

Introduction

Mastitis is in ammation of the mammary gland due to the injury of any type. However, the udder disease of major concern is that associated with microbial infection. e microbes that are associated with mastitis are: S a ____ c. cc species, S. , c. cc species and other Gram positive and Gram negative rods [1]. Mastitis is characterized by physical, chemical and usually bacteriological changes in milk and by pathological changes in the glandular tissue [1]. Loss of milk production, cows premature culling, milk discarded or downgraded as well as veterinary expenses and large of some of money is lost to dairy farming each year through poor udder health [2]. Immunoglobulins in mammary secretion are derived from blood serum or are made locally by cells of the lymphocyte-plasma cell series situated close to the e major immunoglobulin in colostrum and glandular epithelium. milk of ruminants, IgG1 is derived from the blood and is transferred into secretion selectively relative to IgG2, probably by a mechanism requiring speci c receptor sites on the basal or intercellular membrane of the glandular epithelium. Acute in ammation causes suppression of selective transfer of IgG1, but there is a marked increase in the transfer of proteins, such as IgG2 and serum albumin, which enter secretion non selectively. Infusion of antigen into the mammary gland of ruminants some weeks before parturition induces a persisting local production of antibody, most of which is associated with IgA and IgM. IgA antibodies in the mammary gland probably originate in the intestine, and prior antigenic stimulation of the gut may be required for maximal IgA antibody responses in the gland [3].

Substantial increases in immunoglobulin G subclass 1 (IgG1) and IgG2 antibody titers were detected in serum and lacteal secretions of animals immunized through an intestinal stula. IgM and IgA antibody responses were low or undetectable. Low numbers of IgA and IgG1 plaque-forming cells were occasionally detected. It

stimulated IgG lymphoblasts and perhaps of antigen, to spleen and peripheral lymph nodes may be dominant events a er intestinal immunization of ruminants. is is consistent with the predominance of serum-derive IgG antibodies in colostrums and milk. Intramammary infusion of antigen gave rise to increases in antibody titers in all classes which were greater not only in lacteal secretions but also in blood serum than with their systemic route used. Comparison of IgA titers in secretions from the immunized glands with those in serum also suggest that locally synthesized IgA antibodies might have contributed in some measure to serum titers. Local synthesis in both immunized and non immunized glands was also re ected by the presence of increased numbers of IgA and IgG1 plaque-forming cells. It was hypothesized that antibody forming cells responsible for local synthesis originated in lymphoid tissue within the mammary gland or from peripheral lymph nodes, depending upon the route of immunization.

e aim of the study to isolate and identify the bacteria which can cause mastitis in cows and the antibodies forming during the infection by these bacteria and appear in milk and serum.

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Methods

A total of 50 milk samples were collected from all quarters of each cow and 50 serum samples were collected in Khartoum State from dairy farms at Alsalama (Khartoum) and Shambat (Khartoum North). Only milk samples from cows that reacted positively to California Mastitis Test (CMT) were collected and subjected to bacteriological and immunological investigation in the laboratory. e swabs were cultured on blood agar, chocolate gar and MacConkey's agar (Oxoid).

e plates were incubated aerobically at 37°C for 1-2 days. Visual examination for detection of growth, pigmentation and colonial morphology. Isolated bacteria were puri ed by repeated subculture on blood agar and nutrient agar plates and incubated at 37°C for 24 hours until pure colonies were obtained. e puri ed bacteria were stored at 4°C. Identi cation of isolates was carried out according to Barrow and Feltham [4]. Smears were prepared by emulsifying small inoculums of the bacterial culture in a drop of sterile normal saline and spreading them on clean slide. e smears were allowed to dry and xed by gentle heating. Gram stain was done as described by Barrow and Feltham [4]. It was used to study morphology, shape and gram staining reaction of each isolates. Gram-positive bacteria appeared purple, while Gramnegative bacteria appeared red. Biochemical test were conducted and preformed according to Barrow and Feltham [4].

Took 30 ml in two sterile falcon tubes (15 ml to each one) and centrifuged at 4000 rpm for 30 minutes at 28°C to detect milk. е whey and cell depress were removed and the protein was taken and put in clean petri dish and mixed with 20% sodium sulphate, stirred with magnetic stirrer for 30 minutes and centrifuged at 4000 rpm for e deposit dissolved in PBS then concentrated 30 minutes at 28°C. by poly ethylene glycol, kept at -20°C [5]. Whole cell lysate of bacterial isolates prepared from isolate that were grown on blood agar and the colonies, put in 2 ml normal saline, 1 ml of excess uid was transferred to other blood agar plate using pipette. e plate was incubated at 37°C for 24-48 h. e colonies were covered with 0.5% formalin saline and le overnight at 4°C to kill the bacteria, then the pellet was washed 2 times with normal saline at 4000 rpm for 30 minutes at 28°C. e deposit was suspended in PBS and sonicated in sonicator (MSE-England) for 30 seconds stroke and in short intervals, the amplitude kept 18 rpm per cycles, then concentrated by poly ethylene glycol and kept at -20°C. Agar gel immune di usion (AGID) test was done to detect the presence of antibodies in milk and sera using Whole cell lysate of the isolates.

Results

In this study 50 milk samples were collected and bacteria was isolated from 38 (76%) of the milk samples. e bacteria were identi ed to the species level as shown in Tables 1-8. Bacteria were isolated from 29 (64%) of milk samples collected from 37 cows with subclinical mastitis as a previously detected by California mastitis test. Mixed infection was found in 7 (14%) of sample. e main bacteria isolated were Staphylococcus species. Mixed infection was found only 10 milk samples (20%) of both clinical and subclinical mastitis. Most isolates were Staphylococcus 27 (60%) all of them were coagulase positive 10 (22%), 9 (20%) S a ... c cc ... c and 8 (18%) d) (Table 1), S , c cc a a ac a was S.a. c. cc 1 (2%) of the isolate (Table 2), Enterococcus species were 4 (9%) (1, (2%) E., c. cc a, , 2 (4%) E., c. cc d and 1 (2%) E, c, cc a c (Table 3), L, a I, a ... were 2 (4%) (Table 4), Bacillus Species were 3 (7%) (2 (4%) Bac... and 1 C (2%) Bac a c d that show heamolysis on blood agar (Table 5), three strain of C _ bac d, b c (7%) were found with three strains strain 1 (2%) was di ered from the main genus

Biochemical Test	

by acid from xylose and hydrolysis of aesculin the two were found positive in this strain but were negative in the main strain, strain 2 (2%) was di ered from the main genus by acid from xylose, Salicin and

Fi y milk and serum samples were collected and bacteria were isolated from only 38 (76%) milk samples. e most common organism isolated in this study was Staphylococcus spp. Coagulase positive staphylococci (60%) were the most frequently isolated bacteria in this study and all of them were isolated from clinical cases these indings were in agreement with the indings other authors [3,6,7]. In this study $S(a_{1,2}, c_{1,2}, c_{2,3}, d_{2,3})$ isolated and this in agreement with the inding by Cha er [8] who was isolate $S(a_{1,2}, c_{2,3}, c_{3,4}, d_{3,5})$ isolated and this is in agreement with Logan [9]. Also an isolated bacterium was $L_{1,2}(a_{1,3}, d_{2,3})$ from mastitic cow and this is in agreement with Rawool [10]. In this study $Bac_{1,2}(c_{1,3}, d_{2,3})$ was isolated from clinical mastitis and $Bac_{1,3}$.

were isolated from sub-clinical mastitis these ndings C were in agreement with the ndings [11] who reported that Bacillus spp. were isolated in both clinical and subclinical cases. *K*. *b* . . . *a* spp. isolated and this is in agreement with Cullor [12], who found that 20% of bovine mastitic case, in Nordic countries caused by coliform of which about 85% were *E. c.*, and *K. b.*, *a* spp., and other Enterobacteria. In this study we isolated coliform bacteria like: K. b. a а d and this is in agreement with ndings by and C bac Jackson and Bramle [13], mentioned that the Coliform such as E. c., K. b. . a P.a, K. b. ...a. ..., ... ca, E. bac caa, d_ are all associated with E. bac a an and C. bac bovine mastitis. Other isolated bacteria were E c c cc ac d_{1} and E_{2} , c_{2} , c_{2} and c_{3} and this is in agreement E c cc with Jayarao [14]. In this study whole cell lysate of bacterial isolates was tested against sera and milk, in order to detect speci c antibodies against the isolates. Some bacterial isolates gave frank precipitation lines with their respecting serum antibodies but not with milk. In a, c, d gave clear precipitation lines with sera and contrast, Bac. milk although it is not common within the bacteria that cause bovine mastitis and this is in agreement with the nding by Carneiro [15]. e signi cance of these antibodies in protection against mastitis [16-21] and the immunology of the udder were reviewed by Carneiro [15].

Conclusion

is study has shown that Gram positive bacteria, especially Staphylococcus species were the common causative agent of bovine mastitis. e number of cows with subclinical mastitis was higher than cows than cows with clinical mastitis. e bacteria associated with mastitis can induce local and systemic speci c antibodies response as measured by agar gel immune di usion test. e research need further study to detect the other antibodies for other bacterial agents to simplify diagnosis of bovine mastitis without culturing milk to detect the speci c bacteria caused mastitis and apply the method to detect the antibodies to other microorganism causing mastitis like virus and parasites.

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