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Aerobic Bacteria Isolated From Mastitic Milk and Milker's' Hands in Khartoum State, Sudan

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Abstract

A total of 299 microorganisms were isolated from 100 bovine mastitic samples and 62 bacterial isolates from 40 swabs from milker's' hands in different localities of Khartoum State. These isolates were subjected for further confirmation by using API staph, API strep20, Api coryne identification rapid systems. Staphylococci represented 44.5% of the microorganisms isolated from mastitic milk samples and 44.3% of milker's' hands isolates. These findings show a high incidence of staphylococci in dairy herds in Khartoum area. Other microorganisms isolated from mastitic milk samples were Streptococci 13.7%, Escherichia coli 9.4%, Klebsiella pneumoniae 5.4%, Corynebacteria 4.6%, Micrococci 5.4%, Enterobacter spp. 5.7%, Serratia species 5.0% and Fungi 6.4%. Other bacteria isolated from milker's' hands were: Streptococcus spp. 16.1%, Escherichia coli 12.9%, Klebsiella pneumonia 11.3%, Corynebacterium bovis 3.2%, Actinomyces pyogenes 1.6%, Micrococci 8.0% and Enterobacter spp. 6.4%. Sixty percent of staphylococci isolated from milker's' hands samples were also isolated from mastitic milk samples. This may indicate that, milker's may play an important role in the transmission of mastitis, under unhygienic conditions.

Introduction

Bovine mastitis is a large-scale infectious disease with significant impact on the economy of milk production [1]. Subclinical mastitis is an invisible abnormality of milk or udder which characterized by an increase in somatic cell and/or leukocyte count. It is a problem of the herd rather than individual animals [2]. [2] suggested that the total economic losses caused by mastitis are composed of the following items:

Items	Percentage of Total Value
Value of production lost	70
Value of cows lost by premature culling	14
Value of milk discarded or downgraded	7
Treatment and Veterinary expenses	8
Others	1

Several agents are known to be incriminated in mastitis, these include: *Staphylococcus aureus, S. epidermidis, Streptococcus ubris, Str. dysagalactiae, Str. zoepidermicus, Str. faecalis, Str. pyogenes, Str. pneumoniae , Actinomyces pyogenes, Corynebacterium ulcrans, Klebsiella spp, Enterobacter aerogenes, Mycobacterim bovis, M. lacticola , M. fortuitum, Bacillus cereus, Pasteurella maltocida, P. haemolytica, Pseudomonas aeruginosa, Fusibacterium necrophorum, Serratia marcescens, Mycoplasma bovis, Mycoplasma Canadensis, Nocardia asteroids, Nocardia braziliensis, Nocardia farcinicus, Acholeplasma laidlowii, Mycoplasma alkalescens, Mycoplasma bovigenitalium* and *Bacteroid funduformis* [2]. Invasion of the mammary gland by microorganisms is characterized by an increased leukocyte count in the milk, the majority of cells being neutrophils [3]. [4] 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*. [5] isolated *Corynebacterium spp, Micrococcus spp*. Whereas, Keskintep *et al* [6] isolated *Str. lactis* and *Str. faecalis*. *E. coli* might cause acute and peracute form of clinical mastitis [2]. *Enterobacter spp* were found to cause bovine mastitis [7]. *Corynebacterium spp* alone were found to be associated with clinical mastitis [8]. Micrococci were also isolated from bovine milk with subclinical mastitis [9] and from clinical mastitis [10]. *Pasteurella maltocida* and *Pasteurella haemolytica* are rare cause of mastitis among cattle [11]. Yeast mastitis (*Candida spp, Geotrichum spp* and *Trichosporum spp*) is the most commonly found in connection with antibiotic therapy especially if the antibiotic favors the growth of yeasts [12].

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* [9]. 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 [13].

Diagnosis of subclinical mastitis presents a problem due to unapparent signs; however several screening tests were developed beside the culturing method. The culturing method is not suitable for large scale monitoring of udder health [14].

The objective of this research work was to investigate microorganisms implicated in bovine mastitis using conventional and rapid systems and determining the role of milker's in the transmission of mastitis.

Materials and Methods

Area of the Study

A total of 100 bovine milk samples positive for California Mastitis Test (CMT), and 40 milker's hands' swabs, were collected from farms of the three localities in Khartoum State in Sudan. In Bahri and East Nile localities samples were collected from Khartoum university farm, Shigla farms, Sudan university for science and technology farm, Selate farms, Mygoma Farms and Helat kuku farms. In Omdurman locality, samples were collected from El Fetaihab farms, El Ruduan farms and Gabal Toureia Farms. In Khartoum and Gabel Awleia localities samples were collected from El Saig farms, Soba farms and El Azhari farms.

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. Swabs were taken from hands of milker's before milking the cows. 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-freezer and swabs were soaked in tubes containing nutrient broth and incubated at 37C. 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. Swabs were removed from the incubator. Samples were then cultured.

Bacterial Viable Count and Grading of Milk

According to [3] serial ten-fold dilutions of the original fluid of milk were made. Spread plate method was followed and an inoculum of 0.1 ml of each dilution was placed on the surface of an agar plate. The inoculum was spread rapidly over the entire agar surface using a thin, bent glass rod. Inoculated plates were left to dry and then incubated for 24–48 hours at 37C. Twenty to three hundred colonies were counted .Two plates were inoculated per dilution. The total colony count per milliliter of milk was calculated by multiplication of the number of colonies counted by dilution marked. Milk samples with bacterial count that ranged between 50,000 to 100,000 bacteria/ml, were graded as class A, bacterial counts between 100,000 to 200,000 as grade B, bacterial counts between 200,000 to one million as grade C and bacterial counts over one million as grade D, (U.S Department of Health, Education and Welfare, 1953). Baird–Parker medium and Mannitol Salt Agar were used for staphylococcal viable count.

Total Count of Bacterial Cells

According to [3] breed's direct smear method for counting bacteria in milk was used (this method does not distinguish between viable and non–viable and thus the bacterial count will include both living and dead cells). Grease-free microscope slide was placed over a template 1cm ×1cm (area of 100mm^2) and a 0.01ml of sample carefully spread over this area. The smear was allowed to air–dry, fixed by heat and stained with Newman's stain, because this stain conveniently combines both defatting and staining process. The stained smear was examined under the oil–immersion objective. The bacterial cells were counted in at least 50 fields throughout the area of the smear.

$$\text{Bacteria/ml} = \frac{N \times \text{Area of smear } (100cm^2) \times 100}{\text{Area of one field } (3.14 \times r^2)} = \frac{N \times 10^4}{3.14 \times r^2}$$

(Where **N = Average bacterial count/field** and **r = radius of the microscope's oil – immersion field in mm**). The radius of the oil–immersion field is usually about 0.08 mm so that the area of the field will be 0.25 mm^2 and **bacteria / ml = N×4×10^4**

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 staphylococci, 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 4^{o}C.

Biological and Biochemical Identification

The purified isolates were identified as previously described [15] and [16]. 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 17] 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 [18], 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.

Isolation of Fungi from Mastitic Milk Samples

According to Sandholm [19], mastitic milk samples were cultured on Saboraud dextrose agar which contain Chloramphenicol 5.05g/l and cultures were then incubated at 37C. As fungi grow slowly than bacteria then cultures examined over several days for fungal growth. In order to differentiate between moulds and yeasts fungal growth examined visually and under microscopy.

Statistical Analysis

Statistical analysis was done through Microsoft office Excel 2007.

Results

A total of 100 CMT positive mastitic milk samples and 40 swabs of milker's' hands were collected from different localities of Khartoum State.

Results the Viable Count of Mastitic Milk Samples

Forty five out of the 100 mastitic milk samples had a bacterial viable count ranging between 200,000 and 1,000,000 CFU/ml, 27 had a bacterial viable count ranging between 100,000 and 200,000 CFU/ml, 16 had a bacterial viable count ranging between 50,000 and 100,000 CFU/ml and 12 had a bacterial viable count more than one million CFU/ml (Table 1).

Microorganisms Isolated From Mastitic Milk Samples

According to the cultural characteristics, bacterial morphology, fungal morphology, biochemical reactions results, and API rapid systems results, a total of 299 microorganisms were isolated from mastitic milk samples. Staphylococci (figure 1) represented 44.5% of the isolated microorganisms. Other bacteria represented 49.1 of the total isolates (figure 2). Other bacteria included *Streptococcus dysagalactiae* (5.0%), *Str. ubris* (3.7%), *Str. pneumoniae* (2.3%), *Enerococcus faecalis* (26.3%), *Corynebacterium bovis* (2.7%), *Actinomyces pyogenes* (2.0%), *Micrococcus varians* (3.7%), *M. kristini* (1.7%), *Escherichia coli* (9.4%), *Klebsiella pneumoniae* (5.4%), *Enterobacter cloacae* (3.0%), *Serratia marcescens* (3.0%), *E. aerogenes* (2.7%) and *S. liqifacans* (2.0%) (table 2) (figure 3).

Fungal isolates represented 6.4% of the total isolates (figure 2).

Bacterial Isolates from Milker's' Hands

According to the cultural characteristics, bacterial morphology, biochemical reactions results, and API rapid systems results, a total of 62 bacteria isolated from milker's' hands. Staphylococci represented 44.3% of the isolated microorganisms. Staphylococci isolates included *S. epidermidis* (20.0%), *S. hyicus* (8.0%), *S. capitis* (8.0%), *S. saprophyticus* (8.0%), *S. caseolyticus* (4.0%), *S. simulans* (4.0%), *S. sciuri* (4.0%), *S. xylosus* (4.0%), and *S. lentus* (4.0%) (table 3).

Other bacteria represented 59.7 of the total isolates from milker's hands. Other bacteria include *Str. pneumoniae* (1.6%), *E. faecalis* (12.9%), *C. bovis* (3.2%) and *A. pyogenes* (1.6%), *M. kristini* (3.2%) and *M. varians* (4.8%), *E. coli* (12.9%), *K. pneumonia*e (11.3%), *E. aerogenes* (3.2%) and *E. cloacae* (3.2%) (Table 4).

Sixty percent of staphylococci isolated from milker's' hands samples were also isolated from mastitic milk samples (table 5). This may indicates that, milker's may play an important role in the transmission of mastitis, under unhygienic conditions.

Table 1 Result of Bacterial Viable Counts of Mastitic Milk Samples

Farm	No. of Samples	Grade A milk sample	Grade B milk sample	Grade C milk sample	Grade D milk sample
Khartoum university	5	3	1	1	0
Shigla	6	1	3	2	1
Sudan University	5	1	2	2	0
Selate	10	1	3	6	2
Mygoma	10	1	2	5	2
Helat kuku	10	1	2	6	1
El Ruduan	6	1	2	4	1
Gabal Toureia	10	1	3	3	1
El Fetaihab	7	1	2	2	1
El Saig	6	1	3	2	1
Soba	5	1	2	6	1
El Azhari	20	3	2	6	1
Total	100	16	27	45	12

- Grade A milk sample = 50,000 – 100,000 CFU / ml
- Grade B milk sample = 100,000 – 200,000 CFU / ml
- Grade C milk sample = 200,000 – 1,000,000 CFU / ml
- Grade D milk sample > 1,000,000 CFU / ml

Table 2 Identified Streptococci, Micrococci, Corynebacteria and Gram-Negative Bacteria Isolated From Mastitic Milk Samples

Isolated microorganisms	Percentage	Isolated microorganisms	Percentage
Streptococcus pneumoniae	2.3%	*Micrococcus kristini*	1.7%
Streptococcus dysagalactiae	5.0%	*Enterobacter aerogenes*	2.7%
Streptococcus ubris	3.7%	*Enterobacter cloacae*	3.0%
Enterococcus faecalis	2.7%	*Serratia marcescens*	3.0%
Coynebacterium bovis	2.7%	*Serratia liquifacans*	2.0%
Actinomyces pyogenes	2.0%	Moulds	2.7%
Micrococcus varians	3.7%	Yeasts	3.7%

Table 3 Staphylococci Isolated From Hands of Milker's

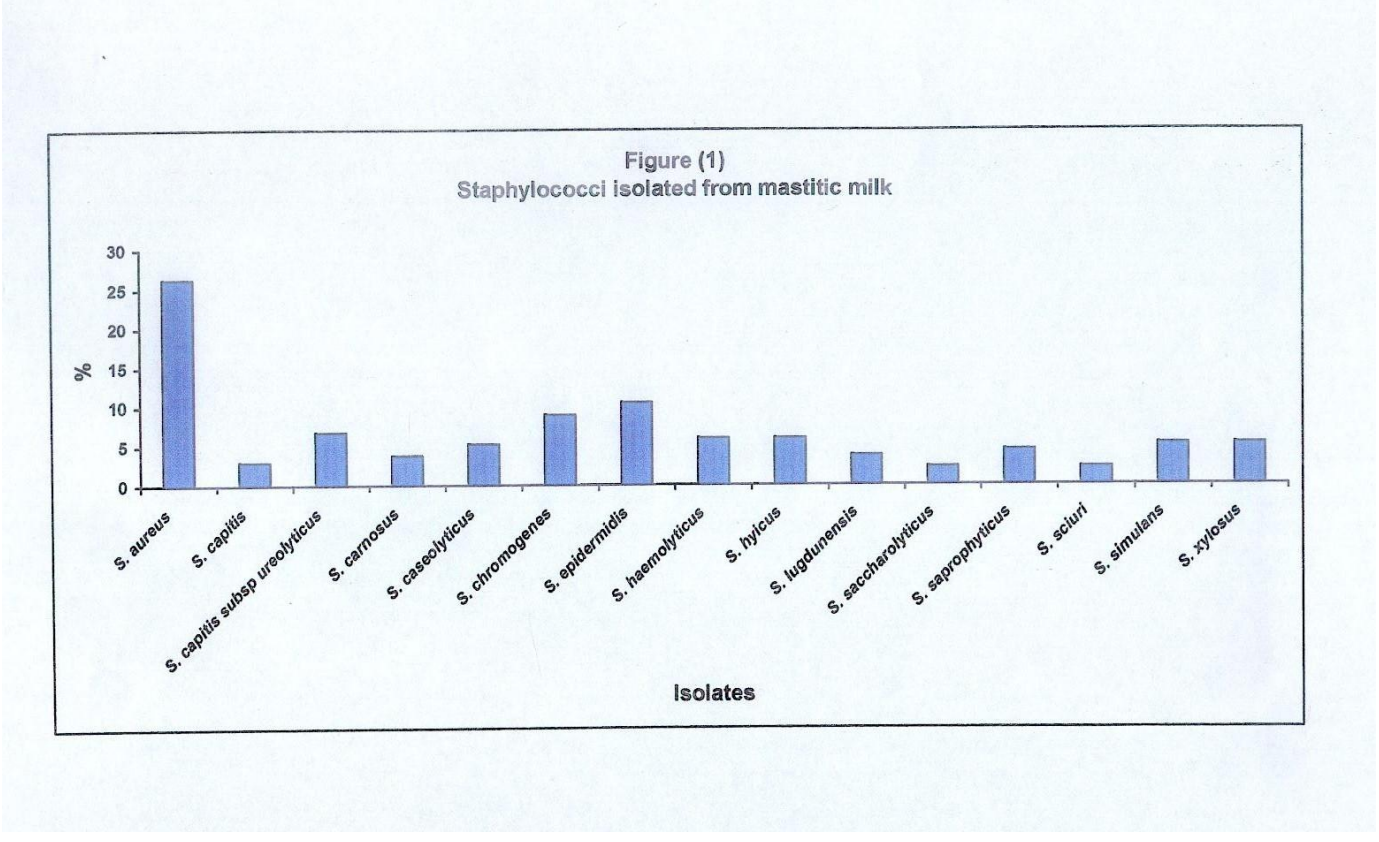
Staphylococcus species	Percentage	Staphylococcus species	Percentage
S. aureus	36.0%	*S. simulans*	4.0%
S. caseolyticus	4.0%	*S. sciuri*	4.0%
S. epidermidis	20.0%	*S. xylosus*	4.0%
S. hyicus	8.0%	*S. saprophyticus*	8.0%
S. capitis	8.0%	*S. lentus*	4.0%

Table 4 Bacteria Isolated from Milker's Hands

Bacterial isolates	Percentage	Bacterial isolates	Percentage
Staphylococcus species	40.3%	*Streptococcus pneumoniae*	16.1%
Escherichia coli	12.9%	*Klebsiella pneumonia*	11.3%
Corynebacterium bovis	3.2%	*Actinomyces pyogenes*	1.6%
Micrococcus varians	4.8%	*Enterobacter aerogenes*	3.2%
Micrococcus kristini	3.2%	*Enterobacter cloacae*	3.2%

Table 5 Comparison between Bacteria Isolated from Mastitic Milk Samples and Hands of Milker's

Bacterial isolates	Mastitic milk isolates (%)	Milker's' hands isolates (%)
Staphylococcus species	44.5%	40.3%
Streptococcus dysagalactiae	5.0%	0.0%
Streptococcus ubris	3.7%	1.6%
Streptococcus pneumoniae	2.3%	1.6%
Enterococcus faecalis	2.7%	12.9%
Actinomyces pyogenes	2.0%	1.6%
Corynebacterium bovis	2.7%	3.2%
Micrococcus kristini	1.7%	3.2%
Micrococcus varians	3.7%	4.8%
Echerichia coli	9.4%	12.9%
Klebsiella pneumoniae	5.4%	11.3%
Enterobacter aerogenes	3.0%	3.2%
Enterobacter cloacae	3.0%	3.2%
Serratia marcescens	3.0%	0.0%
Serratia liquifacans	2.0%	0.0%



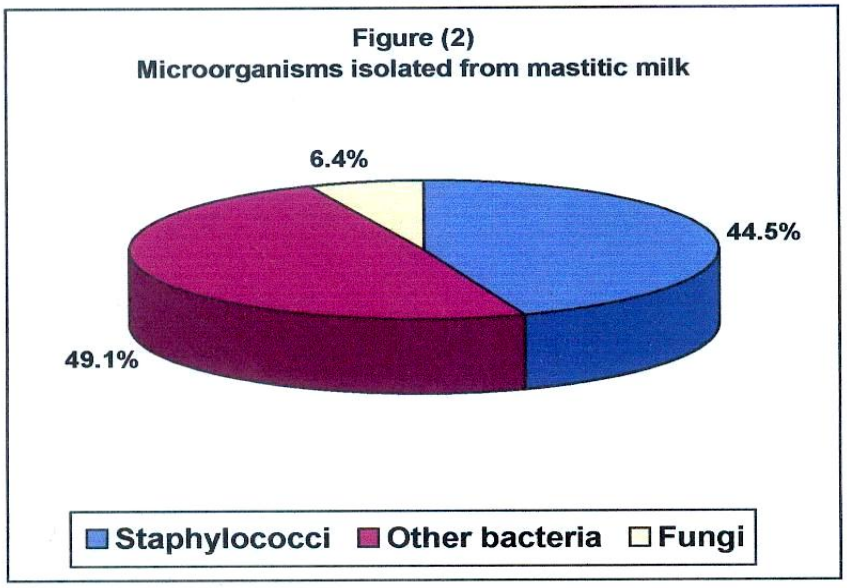


Figure (2)
Microorganisms isolated from mastitic milk
6.4%
44.5%
49.1%
Staphylococci
Other bacteria
Fungi

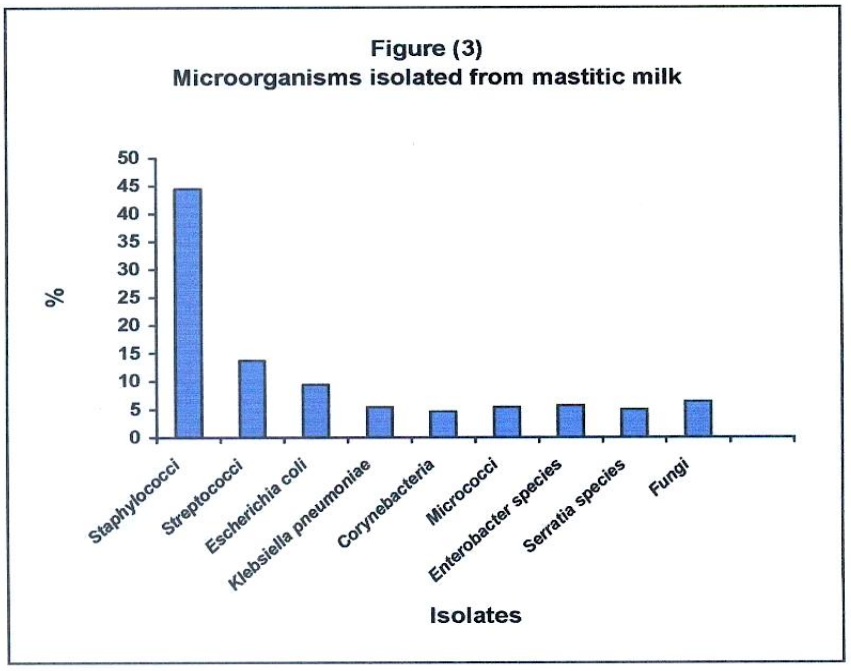


Figure (3)
Microorganisms isolated from mastitic milk
%
50
45
40
35
30
25
20
15
10
5
0
Staphylococci
Streptococci
Escherichia coli
Klebsiella pneumoniae
Corynebacteria
Micrococci
Enterobacter species
Serratia species
Fungi
Isolates

Mastitis is a complex disease caused by several microorganisms [19]. Mastitis is one of the most important destructive infectious diseases of dairy cattle industry. It is considered of quite vital importance to the public health as it is associated with many zoonotic diseases in which milk acts as a vehicle for the infectious agents [20].

In this study 100 bovine mastitic milk samples were collected from different quarters of the 46 producing dairy cows positive for CMT and were further investigated for SCC and bacteriological cultures.

In the 100 bovine mastitic milk samples examined in this study bacterial viable count was more than 50,000 CFU/ml. This reveals a close positive relationship between isolation of bacteria from mastitic milk samples and CMT. This confirms previous findings that CMT is a good diagnostic tool for the detection of subclinical mastitis [21]. The culture method may then be used for confirmation and as an aid to proper treatment [22].

The bacterial analysis provides information on the primary organisms present in the herd which may cause mastitis [23]. Out of 100 bovine mastitic milk samples obtained from 46 dairy cows 299 microorganisms were isolated. Staphylococci represented 44.5% of the total isolates. *S. aureus* represented 73.7% of the staphylococci. These findings show a high prevalence of staphylococci in dairy herds in Khartoum area. [24] found that, *S. aureus* was responsible for 30-40% of subclinical cases of bovine mastitis and over 30% subclinical cases of bovine mastitis were caused by coagulase-negative staphylococci. [2] mentioned that *S. aureus* is the first microorganism incriminated in bovine mastitis. Other staphylococci included *S. epidermidis* (10.5%), *S. chromogenes* (9.0%), *S. haemolyticus* (6.0%), *S. hyicus* (6.0%), *S. simulans* (5.3%) and *S. xylosus* (5.3%), which were implicated in bovine mastitis elsewhere [25].

Other staphylococci: *S. capitis subsp ureolyticus* (6.8%), *S. caseolyticus* (5.3%), *S. carnosus* (3.8%), *S. lugdunensis* (3.8%), *S. saprophyticus* (4.5%), *S. capitis* (3.0%), *S. saccharolyticus* (2.3%) and *S. sciuri* (2.3%), have not been proven to cause mastitis in dairy cattle. However, *S. capitis subsp. ureolyticus, S. caseolyticus* and *S. capitis* were recovered from bovine mastitic milk, whereas *S. carnosus* and *S. lugdunensis* were isolated from normal milk of cows [26] and *S. sciuri* was isolated from normal milk of cows [27].

Other bacteria found in mastitic milk samples in this study included: coliforms, streptococci, micrococci and corynebacteria. Forty one (14.7%) out of 280 bacteria isolated from 100 bovine mastitic milk samples were streptococci. [13] reported a high incidence of bovine subclinical mastitis in Khartoum and the commonest species of bacteria isolated were: *Enterococcus faecalis, Enterococcus faecium, Streptococcus bovis, Str. Pyogenes* and *Lactococcus lactis.* Micrococci were found in 16 (5.7%) out of 280 bacteria in this study and corynebacteria were found in 14 (5.0%) out of 280 bacteria. [9] isolated micrococci and corynebacteria from milk of cows suffering from subclinical mastitis. Coliform bacteria were also high prevalent. In this study *Echerichia coli* constituted 28 (10.0%) out of 280 bacteria isolated. [28] found that 20% of cases of bovine mastitis in Nordic countries were caused by coliforms of which about 85% were *E. coli*; in the rest *Klebsiella spp* and other enterobacteria were isolated. In agreement with [28] *Klebsiella pneumoniae* (5.7%), *Enterobacter cloacae* (3.2%), *Enterobacter aerogenes* (2.9%), *Serratia marcescens* (3.2%) *and Serratia liqifacans* (2.1%) came after *E. coli* as causes of bovine mastitis.

In this study Moulds and Yeasts were found in 19 (6.4%) of the total isolates. [29] mentioned that, the frequency of mycotic mastitis in Nordic countries varied: in some herds it was almost unheard of, whereas in others 20-25% of the mastitis cases were reported to be caused by yeasts and moulds.

Milker's and milking machines act as sources of bovine mastitis. In this study 40 swabs from milker's hands were cultured for bacteria. Sixty percent of staphylococci isolated from milker's hands samples were also isolated from mastitic milk samples. These staphylococci were: *S. aureus, S. capitis, S. caseolyticus, S. epidermidis, S. hyicus, S. saprophyticus S. sciuri, S. simulans* and *S. xylosus.*

Seventy eight point six percent of bacteria other than staphylococci were found in milker's hands samples and mastitic milk samples. These bacteria were: *Str. ubris, Str. Pneumoniae, Enterococcus faecalis, A. pyogenes, C. bovis, M. kristini, M. varians, E. coli, K. pneumoniae, Enterobacter cloacae and E. aerogenes.* [30] found that bacteria isolated from sources of contamination: utensils, milker's hands and teat surface were similar to those isolated from bovine mastitic milk samples.

The majority of bacteria isolated from milker's hands in this finding in order of frequency were: staphylococci (40.3%), coliforms (30.6%) then streptococci (16.1%).

Conclusion

This study clearly revealed the close positive relationship between isolation of bacteria from mastitic milk samples and CMT. This confirms previous findings that CMT is a good diagnostic tool for the detection of subclinical mastitis. The study also revealed that staphylococci are the predominant bacterial spp. isolated from bovine mastitic milk samples and milker's' hands in Khartoum State. Moreover, Staphylococci are involved in bovine mastitis.

Other bacteria found in mastitic milk samples in this study included: coliforms, streptococci, micrococci and corynebacteria which are responsible for bovine subclinical mastitis. According to frequency of isolation, coliforms came in second place to staphylococci as causes of bovine mastitis followed by streptococci.

Milker's hands act as a source of contamination and may transmit bovine mastitis. Further studies should be carried out to investigate the predisposing factors related to the incidence of bovine mastitis and to identify different causes of bovine mastitis. Further studies should include a survey of more animals in different farms and an extensive study of the significance of different microorganisms in bovine mastitis. Moreover the serotyping of isolates obtained from different areas should be given more attention.

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