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Aerobic Bacteria Associated With Subclinical Mastitis in Ewes and Goats in River Nile State, Sudan

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Abstract

In this study a total of 80 milk samples positive for California Mastitis Test (CMT) were collected. Forty samples were collected from ewes and 40 from goats. These samples were obtained from the three localities of River Nile State (Atbara, Barbar and El Damer). Samples were submitted for bacteriological examination and isolates were subjected for further confirmation by using API staph, API strep20, Api coryne and Api20E identification rapid systems. 90 bacterial isolates were isolated from milk samples. The aerobic bacteria isolated and identified from ewes' milk samples were 19 Staphylococci (21.1%), 9 Streptococci (10.0%), 5 Echerichia coli (5.6%), 3 Corynebacterium bovis (3.3%), 3 Enterobacter spp. (3.3%), 2 Klebsiella pneumoniae (2.2%), 2 Enterococcus faecalis (2.2%), 2 Actinomyces pyogenes (2.2%), , 1 Micrococcus varians (1.1%), and 3 Serratia spp. (3.3%). The aerobic bacteria isolated and identified from goats' milk samples were 19 Staphylococci (21.1%), 7 Streptococci (7.8%), 4 Echerichia coli (4.4%), 4 Corynebacterium bovis (4.4%), 4 Enterococcus faecalis (4.4%), and 3 Actinomyces pyogenes (3.3%). 80% of bacteria isolated from ewes' samples were also isolated from goats' milk samples. This may indicates that, the same aetiology may be implicated in the cases of subclinical mastitis in ewes and goats.

Introduction

Mastitis is one of the more common health problems affecting sheep and goats. Severe cases can result in death of the ewe, but more often it takes its toll in the form of treatment costs, premature culling, and reduced performance of lambs and kids [1]. Milk yield losses and increased MSCC in infected goat and sheep udders have been widely documented [2, 3, 4], and it appears that sheep are more vulnerable than goats to milk yield losses due to subclinical mastitis [5]. The most important differences between goats and sheep affecting diagnosis of mastitis are related to the somatic cell count (SCC). These differences are mainly due to the higher SCC in uninfected goat halves, the higher apocrine component of goat milk secretion and the larger number of non-infectious factors that can increase the SCC of goats compared to sheep [6]. Today, most dairy laboratories use SCC methods that are adequate for the apocrine pattern of milk from small ruminants, especially goats. However, given that the SCC is an indicator of milk quality and that bonus/penalty schemes for the dairy manare based on

the bulk tank SCC, it is important that the SCC is as accurate as possible.

The bacteria which are known to cause mastitis in cows, sheep and goats are *Streptococcus sp., Staphylococcus sp., Pasteurella sp.*, and coliforms, such as *E. coli*. The most commonly isolated CNS species in persistent subclinical in goats and sheep are *Staphylococcus epidermidis, S. caprae, S. simulans, S. chromogenes* and *S. xylosus* [3, 7, 8]. Intramammary infections caused by *S. aureus*warrant special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and subclinical mastitis.

[9] found that the *S. aureus* isolates from sheep with subclinical mastitis are less enterotoxigenic (34.4%) than isolates from acute clinical mastitis (70–80%). In an investigation of the leukotoxic actions of *S. aureus* strains isolated from cows, sheep and goats with mastitis [10] found that most isolates were leukotoxic and that strains isolated from small ruminants were more leukotoxic towards bovine polymorphnuclear leukocytes (PMN) than *S. aureus* strains of bovine origin. However, several pathogens can cause mastitis but *Staphylococcus* spp. are the most frequently diagnosed causal microorganisms of mastitis in goats and sheep. Other pathogens such as *Streptococcus* spp., *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Mannheimia haemolytica*, *Corynebacteria* and fungi can produce mastitis in small ruminants, but occurrence rates are lower. In addition, severe cases of mastitis related to incorrect preventative strategies have been attributed to the pathogens *Aspergillus fumigatus*, *Serratia marcescens, P. aeruginosa* or *Burkholdelia cepacia* [11, 12, 13, 7, 14].

In Sudan several agents were isolated from cases of subclinical mastitis, these include: *S. aureus, S. epidermidis, Corynebacterium spp, Pseudomonas spp, Str. agalactiae, Str. dysagalactiae*, and *Micrococcus spp* [15]. High incidence of subclinical mastitis was reported in Khartoum and commonest species of bacteria isolated were: *Enterococcus faecalis, Enterococcus faecium, Str. bovis, Str. equi subsp equi, Lactococcus lactis* and *Str. pyogene*s [16].

Materials and Methods

Area of the Study

A total of 80 milk samples positive for CMT were collected from ewes and goats in River Nile State. 40 ewes' milk samples and 40 goats' were collected from Barbar, Omer Amir farm, El Damer Vet Hospital, Abdelghafarm farm, Food safety Center, Atbara vet hospital, Goats Improving Center-Adamer and Akram farm (table 1).

Sampling Procedure

Before collection of milk samples from the tested cows, the udder was thoroughly cleaned with soap and water, rubbed dry, and the teat area was rubbed thereafter with a piece of cotton soaked in 70% alcohol . The first stream of milk was discarded. The California Mastitis Test was directly applied for quarter's milk and samples were collected from positively reacted milk into sterile bottles.The collection of samples was at (2-5) pm. The collected samples were put in ice box containing ice and transported to the laboratory. In most cases the time between collection and arrival to the laboratory was 1-2 hrs. In the laboratory mastitic milk samples were kept in a deep-freeze. All samples were examined on the next day. On the next day mastitic milk samples were removed from the deep–freezer and left on the bench to thaw. Samples were then cultured.

Isolation, Identification and Characterization of Bacterial Isolates

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of bacteria, a loop full milk sample was streaked onto blood agar, McConkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37oC for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in CHO media and for sediment formation. One half colony from each plate was used for performing gram staining. Colonies which showed Gram positive cocci were sub cultured on nutrient agar. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at $4C^{o}$.

Biological and Biochemical Identification

The purified isolates were identified as previously described [17] and [18]. The identification include: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coaggulase test, oxidation fermentation test, motility test, glucose breakdown test, fermentation of carbohydrates, urease activity, citrate utilization, gelatin hydrolysis test, nitrate reduction test).

Identification of Isolated Bacteria to Species Level

According to [19] API staph (Analytical Profile Index for identification of the Genus *Staphylococcus*) is a standardized system for the identification of the Genera: *Staphylococcus*, *Micrococcus* and *Kocuria*, which uses miniaturized biochemical tests and specially adapted database.

Pure staphylococci isolates were sub cultured on blood agar and incubated at 36oC ± 2 for 18–24 hours. The identification test of staphylococci isolates was conducted according to the manufacturer BIOMERIEUX protocol. Homogeneous bacterial suspension was obtained by using API staph medium. Both tubes and cubules of API staph were filled with the inoculated API staph media. Anaerobiosis was ensured in the ADH, LDC, ODC, URE and H2S tests by filling the cubules with sterile mineral oil to form a convex meniscus. The incubation boxes were closed and incubated at 36oC ± 2 for 18–24 hours. Identification was obtained according to the numerical profile of API staph.

According to [20], isolated bacteria were identified to species level by using API micro-systems. API systems used were: API staph system for identification of Genus: *Staphylococcus*, API strep 20 system for identification of Genus: *Streptococcus*, API coryne system for identification of Genus: *Corynebacterium* and API 20E system for identification of Gram-negative bacteria.

Identification steps (1. Preparation of the strips 2. Preparation of the inoculum 3. Inoculation of the strips 4. Reading of the strips), were the same in all isolated pure cultures of bacteria expected.

In case of API coryne system in step2 dense bacterial suspension with a turbidity greater than 6 Mc Farland was prepared in API GP medium, in step3 sterile mineral oil was added to URE (Urease test), 0 (Negative control), GLU (D-glucose test), RIB (D-ribose test), XYL (Xylose test), MAN (Mannitol test),

MAL (Maltose test), LAC (Lactose test), SAC (Saccrose test) and GLYG (Glycogen test) cupules and in step4 one drop of NIT1 and NIT2 reagents were added to NIT (Nitrate test) cupule, one drop of PYZ reagent was added to PYZ (Pyrazinamidase test) cupule and one drop of ZYM A and ZYM B reagents were added to PAL (Alkaline Phosphatase test), PYRA (Pyrolidonyl Arylamidase test), β-GUR (β-Glucuronidase test), β-GAL (β-Galactosidase test), α-GLU (α-Glucosidase test) and β-NAG (N-acetyl-β-Glucosaminidase test) cupules.

In case of API strep20 system in step2 organisms of β-haemolysis were incubated anaerobically and haemogenous bacterial suspension was prepared in API strep20 medium, in step3 sterile mineral oil was added to ADH (Arginin test) cupule and in step4 one drop of VPI1 and VP2 reagents were added to VP (Voges-Proskauer test) cupule and ZYM A and ZYM B reagents were added to PAL (Alkaline Phosphatase test) cupule.

In case of API 20E in step2 a single well isolated colony of bacterium to be identified was made into a homogenous suspension in 5mlof sterile distilled water, in step3 sterile mineral oil was added to ADH (Arginin test), LDC (Lysine test), ODC (Ornithine test), URE (Urease test) and H2S (Na thiosulphate test) cupules and in step4 one drop of TDA reagent was added to TDA (Tryptophane test) cupule, IND reagent was added to IND (Indol test) cupule, one drop of VP1 and VP2 reagents were added to VP (voges-Proskauer test) cupule and one drop of NIT1 and NIT2 reagents were added to NIT test cupule.

Statistical Analysis

Statistical analysis was done through Microsoft office Excel 2007.

Results and Discussion

A total of 80 CMT positive mastitic milk samples were collected from ewes and goats in different localities of River Nile State.

Aerobic Bacteria Isolated From Ewes' Mastitic Milk Samples

According to the cultural characteristics, bacterial morphology, biochemical reactions results, and API rapid systems results, a total of 49 bacterial isolates were isolated from ewes'mastitic milk samples. Staphylococci (figure 1) represented 21.1% of the isolated bacteria.. Staphylococci species isolated from ewes' mastitic milk included *Staphylococcus aureus* (10.0%), *S. haemolyticus* (3.3%), *S. xylosus* (2.2%), *S. hominis* (2.2%), *S. chromogenes* (2.2%) and *S. epidermidis* (1.1%). Coaggulase negative saphylococci (CNS) represented 11.0% of the total staphlococci isolated (table 2). Other bacteria represented 88.9% of the total isolates Other bacteria isolated included *Escherichia coli* (5.6%), *Streptococcus dysagalactiae* (4.4%), *Str. pneumoniae* (3.3%), *Str. ubris* (2.2%), *Corynebacterium bovis* (3.3%), *Enerococcus faecalis* (2.2%), *Actinomyces pyogenes* (2.2%), *Micrococcus varians* (1.1%), *Klebsiella pneumoniae* (2.2%), *Enterobacter cloacae* (2.2%), *E. aerogenes* (21.1%) *Serratia marcescens* (1.1%), and *S. liqifacans* (2.2%) (table 2) and (figure 1).

Aerobic Bacteria Isolated From Goats' Mastitic Milk Samples

According to the cultural characteristics, bacterial morphology, biochemical reactions results, and API rapid systems results, a total of 41 bacterial isolates were isolated from goats'mastitic milk samples. Staphylococci (figure 2) represented 21.1% of the isolated bacteria. Other bacteria represented 88.9% of

the total isolates. Staphylococci species isolated from goats' mastitic milk included *Staphylococcus aureus* (11.1%), *S. hyicus* (3.3%), *S. epidermidis* (3.3%), *S. chromogenes* (3.3%). Coaggulase negative saphylococci (CNS) represented 9.9.0% of the total staphylococci isolated (table 2). Other bacteria isolated included *Streptococcus dysagalactiae* (4.4%), *Str. ubris* (1.1%), *Str. pneumoniae* (2.2%), *Enerococcus faecalis* (4.4%), *Corynebacterium bovis* (4.4%), *Actinomyces pyogenes* (3.3%), and *Escherichia coli* (4.4%), (table 2) (figure 2).

Eighty percent of bacteria isolated from ewes' samples were also isolated from goats' milk samples. This may indicates that, the same aetiology may be implicated in the cases of subclinical mastitis in ewes and goats.

Table 1 Number of Milk Samples Collected From Ewes and Goats

Farms	No. of ewes' milk samples	No. of ewes' milk samples
BarbarOmer Amir	12	6
El Damer Vet Hospital	5	4
Abdelghafar	5	8
Food safety Center	4	5
Atbara vet hospital	4	7
Goats Improving Center-Adamer	5	0
Akram	5	10
Total	40	40

Table 2 Comparison Between Bacteria Isolated From Mastitic Milk Samples Of Ewes And Goats

Bacterial isolates	Ewes Mastitic milk	Sheep Mastitic milk
Staphylococcus aureus	9 (10.0%)	10 (11.1%)
S. xylosus	2 (2.2%)	0 (0.0%)
S. hyicus	0 (0.0%)	3 (3.3%)
S. epidermidis	1(1.1%)	3 (3.3%)
S. hominis	2 (2.2%)	0 (0.0%)
S. chromogenes	2 (2.2%)	3(3.3%)
S. haemolyticus	3 (3.3%)	0 (0.0%)
Streptococcus dysagalactiae	4 (4.4%)	4 (4.4%)
Streptococcus ubris	2 (2.2%)	1(1.1%)
Streptococcus pneumoniae	3 (3.3%)	2 (2.2%)
Enterococcus faecalis	2 (2.2%)	4 (4.4%)
Actinomyces pyogenes	2 (2.2%)	3(3.3%)
Corynebacterium bovis	3 (3.3%)	4 (4.4%)
Micrococcus kristini	0 (0.0%)	0 (0.0%)
Micrococcus varians	1(1.1%)	0 (0.0%)
Echerichia coli	5(5.6%)	4(4.4%)
Klebsiella pneumoniae	2 (2.2%)	0(0.0%)
Enterobacter aerogenes	1 (1.1%)	0 (0.0%)
Enterobacter cloacae	2 (2.2%)	0 (0.0%)
Serratia marcescens	1 (1.1%)	0 (0.0%)
Serratia liquifacans	2 (2.2%)	0 (0.0%)
Total	49	41 90

Figure 1 The Aerobic Bacteria Isolated and Identified From Ewes' Milk Samples

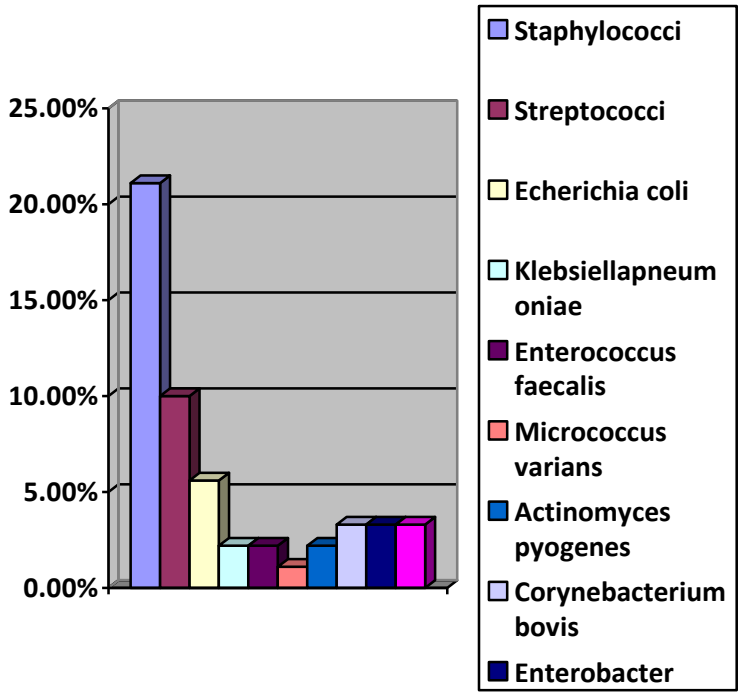
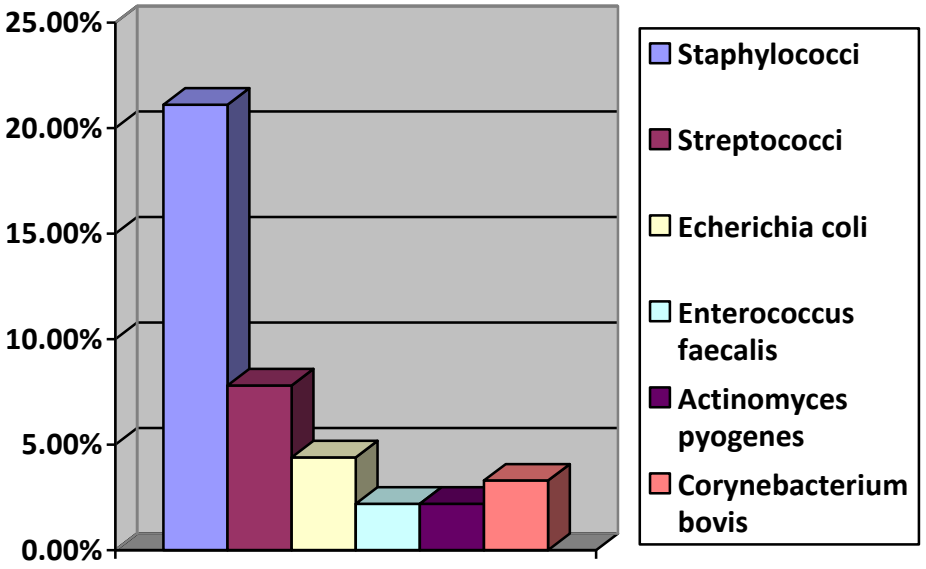


Figure 2 The Aerobic Bacteria Isolated and Identified From Goats' Milk Samples



Mastitis has been recognized as the most important economical factor affecting the dairy animals worldwide [21]. A total of 80 CMT positive mastitic milk samples were collected from ewes and goats in different localities of River Nile State. A total of 49 bacterial isolates were isolated from ewes'mastitic milk samples. Staphylococci represented 21.1% of the isolated bacteria. Staphylococci species isolated from ewes' mastitic milk included *Staphylococcus aureus* (10.0%), *S. haemolyticus* (3.3%), *S. xylosus* (2.2%), *S. hominis* (2.2%), *S. chromogenes* (2.2%) and *S. epidermidis* (1.1%). These findings agree with [9] who mentioned that several pathogens can cause mastitis but *Staphylococcus* spp. are the most frequently diagnosed causal microorganisms of mastitis in goats and sheep. [3] and [7] mentioned that intramammary infections caused by *S. aureu s*warrant special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and subclinical mastitis. Coaggulase negative saphylococci (CNS) represented 11.0% of the total staphlococci isolated. [13] found that the most commonly isolated CNS species in persistent subclinical in goats and sheep are *Staphylococcus epidermidis*, *S. caprae*, *S. simulans*, *S. chromogenes* and *S. xylosus*. Other bacteria represented 88.9% of the total isolates. Other bacteria isolated included *Escherichia coli* (5.6%), *Streptococcus dysagalactiae* (4.4%), *Str. pneumoniae* (3.3%), *Str. ubris* (2.2%), *Corynebacterium bovis* (3.3%), *Enerococcus faecalis* (2.2%), *Actinomyces pyogenes* (2.2%), *Micrococcus varians* (1.1%), *Klebsiella pneumoniae* (2.2%), *Enterobacter cloacae* (2.2%), *E. aerogenes* (21.1%), *Serratia marcescens* (1.1%) and *S. liqifacans* (2.2%). [11, 12, 13, 7, 14] Found that the bacteria which are known to cause mastitis in sheep and goats are *Streptococcus spp., Pasteurella spp., E. coli*, *Pseudomonas aeruginosa*, *Corynebacteria* and *Serratia marcescens*.

A total of 41 bacterial isolates were isolated from goats'mastitic milk samples. Staphylococci represented 21.1% of the isolated bacteria. [22, 23] reported the high prevalence of *Staphylococcus aureus* in cases of mastitis in goats. [24] found that *S. aureus* is at top rank in causing mastitis of dairy goats. Other Staphylococcal species isolated from goats' mastitic milk included *Staphylococcus aureus* (11.1%), *S. hyicus* (3.3%), *S. epidermidis* (3.3%), *S. chromogenes* (3.3%). Coaggulase negative saphylococci (CNS) represented 9.9.0% of the total staphylococci isolated. [22, 23] reported that CNS in a decreasing order of frequency, cannot be considered as minor pathogens in small ruminants. Other bacteria isolated from goats' milk represented 88.9% of the total isolates. Other bacteria isolated included *Streptococcus dysagalactiae* (4.4%), *Str. ubris* (1.1%), *Str. pneumoniae* (2.2%), *Enerococcus faecalis* (4.4%), *Corynebacterium bovis* (4.4%), *Actinomyces pyogenes* (3.3%), and *Escherichia coli* (4.4%). Similar findings were declared by [25] and [21] who found that major bacteria involved in etiology of dairy goat clinical or sub-clinical mastitis are *Staphylococcus aureus, Escherichia coli, Streptococcus sp., Corynebacterium sp*. *Pseudomonas sp. And Bacillus sp.* Streptococci, Enterobacteria, *Arcanobacterium pyogenes*, Corynebacteria, *Pasteurellaceae*, *Pseudomonas* spp. [26, 27] reported Enzootic and epizootic outbreaks due to *S. aureus*, *S. uberis, S. agalactiae, S. suis, Serratia marcescens* and *Pseudomonas aeruginosa* during lactation. Eighty percent of bacteria isolated from ewes' samples were also isolated from goats' milk samples. This may indicates that, the same aetiology may be implicated in the cases of subclinical mastitis in ewes and goats. [10] mentioned that ewes are more susceptible for mastitis than goats.

Conclusion

The study revealed that staphylococci are the predominant bacterial spp. isolated from ovine and caprine mastitic milk samples in River Nile State. Moreover, Staphylococci are involved in bovine mastitis. Other bacteria found in mastitic milk samples in this study included: Coliforms, Streptococci, Micrococci, Enerococci, Actinomyces spp., Klebsiella spp., Serratia spp. and Corynebacteria which are responsible for ovine and caprine subclinical mastitis. According to frequency of isolation, coliforms came in second place to staphylococci as causes of bovine mastitis followed by streptococci. This study indicated that, the same aetiology may be implicated in the cases of subclinical mastitis in ewes and goats. The study also indicated that ewes are more susceptible for mastitis than goats.

Further studies should be carried out to investigate the predisposing factors related to the incidence of ovine and caprine mastitis and to identify different causes of ovine and caprine mastitis. Further studies should include a survey of more animals in different farms and an extensive study of the significance of different microorganisms in ovine and caprine mastitis. Moreover the serotyping of isolates obtained from different areas should be given more attention.

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