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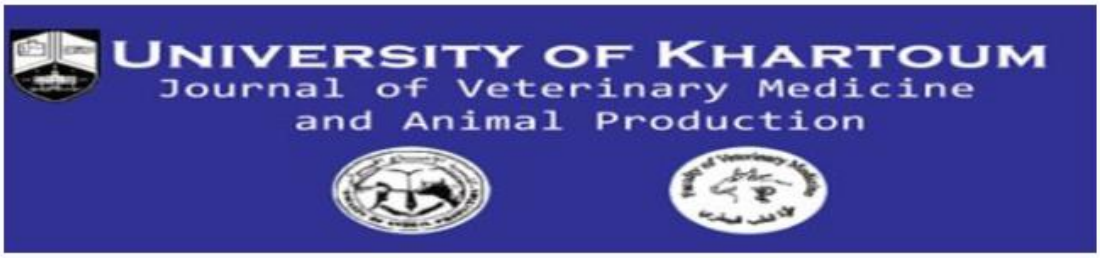

Isolation and Antimicrobial Susceptibility of Bacteria Causing Mastitis from Dairy Cows in Omdurman locality-Sudan

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Abstract

The present study was designed to investigate the sensitivity of tylosin and enrofloxacin against bacteria isolated from mastitic milk collected from Omdurman dairy farms. out of one hundred isolates from mastitic cow milk 70% were Gram +ve, and 30% were Gram -ve bacteria. Among the total of Gram +ve isolates, bacterial examinationshowed57.1%*Staphylococcus* spp., 25.7% *Bacillus* spp, 8.6% *Streptococcus* spp., 5.7% *Corynebacterium* spp. and 2.9%*Actinomyces spp*. while Gram –ve bacteria were found to be 86.7% *Enterobacter* spp., and 4 13.3% *E. coli.* Antibiotic susceptibility tests to Tylosin and Enroflaxcin were performed for the isolated bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter aerogenes, Enterobacter faecalis* were found to be highly sensitive to tylosin and enrofloxacin.

Key words: *Antimicrobial Susceptibility, Bovine Mastitis, Tylosin, Enroflaxin*

المستخلص

هدفت هذه الدراسة لتحديد درجة الحساسية لعقارى الانروفلكساسين والتايلوسين ضد البكتريا المعزولة من لبن أبقار مصابة بالتهاب الضرع من مزارع بمدينة ام درمان-السودان. من مجموع مائة عزلة باكتيرية من لبن أبقارمصابة بالتهاب الضرع. أظهرت العينات 70% عيزة بكتريا موجبه لصبغة الجرام و30% وجدت سالبة لصبغة الجرام. من العينات الموجبة لصبغة الجرام وجد أن 57.1% مكورات عنقوديز ، 25.7% عصويات و 8.6% مكورات سبحيز و 5.7%وتديه و2.9% الباكتيريا الشعيه. العينات السالبة لصبغة الجرام وجدت 86.7% عيزة عبارة عن الإمعائيز الجرثومية 13.3% الاشريكيز القولونيز. تم إجراء إختبارالحساسيز للبكتريا المعزولة (العنقوديز الذهبيز ، العنقوديز ابيبديرمس، انتيروباكترفيكالس ، انتيروباكترايروجينس). كشفت هذه الدراسة أن معظم البكتريا التي عزلت كانت عالية الحساسية للتايلوسين والإنروفلوكساسين.

كلمات مفتاحية: *حساسية مضادات الميكروبات ، إلتهاب الضرع البقري، التايلوسين، الإنروفلوكساسين*

Introduction

Mastitis is a global problem as it adversely affects animal health, quality of milk and the economics of milk production, causes huge financial losses **(**Sharma *et al.,* 2007). It is a multi-etiological and complex disease. It is characterized by physical, chemical and, usually, bacteriological changes in milk, and pathological changes in glandular tissues (Radostis *et al.*, 2000). The occurrence of disease is an outcome of interplay between three major factors: infectious agents, host resistance and environmental factors (Gera and Guha, 2011). There is an agreement among researchers that mastitis is the most widespread infectious disease in dairy cattle (Tiwari *et al.,* 2010; Sharma *et al.,* 2012 and Elango *et al.,* 2010). Variation in prevalence of mastitis might be due to the different regions, breeds, therapeutic practices, management conditions and presence of microorganisms in the environment (Elzubeir, and El Owni, 2006; Sadashiv and Kaliwal, 2013).

Mastitis is a complex disease, and thus there is no simple solution for its control, so understanding its occurrence, the related risk factors, and the mastitogenic pathogens involved, are fundamental elements in developing a control programme (Sharma, 2007).

Mastitis was firstly reported in Sudan in 1953 (Annual Report of the Sudan Veterinary Service, 1953). Then it was described as fairly common (Annual Report of the Sudan Veterinary Service, 1953- 1955; Annual Report of the Department of Animal Production, 1956-1957), later, prevalence of mastitis in dairy herds in the Sudan was thoroughly investigated by Wakeem and Eltayeb 1962; Mamoun and Bakhiet 1992; Elsayed 2000; Ahmed 2003).

In bovine mastitis, bacteria isolated with greatest frequency are *Staphylococcus aureus, Bacillus* spp., *Corynebacterium* spp., *Escherichia coli*, *Streptococcus* spp., *Pseudomonas* spp., and *Klebsiella*spp. (El-Khodery *et al.,* 2008). Bagadi (1970) investigated both clinical and subclinical mastitis in seven herds of cattle in three areas in Sudan and he found that *Staphylococcus aerues* was the most common causative agent representing 92.2% among the isolated bacteria from clinical cases and 44.2% from the subclinical cases.

The success of bovine mastitis therapy basically depends on the understanding of clinical presentation and antimicrobial susceptibility of the etiological agent, among various other factors (Miltenburg *et al.,* 1996). The important reason for therapy failure in the management of mastitis could arise from various factors involving pathological changes in the udder, etiology, lower efficacy of antimicrobials, and improper veterinary services (Adesola, 2012). Identification of mastitis pathogens and their antimicrobial susceptibility is important when selecting appropriate treatment regimen **(**Sadashiv and Kaliwal, 2014).

The Tylosin is used in ruminants to treat various systemic infections such as bronchopneumonia, mastitis, footrot and metritis caused by susceptible microorganisms (Plumb, 2002;

Giguere, 2007), it achieves effective passage from blood into udder and it is a logical candidate for paraenteral treatment to eliminate persistent Gram-positive udder infections.

Enrofloxacin has an activity against some Gram-positive aerobes such as Staphylococci, and a wide range of Gram-negative bacilli and cocci, which include *Klebsiella* spp., *Pasteurella* spp., *Pseudomonas* spp., *Salmonella*, and other organisms such as *Mycoplasma*, and *Chlamydia*.

Therefore, the present investigation was undertaken to study drug sensitivity for tylosin and enrofloxacin against bacteria isolated from mastitic milk to select the suitable drug that is effective against mentioned organisms causing bovine mastitis.

Materials and methods

Sampling

The specimen for the present research work comprised of milk samples obtained from clinical cases of bovine mastitis from Omdurman dairy farms during the period extending from November 2014 to May 2015. The samples were taken under aseptic condition for bacteriological studies by collection in sterile disposable bottles after cleaning the outer surface of the udder and teat with cotton wool soaked in 70% alcohol.

Bacteriological Analyses

Milk samples were cultured on blood agar plates (5% sheep blood), incubated at 37°C for 18-24 h. Growth on the plates was confirmed by additional laboratory tests in accordance with the routines at the laboratory (primary and secondary biochemical tests) using the methods of Barrow and Feltham, (2003).

Sensitivity test

Some of the isolated bacteria were subjected to an *in vitro* antimicrobial susceptibility test. The sensitivity against tylosin and enrofloxacin were determined on Mueller Hinton agar and the antibiotic discs were placed on the surface of the agar plates and then incubated at 37^{o}C for 18-24 hours. Subsequently, the plates were examined for the development of zone of inhibition around the discs.

Results

The diameter of the inhibition zone was measured in mm and compared with the values of quality control limits listed in standard table provided by the manufacturer (Table 1), on the basis of which the isolates were categorized as resistant (R), intermediate sensitive (I) and (S) sensitive.

Table1: Quality control limits for antibiotics used in this study

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

S: sensitive I: intermediate R: resistant

Based on standard biochemical tests, the percentage of the bacteriological examination results were shown in Fig 1. The sensitivity results were found in Table (2).

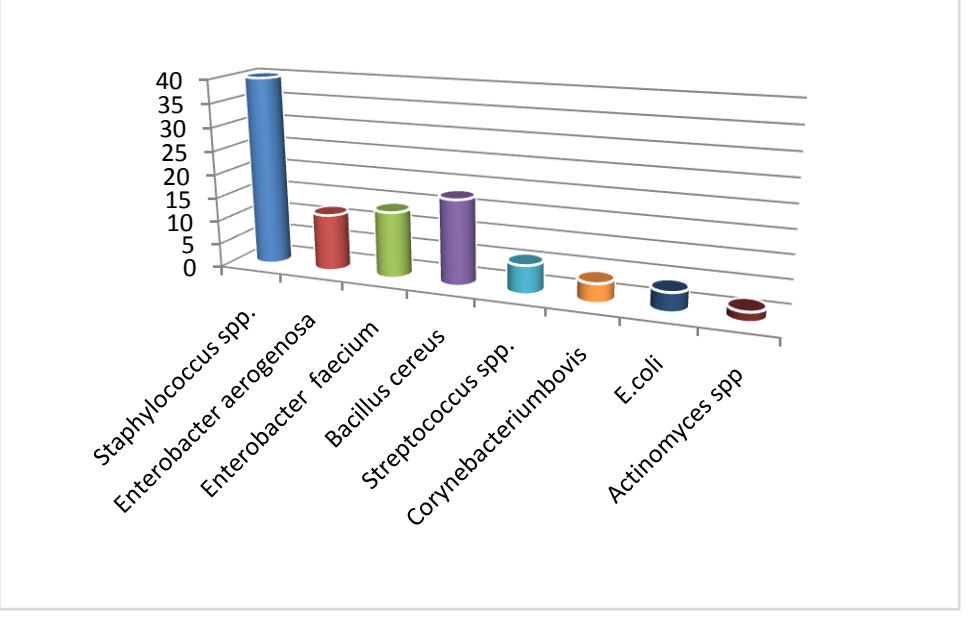

Fig. 1: Percentage of isolated bacteria from mastitic milk

Table 2: The efficacy of tylosin and enrofloxacin against different types of bacteriae

Isolated bacteria	Tylosin		Enrofloxacin	
	Zone of inhibition	Remarks	Zone of inhibition	Remarks
Staph. Aureus	30 mm	S	34 mm	S
Staph. Epidermis	23 mm	I	26 mm	S
Enterobacter aerogenosa	24 mm	I	30 mm	S
Enterococcus faecalsi	27 mm	S	25 mm	S

S: sensitive I: intermediate R: resistant

Discussion

In the current study, *Staphylococcus spp.* were detected in milk samples and *Staphylococcus aureus* was found to be the most frequent organism. This result is in agreement with the result of **El** Zubeir and El Owni, (2009). In Sudan, different researchers (Bagadi, 1970

Adlan *et al.,* 1980; Mamoun and Bakhiet, 1992; Abdelgadir *et al.,* 2001) were also reported that *Staphylococcus aureus* was the most frequent isolated udder pathogen from bovine mastitis in Khartoum state.

In fact, *S. aureus* is a one of the most frequently isolated Staphylococci, supporting the assertion that this microorganism numbers among the main mastitis pathogens (Rysanek *et al., 2007*). This species is isolated as main etiological agents of clinical and subclinical mastitis in mastitic cows and this result is in agreement with Bradley *et al.,* (2004) and Malinowski *et al.,* (2008)

Isolation of *E. coli* and *Enterobacter* spp. might be attributed to the poor or absence of hygiene. This result was also mentioned by El Zubeir and El Owni, (2009) in an investigation of raw milk contamination at Khartoum state.
The isolation of *Bacillus* spp. in this study could be attributed to the failure of sanitary programme. This suggestion was supported by the study of Quinn *et al.,* (2004) who mentioned that *Bacillus cereus* was isolated from mastitic milk of bovine. In addition, coliform bacteria were isolated from the milk samples of clinical mastitis cows. These results collectively supportthe present results in this study. This result supported Sudhan *et al.,* (2005).

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

The *in vitro* susceptibility to antibiotics against the isolated bacteria was found to be similar in comparing to data reported by other researchers, who applied the disc diffusion method (Erskine *et al.,* 2002 and Malinowski *et al.,* 2008).

Mastitis can have serious implications on public health. It 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 Basappa, 2011). The majority of researchers had noted the increase in the bacterial resistance to antibiotics; most of the resistant bacteria were Staphylococcus spp. isolated from mastitis (Malinowski *et al.,* 2008; Schmidt *et al.,* 2015). So that it's necessary to monitor mastitis pathogens to asses any changes in their antibiotic resistant pattern.

From this study, it is clear that *Staph. aureus* strains were the most sensitive (very high *in vitro* susceptibility) to tylosin and enrofloxacin. Also *Enterococcusfacalsi* was found to be sensitive to tylosin and enrofloxacin.The other isolated strains of *Staph. epidermidis* and *Enterobacteraerogenosa* had an intermediate reaction (less susceptible) to tylosin and sensitive to enrofloxacin. These results are supported by the report of du Preez (2000) who found that the tylosin is the drug of choice in parenteral therapy for treatment of mastitis causing by *Staph. aureus*. Other researchers reported that the enrofloxacin was highly sensitive to coagulase negative Staphylococci.

Also du Preez, (2000) found that enrofloxacin is the alternative drug for treating Gram-negative infections.

Finally, success of mastitis therapy is lower in lactating cows than in dry cows especially for Staphylococcal mastitis and the treatment depends on a good identification of etiological agents, proper treatment, proper sanitary measurements and proper veterinary services.

Conclusion

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

The selection of drugs after culture and sensitivity test should be based on their ability to cross blood tissue barrier or mammary parenchyma, lipophilicity and ability to work in alkaline pH.

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