

# COMPARISON BETWEEN THE PERCENTAGE OF INCIDENCE OF MASTITIS CAUSED BY *Bacillus* spp. AND *Staphylococcus* spp. IN WINTER SEASON IN KHARTOUM STATE, SUDAN

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**ABSTRACT:** This study was conducted in certain area at Khartoum State (Eltebna, Falasteen, Shambat, Hilat Kuku, Elhalfaia, Elsamrab and The University of Khartoum farms) in winter season to determine the type of mastitis and to compare between the incidence of mastitis caused by *Staphylococcus* spp and *Bacillus* spp. The total number of dairy cows, which were examined in 34 investigated farms, amounted to 500 animals, but the number of positive cows infected with mastitis were 100. The milk samples were collected from cows due to complain of owners from clinical cases of mastitis. Hundred milk samples were collected from apparent cases of mastitis. All mastitic cases were examined by visual examination and palpation of the udder: 55% acute mastitis, 44% chronic mastitis and 1% gangrenous mastitis were diagnosed. Milk samples were cultured in Blood agar and MacConkey's agar for 24 hours at 37° C. The isolation of *Bacillus* spp. amounted 74% , these constituted 31% *Bacillus coagulans*, 11% *B. cereus*, 9% *B. subtilis*, 9% *B. licheniformis*, 4% *B. circulans*, 2% *B. lentus*, 3% *B. mycoides*, 3% *B. amyloliquefaciens* and 2% *B. megaterium*. The percentages of acute mastitis caused by *B. coagulans* was 14%, *B. subtilis* 8%, *B. lichneformes* 7% and 2% for every followed *Bacillus* spp. (*B. cereus*, *B. circulans*, *B. lentus*, *B. mycoides*, *B. amyloliquefaciens* and *B. megaterium*). The percentage of chronic mastitis caused by *Bacillus* spp. were as follows: *B. coagulans* was 17%, *B. cereus* 9%, 2% for every *Bacillus* spp. (*B. lichneformes*, *B. circulans* and *B. lentus*) and 1% for every followed *Bacillus* spp. (*B. subtilis*, *B. mycoides*, *B. amyloliquefaciens* and *B. megaterium*). *Staph aureus* and *Staph hyicus* amounted to 24% and the percentage of chronic mastitis caused by *Staph aureus* was 44% and that caused by *Staph hyicus* was 8%. The percentage of acute mastitis caused by each species of *Staph* was the same 24%. Other bacteria were isolated from mastitic cows *Corynebacterium* spp. 1% and *Klebsiella* spp. 1% and the last one was isolated from gangrenous mastitis as first report in Sudan.

**Keywords:** Comparison, Incidence, Mastitis, *Bacillus* Spp., *Staphylococcus* Spp., Cattle, Winter, Khartoum, Sudan.

## INTRODUCTION

Mastitis is a multi-factorial disease and very difficult to control. It results from injury, chemical irritation and infection caused by different bacterial species. Mastitis is most expensive disease of dairy animals resulting in the reduction of milk production and quality. These expenses in terms of reduction of production, discarding milk, drug therapy, veterinarian charges, premature culling, and extra use of labour (Anonymous, 1998). Bovine mastitis is the inflammation of the parenchyma cells of the mammary glands of cattle, buffalo and other animals (Radostitis et al., 2007) associated with microbial infections (Schroeder, 1997) and physiological changes (Shouky et al., 1997). Mastitis is caused by a group of infective and potentially pathogenic bacteria (Bezek and Hull, 1995) viruses, fungi and algae (Radostitis et al., 2007).

The bacterial agents responsible to cause inflammation of udder are classified as either contagious or environmental, based upon their primary reservoir and mode of transmission. *Staphylococcus aureus* and *Streptococcus dysagalactiae* are recognized as contagious bacterial species, commonly transmitted among dairy animals through contact with infected milk, but the major pathogen for bovine mastitis is *Staphylococcus aureus* is regarded as being coagulase-positive, although some strains have been suggested in some studies to be coagulase-negative (Fox et al., 1996).

Some other *Staphylococcus* species may also be coagulase-positive (Hajek, 1976; Devriese et al., 1978 and Devriese et al., 2005). Some authors have suggested that infections with minor pathogens like *S. chromogenes* and *Corynebacterium* sp. could be beneficial as they might protect the quarter from mastitis caused by major pathogens such as *S. aureus* (Schukken et al., 1989 and Matthews et al., 1990).

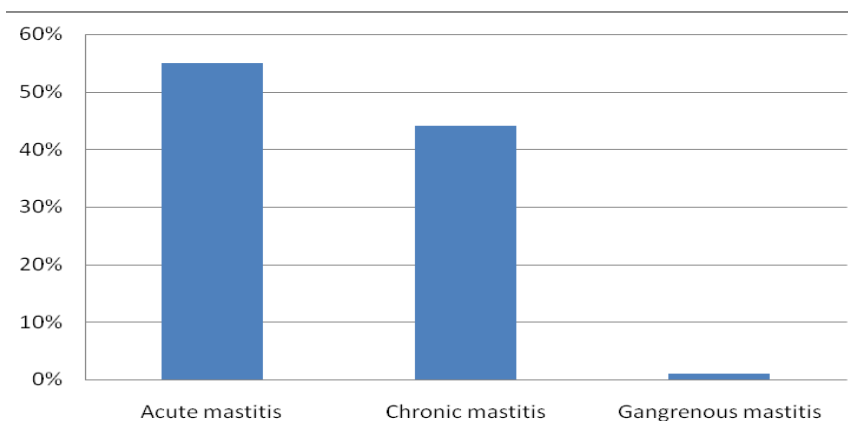
## MATERIAL AND METHODS

One hundred milk samples from clinical mastitis of cows were collected during 2008. Before collection of milk samples, the surroundings of teat canals were cleaned with antiseptics (spirit) and then first few drops of milk were discarded. The milk samples were collected in sterilized bijou bottles and brought to the Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Khartoum. The bacterial culture media were prepared and used for detailed investigation of bacterial organism by (Barrow and Feltham, 2003). Both, solid and broth media were used. In solid media: nutrient, blood and MacConkey's agars and while in broth medium: nutrient broth was prepared; cultured and colony characteristics were recognized.

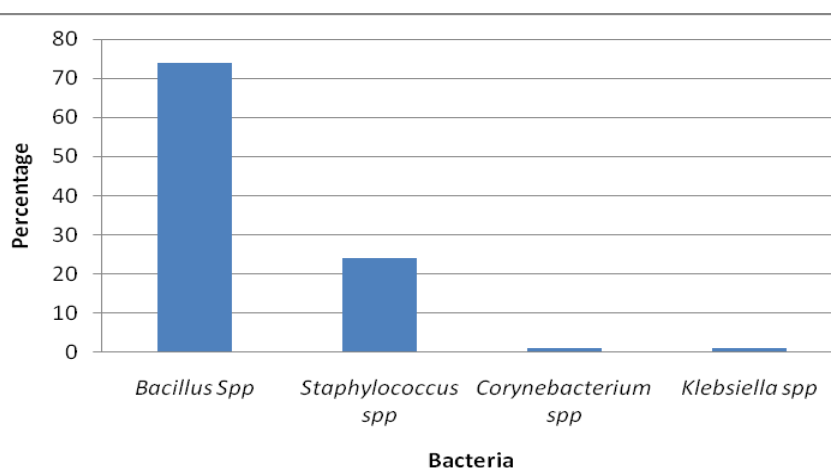
A pure colony from cultured dishes was picked up and smeared on a cleaned glass slide and stained by Gram's Method of staining and staining characteristics were recorded. Furthermore, a few biochemical tests were also conducted to confirm the identification of bacterial organisms, for this purpose, oxidase, catalase, coagulase, indole, oxidation fermentation, urease, methyl red, acid production from sugars (Barrow and Feltham, 2003).

## RESULTS

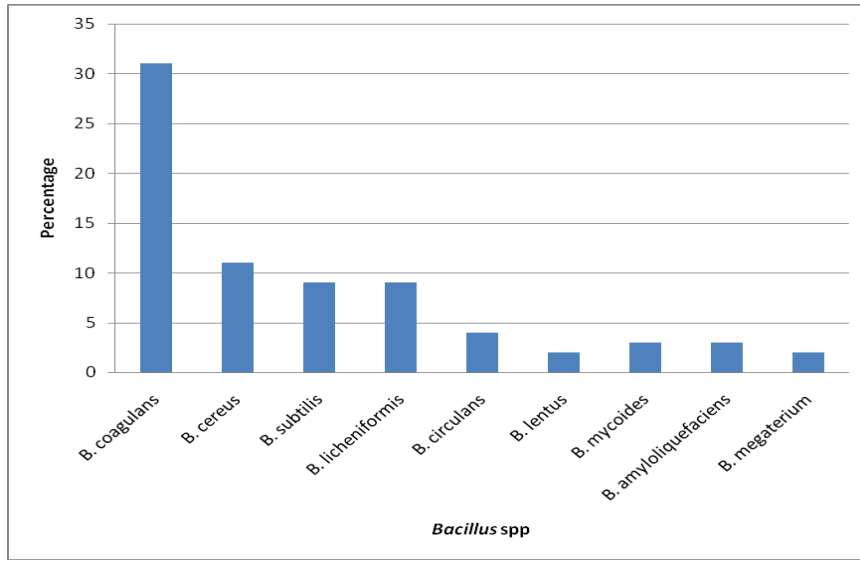
The five hundred samples which were examined and 100 cows were found positive for mastitis. Thirteen bacterial species were recognized from clinical mastitic milk samples of cows. The percentage type of mastitis and bacterial species identified from samples were: Percentage of different type of mastitis diagnosed during this study are shown in Figure 1 acute mastitis 55%, chronic mastitis 44% and gangrenous mastitis 1%. Figure 2 shows the percentage of isolated bacteria compared with *Bacillus* spp. The percentage of *Bacillus* spp. was 74%, *Staphylococcus* spp. 24%, *Corynebacterium* spp. 1% and *Klebsiella* spp. 1%. Figure 3 shows the percentage of isolated *Bacillus* spp. The highest one was *Bacillus coagulans* (31%). Figure 4 shows the percentage of acute and chronic mastitis caused by the isolated *Bacillus* spp. The percentage of acute mastitis caused by *Bacillus* spp. was higher than chronic mastitis. Figure 5 shows the percentage of *Staph* spp. and the type of mastitis caused by the isolated *Staph* spp. In this study the percentage of chronic mastitis caused by *Staph* spp. was higher than acute mastitis. The percentage of acute mastitis was found higher than chronic mastitis.



**Figure 1 - Classification of mastitis according to the clinical state of the mammary gland**

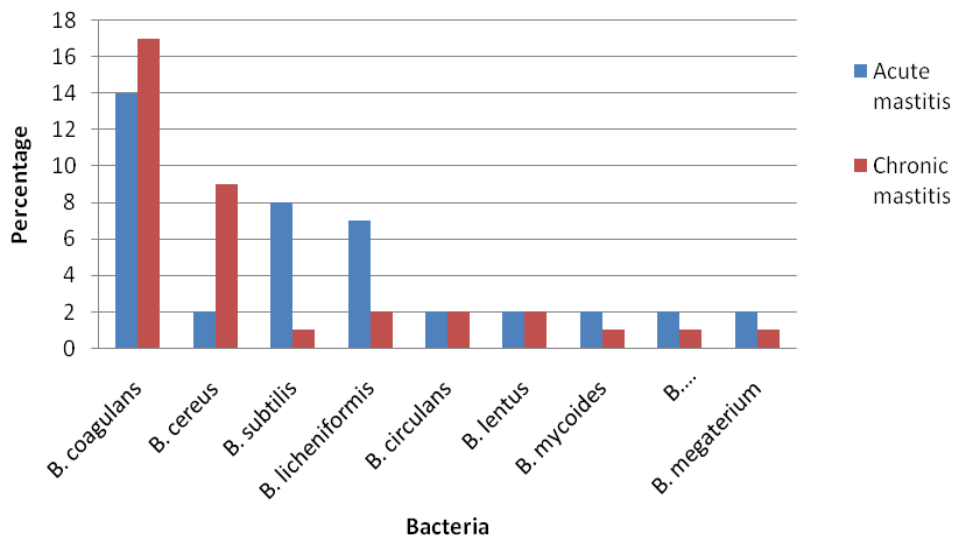


**Figure 2 - Percentage of isolated bacteria compared with *Bacillus* spp. Isolated from 100 mastitic cows**

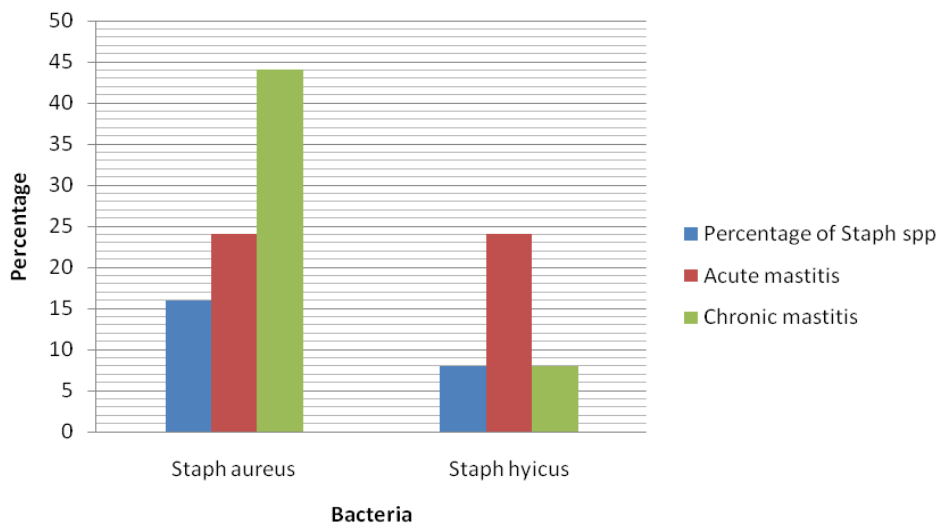


**Figure 3 - The percentage of isolated *Bacillus* spp. from 100 mastitic cows**

Percentage of type of mastitis caused by isolated *Bacillus* spp



**Figure 4 - Percentage of acute and chronic mastitis caused by isolated *Bacillus* spp. from 100 mastitic cows**



**Figure 5. The percentage of *Staph* spp. and the type of mastitis caused by the isolated *Staph* spp.**

## DISCUSSION

Mastitis is an inflammation of the mammary glands regardless of the cause (Blood et al., 1983). It plays a very important role in human health and animal (Kromber and Grabowski, 2002). In this study the types of *Staph* spp. isolated from acute and chronic mastitis were *Staph aureus* and *Staph hyicus*. This agrees with the finding of (DaRong et al., 2010 and Jan et al., 1998). (Radostits et al., 1994) mentioned that *Staph aureus* is the first microorganism incriminated in bovine mastitis. A predominance of *Staph aureus* mastitis in cows has been reported by (Watts, 1988; Falade et al., 1989 and Carlos, 1990). Elsayed, (2000) isolated *Staph aureus* and *Staph hyicus* from 499 milk samples from different domestic animals: cows, sheep, goat and camels. (AlAyies, 2004) isolated *Staph aureus* (73.7%) and *Staph hyicus* (6%) from 100 bovine mastitic milk samples. On conclusion this study confirmed the role of *Staphylococcus* spp. as a cause of mastitis in bovine. The percentage of incidence of bovine mastitis was high according to our findings after examinations.

*Bacillus* species are widely distributed in nature and most species exist in soil, water, dust, air, feces and on vegetation. The first case reported by Brown and Scherer (1957) was attributed to the introduction of the organism during treatment of chronic intra-mammary infections when a single plastic syringe was used by a dairy farmer to infuse the quarters with an antibiotic solution. In this study nine species of *Bacillus* were isolated from acute and chronic mastitis, this in agreement with (Jan et al., 1998). The percentage of incidence of *Bacillus coagulans* was high and this confirms the findings of (Nail et al., 2003). Also *Bacillus cereus* was also isolated by (Nail et al., 2003; Jasper et al., 1972 and Schiefer, 1976). Other species of *Bacillus* were isolated like, *B. licheniformes*, and this in accord with results of (Jones and Turnbull, 1981; Logan, 1988; Nail et al., 2003 and Parvanta, 2000). The isolation of *Bacillus alvei*, *B. subtilis*, *B. megaterium* and *B. cereus* during this study in agreement with (Elgadasi, 2003). *B. licheniformis*, *B. amyloliquefaciens*, *B. circulans*, *B. lentus* and *B. mycoides*, to the best of our knowledge for the first time to be recorded for mastitis in cow in Sudan.

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