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Bacteria Associated with Bovine Mastitis Cows in Khartoum State, Sudan

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Abstract:
Bovine mastitis is the main constrain facing the dairy industry and production in Sudan. Previous surveys and studies conducted in different districts of Sudan showed that the occurrence of bovine mastitis is considerably high. Thus, this study was conducted in some selected dairy farms in Khartoum State in order to isolate and identify the bacteria associated with bovine mastitis. In this study, 150 milk samples were obtained from mastitic cows in East Nile dairy farms; Mahlab-2, Elshigla, El- keraib and El- Selait localities during the period from April 2013 to May 2015. Conventional bacteriological methods for identification were performed for the identification of the isolates. One hundred twenty six pathogenic bacteria were successfully isolated from 150 milk samples of which 100 (79.4%), 23(18.2%) and 3 (2.4%) were Gram positive, Gram negative and yeast respectively. *Staphylococcus* spp. was the most isolated bacteria 67(53.2%). The significance of each categorical variable against positive cases of mastitis was tested by using Chi-square or Fisher Exact test. All statistical tests were conducted using SPSS version 20 (IBM, SPSS) at alpha equal to or less than 0.05 significance level. Our data failed to detect any statistical association between milk source and the characteristic of sampled milk (Chi-square = 1.68, df = 3, p = 0.64). In addition, no association between *Staphylococcus* isolates and their growth pattern was observed in this study (Chi-square = 2.43, df = 3, p = 0.49). This study revealed that a number of pathogenic bacteria of which many are of public health concern were isolated from mastitis cow's milk. Selection of the effective antibiotics for treatment of mastitis together with improvement sanitary conditions during milking may reduce the incidence of mastitis.

Keywords: Mastitis, Bovine, bacteria, conventional methods, Khartoum, State

Introduction

Udder inflammations are still the most frequent and costly diseases affecting dairy cows worldwide (Seeger *et al*., 2003; Losinger *et al*., 2005; Halasa *et al*., 2007; Bar *et al*., 2008). Bovine mastitis is one of the important production diseases of dairy animals, affect the economy of the farmers and ultimately affect the economy of the country by reducing milk production, increase treatment cost, labour and premature culling (Seegers *et al*., 2003, Sharma *et al*., 2007). Nearly 150 species of microrganisms, mostly bacteria are capable to cause mastitis among which are *Staphylococcus* (*Staph*.) *aureus*, coagulase negative *Staphylococcus* (CNS), and *Streptococcus* (*Strep*.) *agalactia.* Environmental *Streptococci* and coliform are predominant etiological agents in both subclinical and clinical mastitis in addition to

Escherichia (*E.*) *coli* the main etiological agent of acute form of the disease (Esron *et al*.,2005; Ferguson *et al*., 2007; Dudko *et al.,*2010). Acute mastitis is in particular the most important bacterial diseases in dairy cattle industries throughout the world resulting in great economical loses to milk producers and milk processing industries (Halasa *et al*.,2007). *Staph*. *aureus*, *Strep*. *agalactia*, *E*. *coli, Klebsiella* (*Kleb*). *pneumonia* and *Enterococcus faecium* are common cause of subclinical mastitis (Ibtisam *et al*., 2006). Recently, a published work has shown that nearly 30% of all animals are infected with *Staph. aureus* (Schukken *et al*., 2009). However, Nuol (2007) found that *Staph aureus*, *Streptococcus*, *Enterobacter*, *Lactobacillus* and Coryneforms organisms are the cause of clinical of mastitis in cows. Furthermore, facultative anaerobic bacteria including *Micrococcus*, *Bacillus*, *Aerococcus*, *E. coli, Acinetobacter, Pseudomonas* (*Pseud.*) and *Proteus* are other causes of clinical mastitis (Ahmed, 2014). *Corynebacterium bovis* is considered as minor pathogen that causing mastitis, the bacterium is frequently isolated from milk samples and is associated with reduced milk production (Watts *et al*., 2000). The objectives of this study were to: isolate the causative agents of bovine mastitis and identify the isolates by conventional bacteriological methods

Materials and Methods

***Sites of the study*:** Dairy farms in Mahlab 2, Elshigla, Elkerib and Elslait located in Khartoum State were selected for samples collection.

***Milk Samples Collection*:** A total of 150 milk samples were collected aseptically from local and cross- breed dairy cattle farms in Khartoum State, Sudan; of which 61(40.7%) from Mahlab-2, 39(26%) from El-Shigla, 24(16%) from El-Kreiab and 26(17.3%) from Seleit with various characteristics (Table 1). The sampled cattle were selected based on a previous or current history of mastitis infection and the willingness of cattle owners to participate in the current study. The teats ends were cleaned with a cotton piece impregnated in 70% alcohol and allowed to dry. The first streams of the milk were discarded then 20 - 25 ml of the milk secretion was collected in sterile plastic containers. All samples were cooled and placed in icebox containing ice and immediately transported to the Department of Bacteriology, Veterinary Research Institute (VRI) and cultured in the same day for bacteriological examination.

***Culturing Procedures*:** The collected milk samples were classified according to their gross appearance into clotted, watery, purulent and bloody (Table 1). A sterile cotton swab was dipped into each of the milk sample then streaked onto 5% sheep blood agar plates (Oxoid, CM 271, UK) and incubated at 37 □C for 24 hours in an aerobic incubator (Scott Science, Model LIB 080M, UK). If no growth was observed, the incubation was continued for 48 hours before the plates were discarded as negative for growth.

Table 1: Distribution and characteristics of the collected samples

Location	Characteristics of the samples				
	Clotted	Watery	Purulent	Bloody	Total
Mahlab-2	30	12	10	9	61(40.7%)
El-Shiga	15	8	12	4	39(26%)
El-Keraib	12	6	6	0	24(16%)
El-Seleit	5	8	10	3	26(17.3%)
Total	62	34	38	16	150(100%)

Isolation and preservation of the isolates: Well isolated representatives of the bacterial colonies were selected according to their morphology and subcultured onto blood agar plate and nutrient agar plates (Oxoid, CM 1, UK) then incubated at 37 □ C for 24 hours. The obtained pure cultures were further subcultured onto blood agar slant, incubated at 37°C for 24 h. then stored in a refrigerator (Coldair, Model H.P, Sudan) at 4 □C for further analysis.

Identification of the isolates:-

***Conventional methods*:-**The purified isolates were identified using standard bacteriological methods as described by Barrow and Felltham (1993) and Quinn *et al,* (1994).

***Primary tests*:** Gram staining reaction, aerobic and anaerobic growth, motility tests, catalase activity, oxidase test, acid from glucose, the oxidation and fermentation (O/F) tests were conducted.

***Secondary biochemical tests*:** Urease activity test, carbohydrates breakdown; sucrose, maltose, lactose, manitol, raffinose, fructose, xylose and glucose, indole production, nitrate reduction, citrate utilization ,H_2S production from TSI, , Methyl red (MR) and Vogues – Proskaur (VP) tests, growth in 6% and 10% Sodium chloride, and tube coagulase tests were performed followed primary tests results where applicable.

***Statistical Analysis*:** Descriptive analysis of milk appearance during collection, the isolated bacteria from the harvested milk and the pattern of their growth were constructed via frequency tables. A cross tabulation between the source of the milk and its characteristic profile was analyzed using univariate analysis. Moreover, univariate analysis was used to examine the association between *Staphylococcus* isolates and their pattern of growth. All statistical tests were conducted using SPSS version 20 (SPSS, IBM) at the alpha level <0.05 and 95% confidence interval.

Results and Discussion

One hundred and twenty six isolates were recovered from 111 samples and 39 samples showed no growth. Of the total isolates 100 (79.4%) were gram positive, 23(18.2%) gram negative bacteria and 3(2.4%) yeast. Among the total isolates 67(53.2%) were *Staphylococcus* spp., 22(17.4%) *Streptococcus* spp. and the least isolated bacteria are *Actinomyces* spp., *pseudomonas aeroginosa* and *Proteus mirabilis* with frequency of isolation of 1(0.7%) (Table. 2).

Table 2: Frequency of bacterial isolates

No.	Organism	Frequencies
1	*S Staphylococcus* spp.	67(53.2%)
2	*S Streptococcus spp.*	22(17.5%)
3	*Enterobacter*	14(11.1%)
4	*Corynebacterium pyogenes*	6(4.7%)
5	*Klebsiella pneumonia*	5(4.0%)
6	*Bacillus spp.*	4(3.2%)
7	*Yeast*	3(2.3%)
8	*E. coli*	2(1.6%)
9	*Actinomyces bovis*	1(0.8%)
10	*Pseudomonas aeroginosa*	1(0.8%)
11	*Proteus mirabilis*	1(0.8%)
	Total	126(100)

Within the *Staphylococcus* spp. *Staph*. *aureus* was the most frequent isolates 30(44.8%) followed by *Staph*. *hyicus* and *Staph*. *chromogenes* 10(14.9%) and the least was *Staph. Sciuri* 1(1.4%), and thirty nine bacteria were isolated in pure cultures (Table 3)

Table 3: *Staphylococcus* isolates and their pattern of growth

No.	Isolates	Pure	Mixed	Total
1	*Staph. aureus*	17(25.3%)	13(19.4%)	30(44.7%)
2	*Staph. hyicus*	6(8.9%)	4(6%)	10(14.9%)
3	*Staph. chromogenes*	8(11.9%)	2(3%)	10(14.9%)
4	*Staph*. s*aprophyticus*	2(3%)	2(3%)	4(6%)
5	*Staph. simulans*	1(1.5%)	3(4.5%)	4(6%)
6	*Staph. hominis*	3(4.5%)	1(1.5%)	4(6%)
7	*Staph. epidermidis*	1(1.5%)	1(1.5%)	2(3%)
8	*Staph. caseuolyticus*	0(0%)	2(3%)	2(3%)
9	*Staph. sciuri*	1(1.5%)	0(0%)	1(1.5)
	Total	**39(58%)**	**28(42%)**	**67(100%)**

Statistical analysis:

there is no statistically significant association between source of milk samples and its Characteristics, when using Chi square test (P-value >0.05). It is also no significant association between Staphylococcus isolates and its pattern of growth (P-value > 0.05), (Table 4 and 5respectively).

Table 4: Association of the sources of milk samples and its characteristics

Milk source	Milk characteristics		Total
	Purulent & clotted	Watery & bloody	
El- Keraib	18 (75%)	6 (25%)	24 (100%)
El- Selait	15 (57.7%)	11 (42.3%)	26 (100%)
El- Shigla	26 (66.7%)	13 (33.3%)	39 (100%)
Mahlab- 2	40 (65.6%)	21 (34.4%)	61 (100%)
Total	99 (66%)	51 (34%)	150 (100%)

Chi-square = 1.68, df = 3, $p = 0.64$

Table 5: Association between *Staphylococcus* isolates and their pattern of growth

Species	Growth		Total
	Mixed	Pure	
Staph aureus	13 (43.3%)	17 (56.7%)	30 (100%)
Staph chromogenes	2 (20%)	8 (80%)	10 (100%)
Staph hyicus	4 (40%)	6 (60%)	10 (100%)
Other *Staph*	8 (50%)	8 (50%)	16 (100%)
Total	27 (40.9%)	39 (59.1)	66 (100%)

Chi-square = 2.43, df = 3, $p = 0.49$

In this study the *Staph. aureus* was the most isolated organism 30(23.8%). This could be attributed to the survival of this bacterium in variety of environments and to its highly infective property to udder. This result was in

agreement to Adlan *et al.,* (1980); Mamoun and Bakhiet (1992), who reported that *Staph. aureus* is the most frequently isolated udder pathogen. Moreover, Abdel Gadir *et al.,* (2001), mentioned that *Staphylococci* were found to constitute the main causes of mastitis which in line with our results in this study. *E. coli*, *Kleb. pneumonia* and *Enterobacter* spp. are recognized as the most common coliform in bovine mastitis (Bannerman *et al*., 2003). However, in another study, *Staph. aureus* represent only 10- 12% of all clinical mastitis (Tenhagen *et al.*, 2009). In this study the prevalence of *Streptococcus* isolates was found to be 22(17.5%). This could be attributed to the contagious nature of the *Streptococci*, so its infection is distributed within the farm quickly. This result was in agreement to the finding of Suheir *et al.,* (2005) who have reported low prevalence rate of bovine mastitis compared to our results and could be attributed to the type of animal husbandry. The isolation of Kleb. *pneumonia*, *Enterococcus* spp., *E. coli* and *Enterobacter spp*. could be due to poor or absence of hygiene. This result was supported by Sudhan *et al.,* (2005) who reported that the high incidence of *Klebsiella* spp. in bovine mastitis was due to poor hygienic conditions. The recovery of *Bacillus* spp. in this study could be attributed to the failure of sanitary program which help in the elimination of the causative agents. This suggestion was supported by the statement of Quinn *et al.,* (1994) who mentioned that *Bacillus cereus* and coliform bacteria are frequently isolated from milk specimens of clinically mastitis cows. These results collectively support our results in this study. The detection of *Actinomyces bovis* mastitis milk in this study was in agreement to Quinn *et al., (*1994) who mentioned that this bacterium among a rarely Gram- positive rod – shaped causing bovine mastitis. A number of pathogenic bacteria of public health concern were isolated in this study. Further epidemiological studies on the occurrence of mastitis and its associated predisposing and environmental factors are needed to adopt the suitable control measures. Sanitation and hygienic standards should be adopted to avoid the contamination at the site of production. Practices of hygienic procedure in dairy farm such as close attention to milking hygiene, the culling of chronically-infected cows, good housing management and effective dairy cattle nutrition to promote good cow health are essential in helping to reduce herd mastitis levels.

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البكتيريا المرتبطة بإلتهابات الضرع في الابقار في ولاية الخرطوم/السودان

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المستخلص

يعتبر إلتهاب الضرع من المشاكل الهامة التي تواجه قطاع الألبان في السودار . أثبتت المسوحات والدراسات السابقة التي اجريت في مناطق مختلفة من انحاء السودانلاوجود المرض بنسبة عالية. لهذا السبب اجريت هذه الدراسة في بعض مزارع الألبان المختارة في ولاية الخرطوم بغرض عزل البكتريا المرتبطة بألتهاب الضرع في الأبقار. في هذه الدراسة تم جمع ، دد 150 عينة لبن من ابقار مصابة بالمرض من مزارع الالبان بمنطقة شرق النيلوكان توزيعها كالاتي: محاب ! ، الشقلة، الكرياب والسليت. تم جمع العينات في الفيرة من ابريل 2013 الى مايو 015. . تم تصنيف هذه المعزولات البكتيرية بإستخدام طرق التشخيص التقليدية. تم عزل 126 كتيريا ممرضة من جملة 150 عينة وكانت منها 00. (9.4 %) موجبة لصبغة الجرام و3! (8.2. %) سالبة لصبغة الجرام بينما ! (4. %) فطر. وكانت أكثر المعزولات من جنس المكورات العنقودية 7; (3.2;). ولمعرفة دلالة المتغيراتلاالمختلفة التي جمعت من العيناتلاتم تحليل البيانات بواسـ ة اختبار كاى تربيع للإستقلالية أو اختبار فيشر الدقيق. :ل الاختبارات الإحصائية تم اجراؤها بواسطة الحزمة الإحصائية للعلوم الإجتماعية (PSS;) اصدارة 0! . وتم اعتبار الفا تساوي او اقل من مستوى الدلالية 1.05 . من تحليل بيانات الدراسة لم يوجد أي علاقة يمكن اثباتها احصائيا بين مصدر البن (خصائص توزيع مربع كاى 0.49 P3 df1.68) كذلك لم تتم ملاحظة اي علاقة بين معزولات المكورات العنقودية المختلفة ونسق نموها(خصاءص توزيع مربع كاي 43.! .P3 df 1.04). كشفت هذه الدراسة عن عزل مجموعة من البكتيريا الممرضة التي تشكل تهديدا للإنسان من عينات اللبن التي جمعت من ابقار مصابة بإلتهات الضرع. أوصت الدراسة بإختبارالمضادات الحيوية الفعالة للعلاج مع تحسين البيئة الصحية اثناء عملية الحلب مما يقلل من حدوث المرض.