. coli and Mannheimia haemolytica were found susceptible to Ampicillin in the ratio of 100%, susceptible to Amoxycillin-Clavulanic Acid in the ratio of 90%, resistant to Penicilin and Kanamycin in the ratio of 100%.

Keywords: Goat, Mastitis, Microorganism, Isolation, Identification

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Introduction

The Turkey takes the 2nd place for production of goat milk in the world. Goat milk forms 12% of our annual milk production according to FAO’s year 1980’s data, 10% according to national statistics and 14-15% according to the researchers and goat milk plays an important role for Turkey economy.

Goat milk is gaining importance in various societies for easy management and feeding of goats and the healthy composition of goat milk. Nowadays in America, France, China and in several European and developing countries, goats are bred professionally for their milk and these milk obtained from goats are sold for high prices by milk products of cheese, yoghurt, cream, butter. Especially in Italy and France, cheese production is developed as another industry branch. The cheese made by only goat milk is valuable and various cheese types are worldwide known (Appleman, 1983; Tao, 1983). In Turkey goats have a potential value for production of meat, milk, fiber and leather and many people make money by breeding goats. In some regions of our country, goat milk and meat is consumed fondly (Yaşlı, 1986).

Goat milk is similar with cow milk because of its composition. It is a good nutrition source for milking babies and gastric patients because its fatty globulins are small. Goat milk is similar with cow milk for its nutritive value. By pointing on different goat breeds, goat milk may have higher fat and dry substance than cow milk. The obtained milk from a high milk capacity goat is approximately 1000 liter in a lactation period of 6-10 months. In some goat breeds in Russia, 1700 liters of milk could be obtained from one goat annually (Yaşlı, 1986).

Milking is the most important issue of milk goat breeding. The intensive care for milking alters the quality of milk and affects the animal health. In our country, goat milking is performed manually with hands in herds (Baştan, 2002; Christensen ve ark, 2003).

Mastitis is generally the inflammation of mammary gland by bacterial or mycotic pathogen agents. The traumatical, pathological and bacteriological changes in goat mammary glands causes the physical and chemical changins in milk and causes mastitis (Shearer and Harris, 2003).

There are many microorganisms that cause mastitis in goats. Unappropriate milking techniques and unsuitable hygiene conditions increases the infections. In most herds, the most important mastitic pathogen is Staphylococcus aureus. Gangrene should be seen in severe cases. Bubbles in milk secretion should be seen with the affiliation of gas producing bacteria. Death should be formed suddenly or in several days according to severity of infection. Some animals should be treated by suspension of necrotic tissue (Shearer and Harris, 2003).

Some Streptococci species (S. agalactia, S. uberis and S. dysgalactia) are generally isolated from infected mammary gland. Mannheimia hemolytica is thought to be transmitted by orally from milking animals to mammary gland. Corynebacterium pseudotuberculosis is isolated from herds with abscess formed mammary tissue inflammations (Shearer and Harris, 2003).

In European countries, mastitis is not a problem for breeder if the human health is taken under guarantee with hygienic and safe production (Shearer and Harris, 2003).

Coagulase negative Staphylococci are found generally in mammary gland and cannal of goats. They cause subclinical infections in goat’s milk in spite of dry period (Poutrel, 1984; East et al., 1987).

The pathogens found generally in goat mammary are coagulase negative Staphylococci (Poutrel and Lerondelle, 1984; Maisi et al., 1987; De La Cruz et al., 1994; Ethenakis, 1994). The bacteria isolated from dairy goats are generally S. aureus. In the studies, S. aureus is rarely isolated from subclinical intramammary infections (Kalogridou-Vassiliadou 1991, Deinhofer and Pernthaner 1995, Contreras et al. 1997). The bacteria isolated from subclinical mastitis cases are S. aureus in the ratio of 37%.

In animals, coagulase negative Staphylococci are isolated from chronic and subclinical intramammary infections. Staphylococcus haemolyticus is an important strain found in coagulase negative Staphylococci. Other strains are S. epidermidis, S. xylosus, S. chromogenes, and S. simulans (Bachmann and Spahr, 1995).

The increase of coagulase negative Staphylococci infections causes loss of capacity and damage of mammary gland (Poutrel et al., 1997; Burriel, 1997; Gonzalo et al., 2002). With this situation, the increase rate of disease continues as intramammary infection for the later lactation period (Larondelleand and Poutrel, 1984; Poutrel, 1984; Watson and Buswell, 1984).

In this study, the subclinical mastitis infections and the antibiotic susceptibility of isolated microorganisms of hair goats found in Aydın region is aimed to be cleared.

Materials and Methods

The teat of goats were disinfected with disinfectants and dried with clean towel. Gloves were worn after disinfection of hands and milk sample of goats were taken out of mammary cannal 3-4 times. And then with 45 degree gradient, milk was milked into sterile tubes (Blowey, 2001). Being 78 samples from Bozdoğan district, 27 samples from Çine district, 47 samples from Karacasu district; in sum of 152 milk sample was taken under cold chain and brought to Adnan Menderes University Faculty of Veterinary Medicine Microbiology Laboratory and were started to examine.
The samples were inoculated to 5% sheep blood agar (Difco) and McConkey agar and incubated at 37°C for 24-48 hours. After that, the morphology, pigmentation and haemolysis of grown colonies were investigated with Gram’s staining method. The identification was made to susceptible colonies according to criteria below.

Gram negative colonies were inoculated to Lassen’s triple tube media (Lassen 1975). The tubes were incubated at 37°C for 24 hours. After incubation, tubes were evaluated and identification of Gram negative strains were performed (Holt et al. 1994, Koneman et al., 1997).

Gram positive strains were performed with catalase reaction by 3% H₂O₂ and positive microorganisms were evaluated as Micrococcaceae family (Koneman et al. 1997).

The microorganism that will be inspected were grown in 1 ml tryptic soy Broth (Oxoid) and inoculated to Mueller-Hinton agar. Bacitracin disc (Oxoid) (0.04 U/ml) were placed onto inoculation zone. After incubation at 37°C for 18 hours, the resistant strains to discs were evaluated as *Staphylococci* (Koneman et al., 1997).

Coagulase test with 1/5 diluated citrated rabbit plasm were performed to the strains seperated as *Staphylococci* and the strains were seperated as coagulase positive and negative.

Coagulase positive *Staphylococci*, were identified as *S. aureus* and *S. intermedius* according to urease activity, hemolysin, mannitol, oxidase and DNAse activity (Koneman et al., 1997; Holt et al., 1994).

Coagulase negative *Staphylococci*, were identified as *S. aureus* and *S. intermedius* according to urease activity, hemolysin, mannitol, oxidase and DNAse activity (Koneman et al., 1997; Holt et al., 1994).

The cultural and biochemical properties of coagulase positive *Staphylococci* strains are shown in Table 2.

### Antibiotic Susceptibility Test

For antibiotic susceptibility tests, Mueller-Hinton Agar (Difco) was used with Kirby-Bauer Disc Diffusion Method (Bauer et al., 1966; Qoronfleh and Wilkinson 1986).

The prepared Mueller-Hinton media were poured into 10 cm diameter plates with 4 mm of thickness and left to freeze. The 0.5 McFarland broth cultures of Staphylococci were inoculated to media and discs were placed after drying of media surface by heat sterilized forceps with 1.5 cm intervals.

The used antibiotic discs and their ingredients are: Amoxicillin- Clavulanic Acid (Oxoid, AMC-10 µg), Oxacillin (OX-5 µg), Kanamycin (K-30 µg), Penicillin (P-10 IU), Ampicillin (AMP-10 µg), Vancomycin (VA-30 µg), Erythromycin (E-15 µg) ve Sulphametaxazol-Trimetoprim (SXT-25 µg).

The plates were incubated at room temperature for 15 min. The inhibition zone diameters were calculated (Bauer et al., 1966; Qoronfleh and Wilkinson, 1986).

### Results

In this study, bacterial isolation was made from 102 (67.1%) of milk sample taken from subclinical mastitic milk of goat that were brought to Adnan Menderes University Faculty of Veterinary Medicine Microbiology Laboratory out of 152 milk samples. No isolation was detected from remaining 50 (32.9%) samples.
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71 (69.6%) S. aureus, 8 (7.8%) S. epidermidis, 5 (4.9%) S. intermedius, 6 (5.9%) S. hyicus, 3 (2.9%) Corynebacterium sp., 4 (3.9%) Klebsiella pneumoniae, 2 (2.0%) Pseudomonas sp., 2 (2.0%) E. coli and 1 (1.0%) Mannheimia haemolytica was identified out of identified 102 samples.

For identified 102 samples, 76 (74.5%) of Staphylococci strains were detected as coagulase positive and 14 (13.7%) of them were detected as coagulase negative.

As the result of antibiogram tests, S. aureus strains showed 100% resistancy against Amoxicillin-Clavulanic Acid, 85% resistancy against Ampicillin and Vancomycin, 65% resistancy against Erythromycin, 60% resistancy against Sulphametaxazol-Trimethoprim, 100% resistancy against Penicilin, 90% resistancy against Kanamycin and Oxacin. It is significant that 11 (15.4%) of S. aureus strains showed resistancy against Vancomycin.

The other isolated Staphylococci strains showed 100% susceptibility against Amoxicillin-Clavulanic Acid, 85% susceptibility against Vancomycin, 75% susceptibility against Ampicillin, 60% susceptibility against Erythromycin and Sulphametaxazol-Trimethoprim, 100% resistancy against Penicilin, 85% resistancy against Kanamycin and Oxacin.

For the identified Corynebacterium sp., Klebsiella pneumoniae, Pseudomonas sp., E. coli and Mannheimia haemolytica strains were found susceptible to Ampicillin in the ratio of 100%, Amoxicillin-Clavulanic Acid in the ratio of 90%, were found susceptible to Vancomycin in the ratio of 85%, were found susceptible to Erythromycin in the ratio of 65%, were found resistant to Penicilin and Kanamycin in the ratio of 100%, were found resistant to Oxacin in the ratio of 90%, were found resistant to Sulphametaxazol-Trimethoprim in the ratio of 85%. The inhibition zone diameters of antibiotics used in the study are shown in Table 3.

The distribution of identified microorganisms by districts is shown in Table 4.

Discussion

Staphylococcus genus is the most of all agents that causes mastitis in goats. Staphylococci are the genus take place in Micrococcaecae family and they have been researched in medical investigations for years (Archer, 1990). Coagulase Negative Staphylococci were evaluated as saprophytes and unimportant, but in recent years, they are being evaluated as very important infectious agents (Abigail and Dixie, 1994; Jawetz et al., 1987; Gur et al., 1998; Ulusoy et al., 1995; Töreci et al., 1985).

S. aureus is an important pathogen but in subclinical mastitis, coagulase negative Staphylococci take place (Castro, 1992; Lima Júnior et al., 1993; Contreras et al., 1995; Contreras et al., 1999; Bedidi-Madani et al., 1998).

In coagulase negative Staphylococci, the most isolated strains are S. epidermidis, S. xylosus, S. chromogenes and S. simulans. After these strains, another strain found in goats is S. caprae.

In addition, both coagulase negative Staphylococci and S. caprae is associated with S. epidermidis in point of

Table 2. The cultural and biochemical properties of coagulase negative Staphylococci strains (Holt et al. 1994).

<table>
<thead>
<tr>
<th>Tests</th>
<th>S. hyicus</th>
<th>S. epidermidis</th>
<th>S. haemolyticus</th>
<th>S. sciuri</th>
<th>S. lentis</th>
<th>S. cohnii subsp. cohnii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>v</td>
<td>v</td>
<td>v</td>
</tr>
<tr>
<td>D-mannitol</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>+</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>D-Trehalose</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>weak +</td>
<td>v</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Arginin hydrolyse</td>
<td>+</td>
<td>weak +</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Urease</td>
<td>d</td>
<td>+</td>
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<tr>
<td>Coagulase</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Clumping factor</td>
<td>-</td>
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<td>unknown</td>
<td>weak +</td>
<td>weak +</td>
<td>weak -</td>
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<tr>
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<td>weak -</td>
<td>unknown</td>
<td>weak +</td>
<td>weak +</td>
<td>weak -</td>
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<tr>
<td>Novobiocin resistancy</td>
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<td>-</td>
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<td>+</td>
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</tr>
</tbody>
</table>

Table 3. The inhibition zone diameters of antibiotics used in the study.

Table 4. The distribution of identified microorganisms by districts.
somatic cell scores. Coagulase negative *Staphylococci* isolated from intramammary infections show alpha, beta and synergistic haemolysis (Bergonier et al. 2003).

In our study, bacterial isolation was made from 102 (67.1%) of milk sample out of 152 milk samples. No isolation was detected from remaining 50 (32.9%) samples.

Out of 102 samples, 71 (69.6%) *S. aureus*, 8 (7.8%) *S. epidermidis*, 5 (4.9%) *S. intermedius*, 6 (5.9%) *S. hyicus*, 3 (2.9%) *Corynebacterium sp.*, 4 (3.9%) *Klebsiella pneumoniae*, 2 (2.0%) *Pseudomonas sp.*, 2 (2.0%) *E. coli* and 1 (1.0%) *Mannheimia haemolytica* was identified.

In a study made with 478 goats in Southeast of Bulgaria, subclinical mastitis was detected in 60 and 100 patient animals. Ninety six *Staphylococcus* strains were isolated in positive milk samples. After isolation, 19 (19.8%) strain were detected as *S. aureus*, other 77 (80.2%) strains were detected as Coagulase negative *Staphylococci* (Bochev and Russenova 2005).

In a farm found in Italy, 156 goats were investigated during lactation period and it was seen that infection caused by coagulase negative *Staphylococci* nearly 80.7% of them. In the first herd, nearly all (96%) infection sources were found as coagulase negative *Staphylococci*. *Staphylococcus caprea* caused 43% of the infections. In the second herd, the infections were caused by coagulase negative *Staphylococci* in the ratio of 67% and the most general pathogen agent was found as *Staphylococcus epidermidis* (Moroni et al. 2005).

Vale et al. (1990) found that staphylococcal enterotoxins exist in *Staphylococcus* strains isolated from healthy goats, and these enterotoxins are important reservoirs.

In our country, there are many studies found about mastitis. Kaya et al. (1993) reported *S. aureus* in the ratio of 39.40% from clinical and subclinical mastitis samples. Plus, Erganis et al. (1995), serotyped 26 of 55 strains as *S. aureus* and 28 of 55 strains as coagulase negative *Staphylococci* in the study made in Konya region for cow and sheep mastitis. In the same study, biotyping of coagulase negative *Staphylococci* was performed too.

In a slaughterhouse found in Elazığ region, 113 mammary lobes owed to 89 sheep and 67 mammary lobes owed to 40 goats were investigated bacteriologically. In the sheep, *Staphylococcus aureus* were isolated in the ratio of 24.06%, *Escherichia coli* were isolated in the ratio of 10.53%, *Actinomyces pyogenes* were isolated in the ratio of 7.52%, *Streptococcus uberis* were isolated in the ratio of 6.01%, *Streptococcus dysgalactiae* were isolated in the ratio of 5.26%, *Staphylococcus epidermidis* were isolated in the ratio of 3.76%, *Mannheimia haemolytica* were isolated in the ratio of 3.76%.

In the goats, in the ratios of 25.37% *Staphylococcus aureus*, 8.96% *Escherichia coli*, 7.46% *Staphylococcus epidermidis*, 7.46% *Streptococcus agalactiae*, 7.46% *Actinomyces pyogenes*, 5.97% *Streptococcus dysgalactiae*, 2.99% *Streptococcus uberis*, 2.99% *Mannheimia haemolytica* was isolated and identified (Gülçü ve Öngör, 2002).

The prophylactic precautions should be taken before milking for sheep and goat mastitis in order to prevent and treat mastitis (Poutrel et al. 1997, Menzies and Ramanoon 2001). Antibiotic therapy is advised for lessening the mastitis infections (Brito and Brito 1998).

With this situation, random used antibiotics may cause the development of resistancy and growth of bacteria may continue. By this reason, random antibiotics should not be used too often (Contreras et al 1995).

In a study by Kuyucuoğlu and Uçar (2001), 152 (92.6%) aerobic bacteria was isolated out of 164 milk samples, and no isolation was seen in 12 (7.3%) samples. 62 (40.1%) of the samples were *S. aureus*, 22 (14.4) of the samples were *S. epidermidis*, 14 (9.2%) of the samples were *S. agalactia*, 6 (3.9%) of the samples were *S. uberis*, 7 (4.6%) samples were *S. dysgalactia*, 5 (3.2%) of the samples were *Acinetobacter spp.*, 3 (1.9%) of the
samples were C. bovis, 7 (4.6%) of the samples were E. coli, 6 (3.9%) of the samples were Micrococcus spp., 4 (2.6%) of the samples were A. pyogenes, 4 (2.6%) of the samples were Enterobacter spp., 2 (1.3%) of the samples were Bacillus spp., 1 (0.6%) of the samples were Pseudomonas aeruginosa, and 9 (5.9%) of the samples were identified as Candida spp. The most effective antibiotics against isolated microorganisms were Amoxicillin-Clavulanic Acid, Ampicillin-Sulbactam, Enrofloxacin, Danofloxacin and Cefaperazone. Different ratios of resistancy were developed against Penicilin G, Erythromycin and Streptomycin and Nystatin were found to be the most effective agent against Candida spp. in the ratio of 77.7%.

In a study by Moroni et al. (2005), the mastitis infections are caused by Coagulase negative Staphylococci in goats in the ratio of 80.7%. The result of this study showed that Benzylpenicillin is a very effective antimicrobial agent against Coagulase negative Staphylococci. Amoxicillin-Clavulanic Acid, Tetracycline and Tilmicocin come after.

As a result of our study, S. aureus strains showed 100% resistancy against Amoxicillin-Clavulanic Acid, 85% resistancy against Ampicillin and Vancomycin, 65% resistancy against Erythromycin, 60% resistancy against Sulphametaxazol-Trimethoprim, 100% resistancy against penicilin, 90% resistancy against Kanamycyn and Oxacillin. It is significant that 11 (15.4%) of S. aureus strains showed resistancy against Vancomycin.

The other isolated Staphylococci strains showed 100% susceptibility against Amoxicillin-Clavulanic Acid, 85% susceptibility against Vancomycin, 75% susceptibility against Ampicillin, 60% susceptibility against Erythromycin and Sulphametaxazol-Trimethoprim, 100% resistancy against penicilin, 85% resistancy against Kanamycyn and Oxacillin.

For the identified Corynebacterium sp., Klebsiella pneumoniae, Pseudomonas sp., E. coli and Mannheimia haemolytica strains were found susceptible to Ampicilin in the ratio of 100%, Amoxicillin-Clavulanic Acid in the ratio of 90%, were found susceptible to Vancomycin in the ratio of 85%, were found susceptible to Erythromycin in the ratio of 65%, were found resistant to Penicillin and Kanamycyn in the ratio of 100%, were found resistant to Oxacillin in the ratio of 90%, were found resistant to Sulphametaxazol-Trimethoprim in the ratio of 85%.

With this study, the subclinical mastitis infections and the antibiotic susceptibility of isolated microorganisms of hair goats found in Aydin region is performed with 152 milk samples. For identification of 102 samples that were seen bacterial growth, 71 (69.6%) S. aureus, 8 (7.8%) S. epidermidis, 5 (4.9%) S. intermedius, 6 (5.9%) S. hyicus, 3 (2.9%) Corynebacterium sp., 4 (3.9%) Klebsiella pneumoniae, 2 (2.0%) Pseudomonas sp., 2 (2.0%) E. coli and 1 (1.0%) Mannheimia haemolytica was identified. No isolation was detected from remaining 50 (32.9%) samples. By the antibiogram tests, S. aureus strains showed 100% resistancy against Amoxicillin-Clavulanic Acid, 85% resistancy against Ampicilin and Vancomycin, 65% resistancy against Erythromycin, 60% resistancy against Sulphametaxazol-Trimethoprim, 100% resistancy against Penicilin, 90% resistancy against Kanamycyn and Oxacillin.

As a result, with the leadership of these data, it is seen that subclinical mastitis effects the milk capacity and quality, and the studies about diagnosis and therapy of subclinical mastitis will help the goat breeders in the future.

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