ISSN 2310-4090

International Journal of Scientific Footprints Open Access

Antibiotic Susceptibility of Major Bacteria Cause Caprine Mastitis in River Nile State, Sudan

Abubaker A. El Ayis[1], Hind A. Elnasri[2]

[1]Department of Medicine, College of Veterinary Medicine, Bahri University - Sudan
[2]Department of Biochemistry, College of Veterinary Medicine, Bahri University - Sudan

Keywords:
Caprine Mastitis; Cephalothin; Khartoum State.

Correspondence:
Abubaker A. El Ayis. College of Veterinary Medicine, Bahri University-Sudan

Funding Information:
No funding information provided.

Manuscript History:
Received: November 2016
Accepted: January 2016

International Journal of Scientific Footprints 2017; 5(1): 24 - 34

Abstract

In this study a total of 40 milk samples positive for California Mastitis Test (CMT) were collected from goats in the three localities of River Nile State (Atbara, Barbar and El Damer). Samples were submitted for bacteriological examination. 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%) and 4 Enterococcus faecalis (4.4%). All isolates were subjected to sensitivity tests using 10 antibiotics in use for treatment of mastitis in Sudan. All Staphylococcal isolates were sensitive to Ampicillin, 57.9% to Kanamycin, 52.6% to Gentamycin, 42.2% to Cephalothin and Chloramphenicol, 36.8% to Ciprofloxacin, 26.3% to Tetracycline, 21.0% to Cloxacillin, 15.8% to Erythromycin and 10.5% to Procaine Penicillin. 75% of Echerichia coli isolates were sensitive to Ampicillin and Ciprofloxacin, 50.0% were sensitive to Cephalothin, Chloramphenicol, Gentamycin, Kanamycin, Tetracycline and Cloxacillin, 25.0% to Erythromycin and all isolates were resistant to Procaine Penicillin. All Corynebacterium bovis isolates were sensitive to Gentamycin, 50.0% to Ampicillin and Tetracycline, 25.0% to Cephalothin, Kanamycin and Chloramphenicol and Cloxacillin, all isolates were resistant to Procaine Penicillin, Ciprofloxacin and Erythromycin. All Enterococcus faecalis isolates were sensitive to Gentamycin and Ciprofloxacin, 75.0% to Chloramphenicol, 50.0% to Cephalothin, Kanamycin and Tetracycline and all isolates were resistant to Ampicillin, Erythromycin, Procaine Penicillin and Cloxacillin.

Introduction

Mastitis is one of the more common health problems affecting sheep and goats. 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* [1, 2, 3]. 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* [4, 5, 6, 7]. [8] isolated many bacteria from cases of subclinical and clinical mastitis. These include: *S. aureus*, *S. epidermidis*, *Str. agalactiae*, *Str. ubris*, *E. coli*, *Ps. aeruginosa* and *K. pneumoniae*. *Enterobacter spp* were found to cause bovine mastitis [2].

Corynebacterium spp alone were found to be associated with clinical mastitis [3].

The choice of antibiotic to be used can be decided by antibiotic sensitivity test [9]. [10] studied the antibiotic sensitivity of 281 *S. aureus* strains isolated from bovine milk in U.S.A, using 21 antimicrobial agents. They reported that more than 90% of *S. aureus* isolates were sensitive to Bacitracin, Cephalothin, Chloramphenicol, Cloxacillin, Erythromycin, Gentamycin, Kanamycin, Lincomycin, Neomycin, Nitrofurazone, Oleandomycin, Tetracycline and Vancomycin. Ampicillin, Penicillin and Polmyxin B were ineffective against the majority of *S. aureus* isolates. [11] studied the antimicrobial susceptibility of coagulase–negative staphylococci isolated from bovine mastitis in Argentina and no resistance was detected for Gentamycin, Cephalothin, Ampicillin, Cloxacillin, Erythromycin and Clindamycin. The results also indicated that coagulase–negative staphylococci isolates in Argentina exhibited the highest degree of resistance to Penicillin of all antimicrobial agents tested. [12] found that *Streptococcus agalactiae* and *S. intermedius* isolates were resistant to tetracycline (61.2%), followed by lincomycin (43.2%), gentamycin (35.3%), oxacillin (34.3%), and erythromycin (28.6%). Cephalothin and penicillin were the only antimicrobial agents to which most of the streptococci (92%) were susceptible. *E. coli* showed variation in their susceptibility to various chemotherapeutic agents in use for treatment of calf diarrhoea such as Amoxicillin, Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamycin, Kanamycin, Nalidixic acid, Streptomycin, Sulphamethoxazole, and Erythromycin [13]. [14] reported that *E. coli* isolates were highly sensitive to Nitrofuran(98%), Colistin sulphate (98%), Gentamycin (97%) and Cotrimoxazole (82%) and resistant to the rest of antibiotics (Streptomycin, Tetracycline, Nalidixic acid and Ampicillin). *Enterococcus faecalis* usually susceptible to Ampicillin and Penicillin. It can acquire resistance to Vancomycin, usually due to van A or van B and canoccasionally produce beta-lactamase [15]. [16] found that coryneform bacteria were 100% resistant to penicillin G except *Corynebacterium ulcers* (60%). In contrast Gentamycin was the drug of choice for Coryneform.

Materials and methods

Area of the Study

A total of 40 milk samples positive for CMT were collected from goats in River Nile State. Milk samples were collected from Barbar, Omer Amir farm, El Damer Vet Hospital, Abdelghafarm farm,ood 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).

Antibiotic Susceptibility Test Discs

This was performed by the standard Disc Diffusion method [19]. The organisms were subcultured onto Blood agar and incubated at 37C for 18-24 hrs. They were diluted in sterile normal saline tubes and homogenous bacterial suspensions were prepared. After drying Nutrient agar and D.S.T agar plates, 2 ml of diluted culture were spread evenly over the surface of the media. Excess fluid was aspirated and the plates were allowed to dry. Oxoid discs (Basingtoke, Hampshire, England) of Novobiocin and other antimicrobial drugs were applied to the surface of the medium and pressed gently using sterile forceps. They were incubated at 37C for 24-48 hours. Zones of inhibition were measured in (mm) to determine whether the organism was sensitive or resistant [20] (Table 2).

Statistical Analysis

Statistical analysis was done through Microsoft office Excel 2007.

Results and Discussion

A total of 40 CMT positive mastitic milk samples were collected from goats in different localities of River Nile State.

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 1) 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 3).

Sensitivity of Bacteria Isolated From Mastitic Milk to 10 Antibiotics

Table (4) presents the sensitivity of 38 bacterial species to different antibiotics in use for treatment of mastitis in Sudan. All Staphylococcal isolates were sensitive to Ampicillin, 57.9% to Kanamycin, 52.6% to Gentamycin, 42.2% to Cephalothin and Chloramphenicol, 36.8% to Ciprofloxacin, 26.3% to Tetracycline, 21.0% to Cloxacillin, 15.8% to Erythromycin and 10.5% to Procaine Penicillin. All Streptococcal isolates were sensitive to Ampicillin, 71.4% to Procaine Penicillin, 57.1% to Cephalothin, Cloxacillin, Gentamycin, Kanamycin and Erythromycin, 42.9 to Ciprofloxacin , 28.6% to Chloramphenicol and 14.3% to Tetracycline. 75% of *Echerichia coli* isolates were sensitive to Ampicillin and Ciprofloxacin, 50.0% were sensitive to Cephalothin, Chloramphenicol, Gentamycin, Kanamycin, Tetracycline and Cloxacillin, 25.0% to Erythromycin and all isolates were resistant to Procaine Penicillin. All *Corynebacterium bovis* isolates were sensitive to Gentamycin, 50.0% were sensitive to Ampicillin and Tetracycline, 25.0% to Cephalothin, Kanamycin and Chloramphenicol and Cloxacillin, all isolates were resistant to Procaine Penicillin, Ciprofloxacin and Erythromycin. All *Enterococcus faecalis* isolates were sensitive to Gentamycin and Ciprofloxacin, 75.0% to Chloramphenicol, 50.0% to Cephalothin, Kanamycin and Tetracycline and all isolates were resistant to Ampicillin, Erythromycin, Procaine Penicillin and Cloxacillin.

Table 1 Number of Milk Samples Collected from Goats

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

Table 2 Zone Size Interpretation Chart (Quinn *et al*, 2011)

Antimicrobial agents	Resistant ≤	Intermediate	Moderately Susceptible	Susceptible ≥
Ampicillin 10µg	28 mm	-	-	29 mm
Cephalothin 30µg	14 mm	-	15-17mm	18 mm
Chloramphenicol 30µg	12 mm	13-17 mm	-	18mm
Ciprofluxacin 15µg	15 mm	-	16-20mm	21mm
Erythromycin 15µg	13 mm	14-22 mm	-	23mm
Gentamycin 10µg	12 mm	13-14 mm	-	15mm
Kanamycin 30µg	12 mm	14-17 mm	-	18 mm
Penicillin 10µg	28 mm	-	-	29 mm
Tetracyclin 30µg	14 mm	15-18 mm	-	19 mm
Cloxacillin 10µg	14 mm	15-20 mm	-	21mm

Table 3 Aerobic Bacteria Isolated from Mastitic Milk Samples of Goats

Bacterial isolates	Goats' Mastitic milk
Staphylococcus aureus	10 (11.1%)
S. hyicus	3 (3.3%)
S. epidermidis	3 (3.3%)
S. chromogenes	3(3.3%)
Streptococcus dysagalactiae	4 (4.4%)
Streptococcus ubris	1(1.1%)
Streptococcus pneumoniae	2 (2.2%)
Enterococcus faecalis	4 (4.4%)
Corynebacterium bovis	4 (4.4%)
Echerichia coli	4(4.4%)
Total	38

Figure 1 The Aerobic Bacteria Isolated and Identified from Goats' Milk Samples

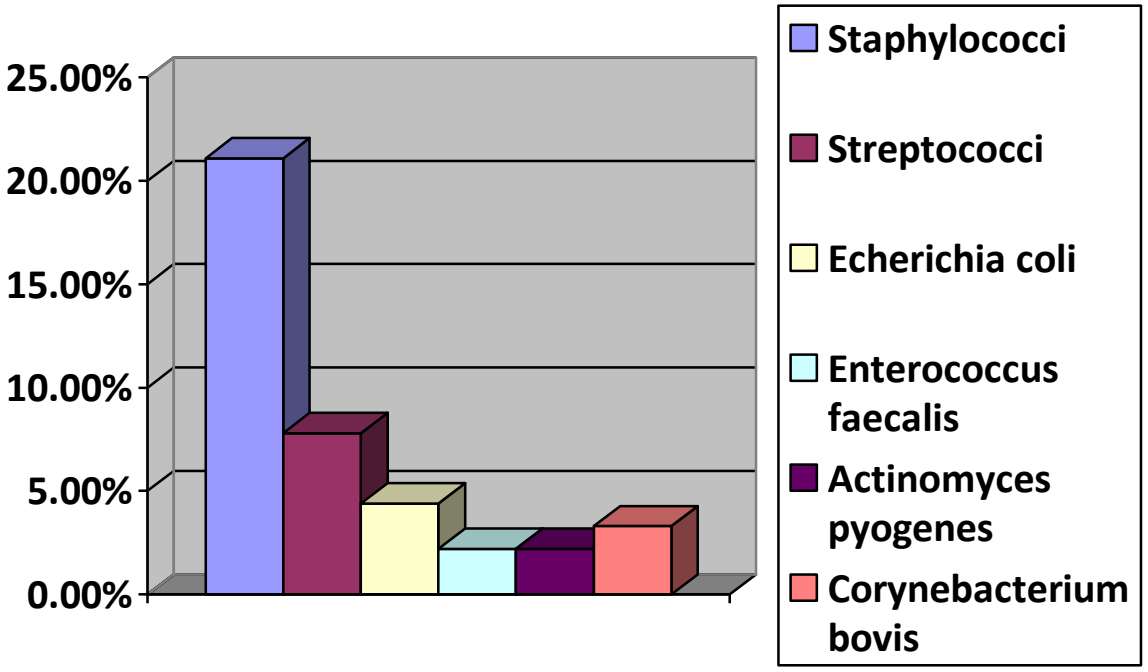

Table 4 Sensitivity of Different Staphylococci from Mastitic Milk to 10 Antibootics

Species	A	KF	CH	CI	E	CN	K	P	T	CL
S. aureus	38	19	15	22	22	16	18	31	19	22
S. aureus	36	14	13	12	12	14	19	14	12	14
S. aureus	35	16	18	23	11	16	16	16	10	12
S. aureus	36	18	15	10	16	15	18	17	20	18
S. aureus	38	10	11	10	19	15	17	16	12	11
S. aureus	35	11	19	19	10	13	15	18	11	13
S. aureus	40	18	15	11	23	14	18	30	14	11
S. aureus	40	11	18	12	14	13	16	20	12	25
S. aureus	35	13	19	15	20	17	18	19	14	14
S. aureus	29	10	16	12	17	13	19	17	12	10
S. epidermidis	29	15	19	19	18	18	19	11	19	28
S. epidermidis	32	18	10	15	14	12	10	16	11	15
S. epidermidis	30	17	16	15	16	13	18	22	15	18
S. chromogenes	29	11	18	19	19	10	11	26	14	22
S. chromogenes	33	18	15	11	11	18	20	15	19	19
S. chromogenes	29	10	10	10	16	17	19	19	15	16
S. hyicus	31	19	19	18	24	11	10	30	25	27
S. hyicus	30	17	20	12	14	16	21	12	11	18
S. hyicus	29	16	18	15	17	15	22	22	19	15
Streptococcus dysagalactiae	29	15	10	18	23	16	19	29	17	17
Streptococcus dysagalactiae	33	12	19	13	15	11	10	16	11	23
Streptococcus dysagalactiae	32	11	11	15	17	18	15	22	10	18
Streptococcus dysagalactiae	29	18	22	21	28	12	18	16	17	15
Streptococcus ubris	29	10	16	11	10	15	11	30	10	25
Streptococcus pneumoniae	31	13	21	15	16	10	19	18	13	22
Streptococcus pneumoniae	30	10	19	18	10	15	18	16	12	18
Enterococcus faecalis	10	18	18	23	22	16	15	21	21	16
Enterococcus faecalis	22	12	12	22	21	16	15	19	19	17
Enterococcus faecalis	18	15	19	25	20	18	19	25	13	15
Enterococcus faecalis	15	19	21	23	19	17	17	20	15	17
Corynebacterium bovis	30	11	19	10	19	15	15	22	21	15
Corynebacterium bovis	17	15	11	12	21	16	14	23	16	23
Corynebacterium bovis	17	10	15	14	19	18	19	19	15	16
Corynebacterium bovis	29	11	12	11	22	20	11	18	19	15
Echerichia coli	30	11	19	22	19	20	20	22	19	21
Echerichia coli	31	18	11	15	18	10	15	19	12	12
Echerichia coli	29	13	15	21	25	15	19	25	15	19
Echerichia coli	25	19	20	25	19	11	15	27	21	25

Key:

- **A**: Ampicillin 10µg
- **KF**: Cephalothin 30 µg
- **CN**: Gentamycin 10µg
- **CH**: chloramphenicol 30µg
- **P**: penicillin 10µg
- **CL**: Cloxacillin 10µg
- **CI**: Ciprofluxacin 5µg
- **E**: Erythromycin 15µg
- **K**: Kanamycin 30µg
- **T**: Tetracycline 30 µg
- Inhibitory zones measured in millimeters

Mastitis has been recognized as the most important economical factor affecting the dairy animals worldwide [21]. 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%), 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. 38 bacterial species were tested for their susceptibility to 10 different antibiotics in use for treatment of mastitis in Sudan. All Staphylococcal isolates were sensitive to Ampicillin, 57.9% to Kanamycin, 52.6% to Gentamycin, 42.2% to Cephalothin and Chloramphenicol, 36.8% to Ciprofloxacin, 26.3% to Tetracycline, 21.0% to Cloxacillin, 15.8% to Erythromycin and 10.5% to Procaine Penicillin. These findings agree with [28] findings who reported resistance of *S. aureus* to Penicillin and Tetracycline. And [10] who reported sensitivity of *S. aureus* to Cephalothin, Cloxacillin, Gentamycin, Kanamycin and Ampicillin, Also [11] reported sensitivity of coagulase-negative staphylococci to Gentamycin and Ampicillin. All Streptococcal isolates were sensitive to Ampicillin, 71.4% to Procaine Penicillin, 57.1% to Cephalothin, Cloxacillin, Gentamycin, Kanamycin and Erythromycin, 42.9 to Ciprofloxacin , 28.6% to Chloramphenicol and 14.3% to Tetracycline. [12] found that Streptococcus agalactiae isolates were resistant to tetracycline (61.2%), followed by lincomycin (43.2%), gentamycin (35.3%), oxacillin (34.3%), and erythromycin (28.6%). Cephalothin and penicillin were the only antimicrobial agents to which most of the streptococci (92%) were susceptible. 75% of *Echerichia coli* isolates were sensitive to Ampicillin and Ciprofloxacin, 50.0% were sensitive to Cephalothin, Chloramphenicol, Gentamycin, Kanamycin, Tetracycline and Cloxacillin, 25.0% to Erythromycin and all isolates were resistant to Procaine Penicillin. According to [13] *E. coli* showed variation in their susceptibility to various chemotherapeutic agents such as: Amoxicillin, Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamycin, Kanamycin, Nalidixic acid, Streptomycin, Sulphamethoxazole, and Erythromycin. [29] reported that all *E. coli* isolates were sensitive to choramphenicol and Erythromycin. 97% of the isolates showed different patterns of sensitivity to other antibiotics used (Nalidixic acid, Neomycin, Tetracycline, Ampicillin, Gentamycin, Sulfamethoxazole/ trimethoprim, and Streptomycin). All *Corynebacterium bovis* isolates were sensitive to

Gentamycin, 50.0% were sensitive to Ampicillin and Tetracycline, 25.0% to Cephalothin, Kanamycin and Chloramphenicol and Cloxacillin, all isolates were resistant to Procaine Penicillin, Ciprofloxacin and Erythromycin.The same findings were reported by [16] who found that coryneform bacteria were 100% resistant to penicillin G except *Corynebacterium ulcer's* (60%). In contrast Gentamycin was the drug of choice for Coryneform. All *Enterococcus faecalis* isolates were sensitive to Gentamycin and Ciprofloxacin, 75.0% to Chloramphenicol, 50.0% to Cephalothin, Kanamycin and Tetracycline and all isolates were resistant to Ampicillin, Erythromycin, Procaine Penicillin and Cloxacillin. The findings disagree [15] who reported that *Enterococcus faecalis* usually susceptible to Ampicillin and Penicillin.

Conclusion

The study revealed that the predominant bacteria isolated from mastitic goats' milk were Staphylococci (21.1%), Streptococci (7.8%), *Echerichia coli* (4.4%), *Corynebacterium bovis* (4.4%) and *Enterococcus faecalis* (4.4%). All Staphylococcal isolates were sensitive to Ampicillin, 57.9% to Kanamycin, 52.6% to Gentamycin, 42.2% to Cephalothin and Chloramphenicol, 36.8% to Ciprofloxacin, 26.3% to Tetracycline, 21.0% to Cloxacillin, 15.8% to Erythromycin and 10.5% to Procaine Penicillin. All Streptococcal isolates were sensitive to Ampicillin, 71.4% to Procaine Penicillin, 57.1% to Cephalothin, Cloxacillin, Gentamycin, Kanamycin and Erythromycin, 42.9 to Ciprofloxacin , 28.6% to Chloramphenicol and 14.3% to Tetracycline. 75% of *Echerichia coli* isolates were sensitive to Ampicillin and Ciprofloxacin, 50.0% were sensitive to Cephalothin, Chloramphenicol, Gentamycin, Kanamycin, Tetracycline and Cloxacillin, 25.0% to Erythromycin and all isolates were resistant to Procaine Penicillin. All *Corynebacterium bovis* isolates were sensitive to Gentamycin, 50.0% were sensitive to Ampicillin and Tetracycline, 25.0% to Cephalothin, Kanamycin and Chloramphenicol and Cloxacillin, all isolates were resistant to Procaine Penicillin, Ciprofloxacin and Erythromycin. All *Enterococcus faecalis* isolates were sensitive to Gentamycin and Ciprofloxacin, 75.0% to Chloramphenicol, 50.0% to Cephalothin, Kanamycin and Tetracycline and all isolates were resistant to Ampicillin, Erythromycin, Procaine Penicillin and Cloxacillin.

Further studies should include a survey of more animals in different farms and an extensive study of the significance of different bacteria in caprine mastitis. A comprehensive study of *in vitro* susceptibility of pathogenic bacteria which cause mastitis to different antimicrobial drugs must be done. This should be followed with *in vivo* studies using single and combined drugs, systemic and udder therapy in order to determine the most efficient procedures for mastitis therapy.

Acknowledgement

Authors are thankful to the University authorities for providing necessary facilities to carry out this research work.

References

[1] Barrow GI, Feltham RK, Cowan K J and Steel GI. (2004). *Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd. ed*. Cambridge University Press. Cambridge.

[2] Ali, M. A., M. D. Ahmad, K. Muhammd, and A. A. Anjum (2011). Prevalence of subclinical mastitis in dairy buffaloes of Punjab, Pakistan. J. Anim. Plant Sci. 21(3): 477-480.

[3] Ameh J.A., Tari I.S., Observations on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri, Small Rumin. Res. 35 (1999) 1–5.

[4] Aydin, I., K. Kav and H.A. Celik (2009). Identification and antimicrobial susceptibility of subclinical mastitis pathogen isolated from Hair goats milk. J. Anim. Vet. Adv. 8: 1086-1090.

[5] Bergonier D., Berthelot X., New advances in Epizootiology and control of ewe mastitis, Livest. Prod Sci. 79 (2003) 1–16.

[6] Bergonier, D., Berthelot, X., (2003). New advances in epizootiology and control of ewe mastitis. Livest. Prod. Sci. 79, 1–16.

[7] Bergonier, D., de Cremoux, R., Rupp, R., Lagriffoul, G., Berthelot, X., (2003). Mastitis of dairy small ruminants. Vet. Res. 34, 689–716.

[8] Berriatua E., Ziluaga I., Miguel Virto C., Uribarren P., Juste R., Laevens S., Vandamme P., Govan J.R.W., Outbreak of subclinical mastitis in a flock of dairy sheep associated with *Burkholderia cepacia* complex infection, J. Clin. Microbiol. 39 (2001) 990–994.

[9] Berriatua, E., Ziluaga, I., Miguel-Virto, C., Uribarren, P., Juste, R., Laevens, S., Vandamme, P., Govan, J.R., (2001). Outbreak of subclinical mastitis in a flock of dairy sheep associated with *Burkholderia cepacia* complex infection. J. Clin. Microbiol. 39, 990–994.

[10] Buxton, A. and Frazer, G. (1977). Animal Microbiology. Vol. 1. Immunology, Bacteriology, Mycology, Diseases of fish and laboratory methods. 1st ed. Blackwell scientific publication. London. UK. pp. 93-102.

[11] Contreras, A., Corrales, J.C., Sierra, D., Marco, J., (1995). Prevalence and aetiology of nonclinical intramammary infection in Murciano–Granadina goats. Small Rumin. Res. 17, 71–78.

[12] Contreras, A., Luengo, C., Sanchez, A., Corrales, J.C., (2003). The role of intramammary pathogens in dairy goats. Livest. Prod. Sci. 79, 273–283.

[13] Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. A. (2007). Medical Microbiology. 12th. ed. Vol.2. The practice of medical microbiology. Churchill Livingstone. London. UK. pp. 133.

[14] Ellaithi, S. O. A. (2004). Characterization of *E. coli* isolated from diarrhoeic calves in the Sudan. Ph.D. Thesis. Univ. Khartoum. Sudan.

[15] Gentilini, E.; G. Denamiel; A. Betancov; M. Rebuelto; M. Rodrigues; Fermepin and R. A Detorres. (2002). Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. Am. J. Dairy. Sci. Assoc., 85, 1913-1917.

[16] Gonzalo, C., Ariznabarreta, A., Carriedo, J.A., San Primitivo, F., (2002). Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. J. Dairy Sci. 85, 1460–1467.

[17] Gonzalo, C., Tardaguila, J.A., De la Fuente, L.F., San Primitivo, F., (2004). Effects of selective and complete dry therapy on prevalence of intramammary infection and on milk yield in the subsequent lactation in dairy ewes. J. Dairy Res. 71, 33–38.

[18] Hyang-mi Nam,1 Suk-Kyung Lim, Hyun-Mi Kang, Jae-Myoung Kim, Jin-San Moon, Keum-Chan Jang, Yi-Seok Joo, Mun-il Kang, Suk-Chan Jung (2009). Antimicrobial resistance of streptococci isolated from mastitic bovine milk samples in Korea. J Vet Diagn Invest 21:698–701.

[19] Jacoby, G. A. and Archer, G. L. (1991). New mechanisms of bacterial resistance to antimicrobial agents, N. *Eng. J. Med*., 324: 601- 612.

[20] Las Heras, A., Dom´ınguez, L., Fern´andez-Garayz´abal, J.F., (1999). Prevalence and aetiology of subclinical mastitis in dairy ewes of the Madrid region. Small Rumin. Res. 32, 21–29.

[21] Leitner, G., Chaffer, M., Shamay, A., Shapiro, F., Merin, U., Ezra, E., Saran, A., Silanikove, N.,(2004). Changes in milk composition as affected by subclinical mastitis in sheep. J. Dairy Sci. 87, 46–52.

[22] Marie B. Coyle (2005). Manual of Antimicrobial susceptibility testing. American society for micro biology. Library of Congress Cataloging-in-Publication Data. ISBN 1-55581-349-6.

[23] Mc Donald, J. S. and Anderson, A. J. (1981). Antibiotic sensitivity of *Staphylococcus aureus* and coagulase-negative staphylococci isolated from infected bovine mammary glands. Cornell. Vet., 71 (4), 391-396.

[24] Mohamed, S. M. E.(2009). *Eschericha coli* associated with neonatal calf diarrhoea in Khartoum North, Sudan. M. Sc. Thesis. Univ. Khartoum. Sudan.

[25] Nuol Aywel Madut and Atif Elamin Abdel Gadir. Susceptibility of *Corynebacterum spp.* responsible for bovine mastits against commonly used antibiotics in Kuku dairy farms, Sudan Journal of Cell and Animal Biology Vol. 5(1), pp. 6-10, January 2011.

[26] Quinn, P. J.; Markey, B. K.; Leonard, F. C.; Fitzpatrick, E. S.; Fanning, S. and Hartigan, P. J. (2011). Veterinary Microbiology and Microbial diseases.Virginia. Willey – Blackwell. U.S.A.

[27] Radostits, O. M.; Gay, C.C.; Hinchcliff, K. W. and Constable, P. D. (2012). Veterinary Medicine, A text book of the diseases of cattle, sheep, pigs and goats. 10th ed. Philadelphia. U.S.A.
[28] Smith, P. H. A. N. S.; Mair, M. E.; Sharp and J. G. Holt (1986). Bergy's manual of systemic bacteriology. 9th ed. Williams and Walkins, London. U.K.
[29] White E.C., Hinckley L.S., Prevalence of mastitis pathogens in goat milk, Small Rumin. Res. 33 (1999) 117–121.