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Aerobic Bacteria Isolated from Cases of Bovine Subclinical Mastitis in White Nile State, Sudan

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Abstract:

In Sudan clinical and subclinical mastitis leads to a substantial drop in milk production which may reach 20%. Clinical mastitis can be detected by visual examination of both milk and mammary glands as any abnormalities can be detected easily. Diagnosis of subclinical mastitis presents a problem due to unapparent signs; however several screening tests are in use besides culturing methods. The study was aiming for isolation and identification of aerobic bacteria implicated in dairy cattle mastitis in White Nile State (Sudan). In this investigation a total of 83 bacterial isolates were obtained from 80 mastitic milk samples collected from different localities of White Nile State. According to the cultural characteristics, bacterial morphology and biochemical reactions results, the identified bacteria were: 15 Staphylococcus aureus (18.3%), 7 Staphylococcus epidermidis (8.4%), 4 Staphylococcus chromogenes (4.8%), 7 Streptococcus uberis (8.4%), 9 Bacillus subtilis (10.8%), 5 Micrococcus variens (6.0%), 3 Micrococcus luteus (3.6%), 13 Escherichia coli (15.7%), 11 Pseudomonas aerogenosa (13.3%) and 9 Klebsiella pneumoniae (10.8%). Gram positive Bacteria represented the higher

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percentage (60.2%) compared to gram negative bacteria which represented 39.8% of the total bacteria isolated from mastitic milk samples collected from White Nile State. Coagulase-positive and coagulase-negative staphylococci are involved in bovine mastitis in White Nile State. Coliforms came in first place (39.9%) as a cause of bovine mastitis in White Nile State followed by Staphylococci (31.5%), B. subtilis (10.8%), Micrococci (9.6%) and Str. uberis (8.4%).

Key words: aerobic bacteria, bovine subclimical mastitis, White Nile State, Sudan

I. INTRODUCTION:

Mastitis is defined as inflammation of the udder irrespective of the cause. Two forms of mastitis are known viz clinical and subclinical mastitis. Despite extensive research and control efforts, mastitis remains a major problem for the dairy industry. The disease is complex and may be caused by a large number of organisms [1]. Over 130 different microorganisms have been isolated from bovine mastitic milk samples, but *S. aureus*, Streptococcus spp. and members of *Enterobacteriaceae* are among the most common aetiological agents in cows and in other animal species. Invasion of the mammary gland by microorganisms is characterized by an increased leukocyte count in the milk, the majority of cells being neutrophils [2]. Over 95% of subclinical and more than 60% of clinical cases of mastitis in the Nordic countries is caused by Gram–positive cocci. Of these, the most common pathogen is *S. aureus*, which was found to be responsible for 30–40 % of subclinical cases and 20–30% of acute cases. The importance of coagulase – negative staphylococci (CNS) has increased during recent years. Over 30% of subclinical cases and nearly 20% of acute cases were found to be caused by CNS [3]. 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* [4]. High incidence of subclinical mastitis was reported in Khartoum and commonest species of bacteria isolated were: *Ent. faecalis, Ent. faecium, Str. bovis, Str. equi subsp equi, Lactococcus lactis* and *Str. pyogene*s. *S. aureus* was considered as the major bacterium isolated from bovine clinical mastitis followed by *Str. agalactiae* [5]. Other organisms isolated include: *B. cereus* [6], *E. coli* [7], *K. pnumoniae* and *S. epidermidis* [8]. [9] isolated *S. aureus* from 20.48% of mastitic bovine milk samples and *S. epidermidis from* 28.7% samples. [10] reported that staphylococci were responsible for 24.5% of mastitis in cows. Bovine *S. aureus* strains express many The economic consequences of mastitis are related to increased treatment cost, reduction in milk yield, discarded milk, increase in culling and replacement rates and financial penalties for exceeding legal milk quality limits. [11] reported that Coagulase-negative staphylococci are suspected to be significant as a cause of mastitis especially in quarters with high SCC. The coagulase-negative staphylococci isolated in order of frequency were: *S. epidermidis* (10.3%), S. chromogenes (9.0%), *S. capitis subsp. ureolyticus* (6.8%), *S. haemolyticus* (6.0%), *S. hyicus* (6.0%), S. caseolyticus (5.3%), *S. simulans* (5.3%), *S. xylosus* (5.3%), *S. saprophyticus* (4.5%), *S. carnosus* (3.8%), S. lugdunensis (3.8%), *S. capitis* (3.0%), *S. saccharolyticus* (2.3%) and *S. sciuri* (2.3%).

This study was aiming for solation and identification of aerobic bacteria implicated in dairy cattle mastitis in White Nile State (Sudan).

II. MATERIALS AND METHODS:

Area of Study:

This study was conducted out in White Nile State during the years 2017 and 2018.

Source of samples:

A total of 80 milk samples from mastitic cows were collected from different localities of White Nile State (Sudan).

Sampling procedure:

Before collection of milk samples from the tested cows, the udder was thoroughly be 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 collected samples were put in ice box containing ice and transported to the laboratory. In the laboratory mastitic milk samples were kept in a deep-freezer. 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.

California Mastitis Test (CMT):

The California Mastitis Test was carried out at the side of cows to diagnose the presence of subclinical mastitis based on the method described by [12]. Accordingly, udder and teat were washed with water and dried before milk sample collection; first few strips of milk were removed and discarded, A squirt of milk from each quarters of the udder was placed in each of four shallow cups in the CMT paddle. Mixture of 2ml of milk with an equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane and a positive gelling reaction occurred in a few second with positive samples. The result was scored from negative without gelling to positive from slight gelling to viscous form.

Primary isolation:

Three loopfull from milk sample were streaked on Blood agar, McConkey'sagar, and Nutrient agar and then the streaking over the plate was completed using the wire loop.

Incubation of culture:

All inoculated solid and liquid media were incubated aerobically at 37C^{o} for 18-24 hours.

Examination of cultures:

Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis whereas, broth media were checked for turbidity, change in colour, accumulation of gases in Carbohydrates media and for sediment formation.

Preparation and staining of smears:

From one colony on each plate one half was taken with a sterile loop, emulsified in a drop of normal saline on a clean microscopic slide. The smear was allowed to dry and then fixed by passing the slide over a flame. The slides were placed on the rack and flooded with crystal violet stain for one minute and rinsed with water. They were then covered by iodine for a minute and rinsed with water. Alcohol was poured and immediately the slides were rinsed with water. The slides were counter stained with neutral red for two minutes and rinsed with water again and allowed to dry by blotting with filter paper. A drop of immersion oil was added to each slide and examined under microscope. Colonies which showed Gram–positive cocci, Gram positive bacilli and Gram-negative bacilli were subcultured on nutrient agar.

Subculturing and purification:

Purification was based on the characteristics of colonial morphology and smear. Discrete colonies were picked, smeared,

fixed, and Gram–stained. Then the same colonies were subcultured on nutrient agar.

Biological and biochemical identification:

The purified isolates were identified as previously described [13] and [14]. 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, gelatine hydrolysis test, nitrate reduction test).

III- RESULTS:

Bacteria isolated from mastitic milk samples collected from White Nile State:

In this investigation a total of 83 bacterial isolates were obtained from 80 mastitic milk samples collected from different localities of White Nile State. According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 2) the identified bacteria were: 15 *S. aureus* (18.3%), 7 *S. epidermidis* (8.4%), 4 *S.* chromogenes (4.8%), 7 *Str. uberis* (8.4%), 9 *B. subtilis* (10.8%), 5 *M. variens* (6.0%),3 *M. luteus* (3.6%),13 *E. coli* (15.7%), 11 *Ps. aerogenosa* (13.3%) and 9 *K. pneumoniae* (10.8%) (Table 1). Gram positive Bacteria represented the higher percentage (60.2%) compared to gram negative bacteria which represented 39.8% of the total bacteria isolated from mastitic milk samples (Figure 1). Staphylococci represented the predominant bacteria (31.5%) isolated from mastitic milk samples compared to other bacteria *E. coli* (15.7%), *Ps. aerogenosa* (13.3%), *K. pneumoniae* (10.8%), *B.*

subtilis (10.8%), Micrococci (9.6%) and *Str. uberis* (8.4%) (Figure 2).

Table (1)
Bacteria isolated from mastitic milk samples collected from different localities of White Nile State.

Bacterial Isolates	Kosty locality	Asalaya locality	Rabak locality	Kenana locality	Elgitainah locality	Total
B. subtilis	1	2	3	2	1	9
S. aureus	2	3	5	2	3	15
S. epidermidis	1	2	2		2	7
S. chromogenes	1		1	2		4
Micrococcus spp.	1	2	2	1	2	8
Str. uberis	1	2	2	1	1	7
Ps. Aerogenosa	2	3	2	2	2	11
K. pneumoniae	1	2	2	2	2	9
E. coli	2	2	3	4	2	13
Total	12	18	22	16	15	83

Table (2)
Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	*E. coli*	*S. aureus*	*S. epidermidis*	*S. chromogenes*	*Str. uberis*
Aerobic growth	+	+	+	+	+
Colonies on MacConkey	Bright pink	Pink	Pink	Pink	Pink
Haemolysis on blood agar	+	+	-	-	-
Gram reaction	-	+	+	+	+
Shape	Rods	Cocci	Cocci	Cocci	Cocci
Spore	-	-	-	-	-
Motility	+	-	-	-	-
Catalase	+	+	+	+	-
Oxidase	-	-	-	-	-
Indole	+	-	-	-	-
Methyl red	+	+	+	+	-
VP	-	-	+	-	+
Citrate	-	-	-	-	-
H_2S	-	-	-	-	-
O/F	+	+	+	-	+
Glucose	+	+	+	-	+
Lactose	+	+	+	+	+
Coaggulase	-	+	-	-	-

Table 2 (continued)

Test	Ps. *aerogenosa*	*K. pneumoniae*	*M. luteus*	*M. variens*	*B. subtilis*
Aerobic growth	+	+	+	+	+
Colonies on MacConkey	Bright pink	Pink	Pink	Pink	Pink
Haemolysis on blood agar	+	-	+	-	+
Gram reaction	-	-	+	+	+
Shape	Rods	Rods	Cocci	Cocci	Rods
Spore	-	-	-	-	+
Motility	+	-	-	-	+
Catalase	+	+	+	+	+
Oxidase	+	-	+	+	-
Indole	-	+	-	-	-
Methyl red	-	-	-	-	-
VP	-	-	-	-	-
Citrate	+	+	-	-	-
H_2S	-	-	-	-	-
O/F	+	+	-	+	+
Mannose	-	+	+	+	+
Nitrate	-	+	+	+	-
Coaggulase	-	-	-	-	-

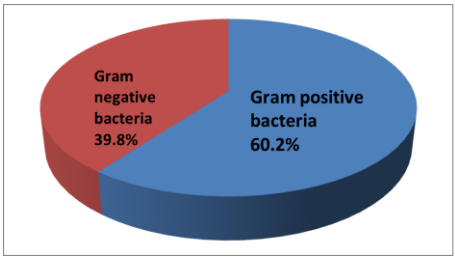

Fig (1): gram positive and gram negative bacteria isolated from mastitic milk samples collected from White Nile State.

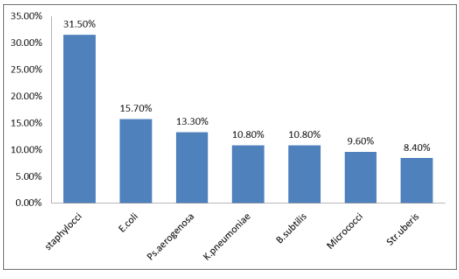

Fig (2): Bacteria isolated from mastitic milk samples collected from White Nile State.

IV. DISCUSSION:

Infection or injury of the udder results in an increase in somatic cells. Somatic cells are composed of approximately 75 percent leucocytes and 25 percent epithelial cells. Epithelial cells are in fact dead cells which have been sloughed from alveoli and canals within the udder. Somatic cells are useful in detecting subclinical mastitis which has been shown to be the most costly form of mastitis. Rising levels of somatic cells act as an early indicator of future clinical mastitis. As a cow-side test, the California Mastitis Test (CMT) can be useful in detecting and controlling mastitis since it focuses attention on the individual quarters that are secreting milk with high number of leucocytes. It is practically useful in detecting subclinical and chronic mastitis. Most clinical cases begin as subclinical mastitis while the chronic cases serve as constant reservoir of mastitis causing organisms. Problem cows can be identified by the Somatic Cell Count (SCC) score [15]. In this study a total of 83 bacterial isolates were obtained from mastitic milk sample collected from different localities of White Nile State. The identified bacteria were: *S.aureus* (18.3%), *S. epidermidis* (8.4%), *S.* chromogenes (4.8%), *Str.uberis* (8.4%), *B. subtilis* (10.8%), *M. variens* (6.0%), *M. luteus* (3.6%), *E. coli* (15.7%), *Ps. aerogenosa* (13.3%) and *K. pneumoniae* (10.8%). Staphylococci represented the predominant bacteria isolated from White Nile State (31.5%). [16] isolated many bacteria from cases of subclinical and clinical mastitis. These include: *S. aureus*. [17] reported that Staphylococci represented 44.5% of the microorganisms isolated from mastitic milk samples collected from Khartoum State. [11] reported that coagulase-positive *Staphylococcusaureus* represented 26.3% of the total bacteria solated from River Nile State and coagulase-negative Staphylococc were 73.7%. The isolated coagulase-negative staphylococci from River Nile State included *S. epidermidis* (10.3%) and *S. chromogenes* (9.0%). [7] found that coagulase-

negative staphylococci represented 70.7% of the total Gram-positive bacteria isolated from bovine mastitic milk. *E. coli* represented 15.7%, *Ps. aerogenosa* 13.3% and *K. pneumoniae*10.8% of the total bacteria isolated from the State. [18] 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. Ribeiro *et al.* [1] mentioned that *E. coli* and *K. pneumonia* are worldwide recognized as the predominant coliform microorganisms involved in bovine mastitis. [19] mentioned that Pseudomonas spp. is environmental mastitis-causing pathogens. [20] reported that coliform bacteria represented 28.4% of the total bacteria isolated from bovine mastitic samples collected from River Nile State. The isolated coliforms were *E. coli* (12.4%) and *K. pneumoniae* (8.4%). [16] isolated *K. pneumonia* from cases of subclinical and clinical mastitis. *Str. uberis* represented 8.4% of the total bacteria isolated from the State. [21] isolated *Str. uberis* from mastitic bovine milk samples. [22] mentioned that *Stre. uberis* is worldwide known as an environmental pathogen responsible for a high proportion of cases of clinical, mostly subclinical mastitis in lactating cows and is also the predominant organism isolated from mammary glands during the non-lactating period. [16] isolated *Str. ubris* from cases of subclinical and clinical mastitis. Micrococci represented 9.6% of the total bacteria isolated from the State. [23] isolated Micrococcus spp. From mastitic milk samples. [24] isolated Micrococci from clinical mastitis. [17] reported that Micrococci represented 5.4% of the microorganisms isolated from mastitic milk samples collected from Khartoum State. *B. subtilis* represented 10.8% of the total bacteria isolated from the State. [19] reported that most strains of Gram positive spore forming bacteria can cause bovine mastitis like *Bacillus* spp. [25] isolated *B. subtilis* (9%) from mastitic milk samples. [26] reported that *B. subtilis* can cause mastitis.

V. CONCLUSION:

From this study we conclude the following:

1. CMT and SCC are valuable in detecting subclinical cases of bovine mastitis.

2. Staphylococci constituted 31.5% of the total bacterial isolates from mastitic samples collected from White Nile State.

3. Coagulase-positive and coagulase-negative staphylococci are involved in bovine mastitis. Coagulase-negative staphylococci should be suspected as a cause of bovine mastitis when accompanied with a high SCC.

4. Coliforms came in first place (39.9%) as a cause of bovine mastitis in White Nile State followed by Staphylococci (31.5%), *B. subtilis* (10.8%), Micrococci (9.6%) and *Str. uberis* (8.4%).

VI. ACKNOWLEDGEMENT

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