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IN VITRO EFFICACY OF TYLOSIN AND ENROFLOXACIN IN TREATMENT OF BOVINE MASTITIS CAUSING BACTERIA IN OMDURMAN LOCALITY

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ABSTRACT: Dairy industry has recently grown as a very important economic national source of income. In Sudan, many dairy owners introduced foreign blood. This might result in a progeny of mixed blood cows with lowered resistance to endogenous and locally prevailing diseases such as mastitis. In this study 60 milk samples were obtained from Frisian cows in Elrudoan and Elmouileh Convention in Omdurman, Khartoum State, Sudan. Samples positive for bacterial growth were identified using the gram stain and various conventional biochemical tests. Hundred species of bacteria were isolated from 60 samples of milk. A total of 70 (70%) were gram positive, and 30 (30%) were gram negative bacteria. Among the total of the gram positive isolates, 40 (57.1%) were *Staphylococcus spp*., 18 (25.7%) were *Bacillus spp.*, 6 (8.6%) *Streptococcus spp.*, 4 (5.7%) *Corynebacterium spp*., and 2 (2.9%) were *Actinomyces spp.* and from gram negative isolates, 26 (86.7%) were Enterobacter *spp*. and 4 (13.3%) were *E. coli*. Antibiotic susceptibility tests to Tylosin and Enroflaxcin were performed for the isolated bacteria (*Staphylococcus aureus, Staph. epidermidis, Enterobacter aerogenes* and *Enterobacter* faecalis). The isolated bacteria were found to be highly sensitive to Tylosin and Enrofloxacin.

Keywords: Tylosin, Enroflxacin, Bovine, Mastitis, *In vitro*

INTRODUCTION

Bovine Mastitis is a multi-etiological and complex disease, which is defined as inflammation of parenchyma of mammary glands. Mastitis is considered the main disease in dairy herds (Kaneen and Bandhard, 1990). The occurrence of disease is an outcome of interplay between three major factors: infectious agents, host resistance, and environmental factors (Gera and Guha, 2011). It is characterized by physical, chemical and, usually, bacteriological changes in milk and pathological changes in glandular tissues (Radostitis et al., 2000). Mastitis is a global problem as it adversely affects animal health, quality of milk and the economics of milk production, affecting every country, including developed ones and causes huge financial losses (Sharma et al., 2007). It is the most important disease in dairy cattle and more affect in economic, the most damaging (Ashish et al. 2000; Sharma et al. 2012; Elango et al. 2010; Mostert et al. 2004). Mastitis is caused by several species of common bacteria, fungi, mycoplasmas and algae (Batavani et al., 2007). Most mastitis is of bacterial origin, with just a few of species of bacteria accounting for most cases.

Mastitis pathogens are categorized as contagious or environmental (Kivaria, 2006). Contagious pathogens live and multiply on and in the cow's mammary gland and are spread from cow to cow, primarily during milking. Contagious pathogens include: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* spp. and *Corynebacterium bovis* (Radostitis et al., 2000). Environmental mastitis can be defined broadly as those intra-mammary infections (IMI) caused by pathogens whose primary reservoir is the environment in which the cow lives (Smith et al., 1985). The most frequently isolated environmental pathogens are Streptococci, other than *S. agalactiae*, commonly referred to as environmental streptococci usually *S. uberis* and *S. disagalactiae* and gram-negative bacteria such as *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. (Hogan et al., 1999).

Tylosin is macrolide antibiotic produced from Streptomyces fradiae and related structurally to erythromycin (Plumb, 2002; Giguere, 2013). It is the first antimicrobial of the fluoroquinolones group available to veterinarians, they are bactericidal, their wide spectrum of antimicrobial activity includes various microorganisms such as gram positive, gram negative bacteria , mycoplasma, and chlamydiae (Pyorala et al., 1994). Enrofloxacin is alternative

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drug. The aim of this study to identify the most common causes of bacterial mastitis in cows in Omdurman locality and to measure the effectiveness of Tylosin and Enrofloxacin in the treatment of bacterial mastitis in vitro.

MATERIALS AND METHODS

Study area

Study area is Omdurman city which located at the intersection of latitude 15 degrees 41 minutes north, longitude 32 degrees 37 minutes east, on the west bank of the Nile opposite the coupler with a tributary of the White Nile, and off both Khartoum and Khartoum North, which are linked by the bridge of the White Nile and Shambat bridge. The numbers of dairy cows were more in this locality than the other sites of Khartoum province.

Sampling

A total of 30 suspected cows were examined clinically: took the body temperature, pulse, heart rate, respiration, auscultation and palpation the last examination especially for mammary gland and supra mammary lymph nodes for presence of mastitis. Sixty milk samples from mastitic cows were collected. Mastitis was diagnosed when there were visible or palpable singes of udder, inflammatory changes in milk secretion, or through bacteriological examination of milk. During the study 60 milk sample were encountered from 30 cows suffering from clinical and subclinical mastitis. Milk sample were taken under from infected quarters only under aseptic condition for bacteriological studies. The fore milk was stripped off and about 5 ml of milk were drawn in sterile disposable bottle. All samples collected were immediately placed on ice in a thermo flask after collection.

Isolation and identification of bacteria

Culture. Milk samples were collected from mastitic cows were cultured in two media: Blood agar and MacConkey's agar. After culturing the plates were incubated for 24 hours at 37ºC. Purification was achieved by further subculturing on nutrient agar and incubated at 37ºC for 24 hours. After purification, a full loop from purified culture was taken and a smear was made and stained with Gram´s stain to differentiate between Gram's positive and Gram's negative bacteria and to see the shape of bacteria. Plates were examined for cultural characteristics and biochemical reactions according to standard keys (Barrow and Feltham, 2003). Staphylococci were studied in particular for haemolysis and coagulase production using human plasma. A positive coagulase test was judged as any degree of clotting from a loose clot suspended in plasma to a solid clot (Barrow and Feltham, 2003).

Purification of cultures. Purification of culture was made by sub-culturing a part of a typical and well isolated colony on nutrient agar. This process was repeated twice. The resulting of growth was checked for purity by examining smears stained with Gram´s stain method.

Identification of bacteria. The purified isolated bacteria were identified according to criteria outlined by Barrow and Feltham (2003) which included of: Reaction of Gram's stain, shape of the bacterial colonies, presence or absence of spores, motility, the colonial characteristics on different media, haemolysis of blood agar and biochemical tests. All biochemical tests for identification of isolated bacteria were performed according to Barrow and Feltham (2003).

Antibiotic sensitivity test. Some of the bacteria that isolated through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to identify the effectiveness of the Tylosin and Enrofloxacin. The sensitivity against Tylosin and Enrofloxacin were determined on Mueller Hinton agar as described by National Committee for Clinical Laboratory Standards (NCCLS, 2002). The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain and recorded as sensitive, intermediate and resistant.

RESULTS

Identification of isolates

Out of the 100 isolates 70 (70%) were gram positive, and 30 (30%) were gram negative bacteria. Among the total of the gram positive isolates, 40 (57.1%) were *Staphylococcu spp*., *Bacillus spp*. were 18 (25.7%), *Streptococcus* spp. were 6 (8.6%), *Corynebacterium* spp. were 4 (5.7%), *Actinomyces* spp. were 2 (2.9%) and from gram negative isolates, *Enterobacter* spp. were 26 (86.7%) and *E. coli* were 4 (13.3%) (Figure, 1). The isolated *Staphylococcus spp*. in this study was divided into two groups: Coagulase – positive included *Staph. aureus* and *Staph. hyicus*. Coagulase – negative were *Staph. epidermidis, Staph. chromogens, Staph. simulans* and *Staph.*

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hominis. The total 70 isolates: 18 (25.7%) were found to be bacillus species. There were (6%) isolates of *Streptococcus* spp obtained in this study. Other isolates were: *Corynebacterium spp*., *Actinomyces spp*., *Enterobacter spp*. and *E. coli*. Table 1 shows the quality control limits for antibiotics. The results of sensitivity tests by used Tylosin and Enrofloxacin are shown in Tables 2 and 3. They were affected in *Staph. aureus, Staph. epidermidis, Enterobacter aerogenosa, Enterococcus faecalis*.

Table 1 - Quality control limits for antibiotics

Antimicrobial agent			Zone diameter in mm			*Staph. aureus*
	Potency	Code	S	I	R	
Enrofloxacin	10Mg	EX	>23	22-17	<16	27-34
Tylosin	15Mg	TY	>26	25-23	<23	24-31

S: sensitive; I: intermediate; R: resistant

Table 2 - The efficacy of Tylosin against different types of bacteria.

Isolated bacteria	Zone of inhibition	Remarked
Staph. aureus	30mm	S
Staph. epidermidis	23mm	I
Enterobacter aerogenosa	24mm	I
Enterococcus faecalis	27mm	S

S: Sensitive; I: Intermediate

Table 3 - The efficacy of Enrofloxacin against different types of bacteria

Isolated bacteria	Zone of inhibition	Remarked
Staph. aureus	34 mm	S
Staph. epidermidis	26 mm	S
Enterobacter aerogenosa	30 mm	S
Enterococcus faecalis	25 mm	S

S: sensitive

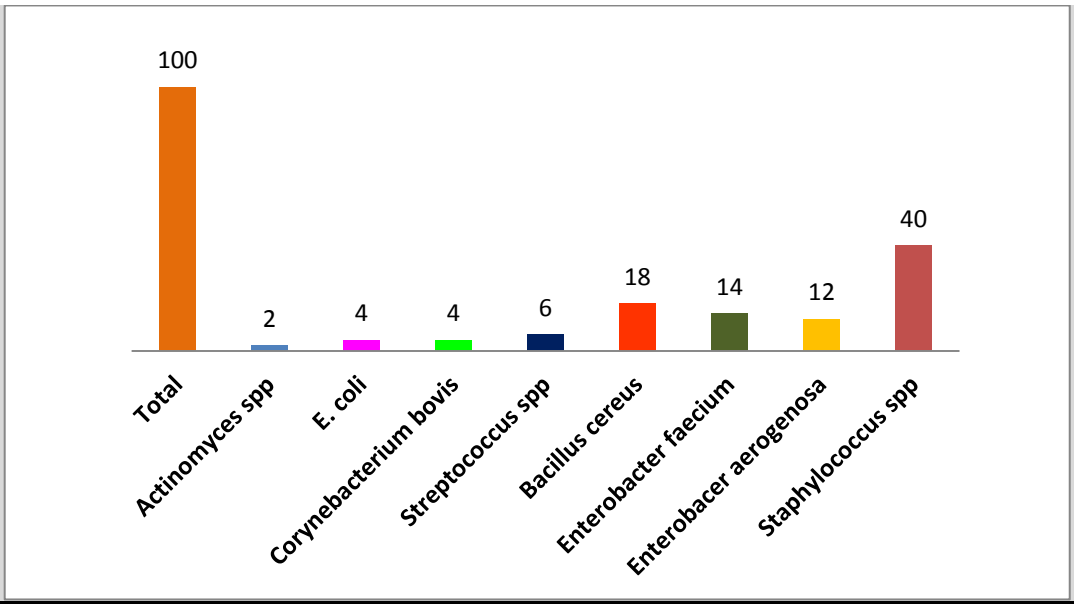

Figure 1 - Total of different isolated bacteria from mastitic milk samples

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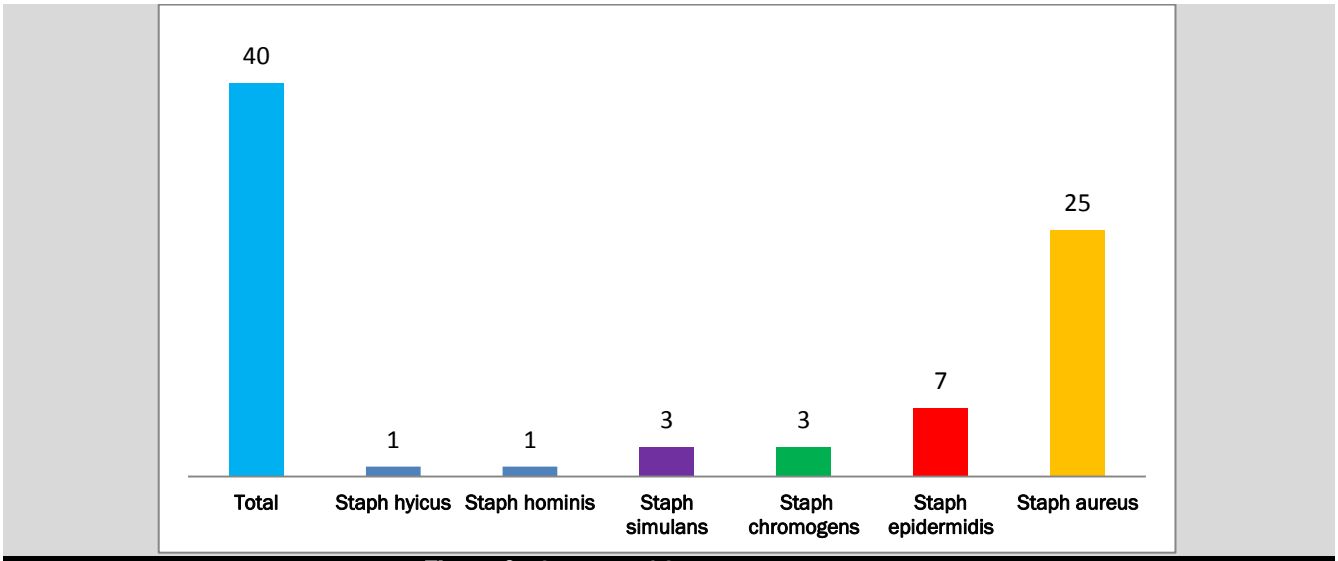

Figure 2 - Species of Staphylococci isolates

DISCUSSION

Bovine mastitis is a common disease entity of dairy cows, accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). It is a harmful disease affecting the dairy industry worldwide and is a matter of great concern for leading milk producing country because of the losses incurred due to high morbidity, discarded milk, treatment costs and reduced milk production, thus drawing in more attention towards its treatment and control (Mohanty et al., 2013).

Apart from the economic losses, mastitis can have serious implications on public health. Mastitis which is mostly caused by the interaction of multiple pathogenic agents (primarily bacteria), can expose human beings to various organisms through infected milk, thus serving as a media for transmission of various zoonotic diseases like T.B, brucellosis, diphtheria, scarlet fever and Q fever (Mahantesh and Kaliwal, 2011).

In fact, *S. aureus* was one of the most frequently isolated staphylococci, supporting the assertion that this microorganism numbers among the main mastitis pathogens in the Czech Republic (Rysanek et al., 2007). In this study the isolation of *E. coli*, *Bacillus* spp., and *Enterobacter* spp., might be attributed to poor or absence of hygiene. This suggestion was supported by the statement of Quinn et al. (2004) who mentioned that *Bacillus cereus* and *E. coli* were isolated from mastitic milk of bovine. These results collectively support our results in this study also the result is in agreement with Sudhan et al. (2005).

The detection of *Actinomyces bovis* in the mastitic milk in this study was in agreement with Quinn et al. (2004) who mentioned that this bacterium among a rarely Gram- positive rod – shaped causing bovine mastitis.

In this study found the isolates were sensitive to tylosin and enrofloxacin sensitive, these antibiotics are used either for treatment of clinical cases to avoid the spreading of the causative agent, in prophylactic measures to eliminate the susceptibility of animals for prevention of new infection or growth promotion in weight gain for fattening programmes. This study is an agreement with Anon (2011), who reported that indicated for the treatment of local signs (inflammation, milk quality and yield) associated with per acute/acute mastitis in lactating dairy cattle. Also the isolates in this study were affected with Tylosin and this is an agreement with (Pyorala et al., 1994).

CONCLUSION

In this study we have showed that the incidence of bovine mastitis is high in Omdurman locality. The most frequent isolated bacteria are *Staphylococci*. In addition, other opportunistic and environmental organisms were isolated from mastitic milk samples. Inadequate stall or pasture management e.g. dirty and wet bedding material or muddy areas as well as in proper milking procedures lead to an increased infection risk.

Recommendation

1- In dairy farm hygienic procedure must be from Practice sustainable.

2- Antimicrobial sensitivity testing should be practiced before treatment of mastitis with antibiotics.

3- Farmers should be aware about what suitable antibiotics to be used for specific mastitis case.

4- The usage of antibiotics in dairy farm should be under supervision of veterinarian to avoid missed used which leading to the development of antibiotic resistance bacteria.

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DECLARATIONS

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Authors' contribution
All authors contributed equally to this work.

Availability of data
The data can be available to the journal upon request.

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Conflict of interest
The authors declare they have no competing of interests.

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