Mastitis pathogens prevalent in dairy cattle at Magadu farm, Morogoro-Tanzania

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SUMMARY

Mastitis is among the major factors contributing to reduced milk production in dairy cattle. This study was carried out to determine the prevalence of mastitis pathogens at the Magadu Dairy Farm, Sokoine University of Agriculture. A total of 19 milk samples were collected and cultured on blood agar and MacConkey agar followed by identification of bacteria colonies and cells using standard laboratory techniques. Antimicrobial susceptibility test of the isolates was done by using disk diffusion method. All samples tested had bacterial growth with *Staphylococcus* spp. being the most common (44.6%). Kanamycin and Norfloxacin showed to be the best drugs of choice for treatment. The economic losses due to reduction in milk yield, changes in milk composition and discarded milk are serious burden to the dairy industry. Mastitis can be subclinical, therefore, it is important to screen dairy animal frequently to determine the health and milk production.

Key words: Mastitis, dairy cattle, bacteria, antimicrobial susceptibility

INTRODUCTION

Mastitis is the inflammation of the parenchyma of the mammary gland and is characterized by physical, chemical and bacteriological changes of the milk in dairy animals and pathological changes in udder (Katsande and Matope, 2013). Mastitis is among the major factors contributing to reduced milk production in dairy cattle. The economic losses due to reduction in milk yield, changes in milk composition and discarded milk are serious handicaps for the farmers and the dairy industry. Clinical mastitis, in which abnormal milk is easily detected, and sub-clinical mastitis, in which no change in the milk is apparent, both reduce milk production (Gianneechini *et al*., 2002).

Inflammation of the mammary gland is mostly caused by infectious agents, especially bacteria, but it can also be due to physical or chemical agents. Somatic cell counts (SCC) in milk are commonly used as indicators of mastitis, on the basis that their increase reflects an immune response to the presence of infection in the mammary (Gianneechini *et al.*, 2002). The increase of somatic cell count is mostly due to increased leukocyte levels which are involved in the removal of the infectious agents, and also of exfoliated epithelial cells. The enzymes left behind in the milk by the phagocytic process significantly reduce the shelf life of milk and milk products. However, animals with low SCC can also have the mastitis pathogen present (Katsande and Matope, 2013).

Transmission of mastitis is normally through infection but can also be environmental. Cows with mastitis are the main source of infection. The spread of the bacteria that cause the infection primarily happens during milking (Sudhan and Sharma, 2010). This study was designed to identify the bacteria which are the potential cause of mastitis at the Magadu Dairy Farm Sokoine University Agriculture (SUA) and to demonstrate the drugs which are suitable to treat the animals in mastitis cases.

MATERIAL AND METHODS

Study site and animals

The study was conducted at Magadu Dairy Farm, Sokoine University of Agriculture. Laboratory studies were conducted at the Tanzania Veterinary Laboratory Agency (TVLA) in Dar es Salaam. The study animals were cross of Friesian and Holstein cattle that were lactating during the study. Nineteen cattle were conveniently selected for milk sampling. The age of the animal ranged from 30 to 60 months.

Sample collection and laboratory analysis

The collection of milk samples for bacterial culture was done under sterile condition. Teats were washed using clean running water, wiped dry with disposable paper towels and disinfected using 70% ethyl alcohol. To minimize contamination with

bacteria from the skin around the teat canal, the first streams of milk were discarded. Milk samples were collected into a sterile and clean bijou bottles. After sampling, the samples were stored in a cool box with ice packs and subsequently transported to TVLA microbiology laboratory for analysis.

Milk samples were cultured on blood agar and MacConkey agar and incubated aerobically at 37 ºC for up to 48 hours. A milk sample was considered positive for mastitis pathogen(s) if at least a single bacteria colony of a potential pathogen was detected. Macromorphology assessment of colonies was used as a preliminary means of identification followed by Gram stain. Then the bacteria of interest were subcultured to blood agar and MacConkey agar for purification. Standard biochemical tests like oxidase test, lactose fermentation on MacConkey agar, indole production test and citrate utilization and the IMVIC were done. *Streptococcus* spp. was differentiated from *Staphylococcus* spp by catalase test, where catalase positive were *Staphylococcus* spp. while catalase negative were *Streptococcus* spp. To identify the *Staphylococcus* spp. the slide coagulase test was done using rabbit plasma. Coagulase positive were grouped as *Staphylococcus aureus*, while coagulase-negative were grouped as other *Staphylococcus* spp. The CAMP test was done to identify the *Streptococcus agalactiae*, where *Staphylococcus aureus* was streaked against β-hemolytic *Streptococcus* spp. on sheep blood agar.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was done using the disk diffusion method. Seven antibiotic discs (Oxoid, Basingstoke, Uk) and their concentrations in brackets for *E. coli* were: Kenamycin (30 µg), Cloxacillin (1 µg), Tetracycline (30 µg), Amoxilline (30 µg), Norfloxacin (10 µg), Streptomycin (25 µg) and Neomycin (30 µg) were used.

The isolates were grown on blood agar, and then antibiotics disks were laid on top of strikes of bacteria as described by Clinical and Laboratory Standards Institute (CLSI, 2007). The plates were incubated at 37°C for 24 and 48 hours. The plates were examined for zones of inhibition around the discs. Diameters of inhibition zones around the discs were measured in millimeter (mm) using a metal caliper, and the results were recorded. The results were compared with standard provided by the manufacturer (Oxoid) and classified as resistant (R), intermediate (I) and sensitive (S) according to the general guidelines by CLSI (2007).

Statistical Analysis

The prevalence of mastitis in individual cow herds calculated by the proportion of mastitis-positive cows against the total number of animals of the herd (n=34). A sample was denoted as positive for mastitis if at least a single colony of potential pathogen grew on plate culture, during isolation. The proportions of most common bacterial isolates were calculated against the total number of isolates.

RESULTS

Isolated bacteria

All the 19 milk samples had bacteria grown on culture. The bacteria isolated were Enterobacter spp., *Staphylococcus aureus*, *Staphylococcus* spp., *Streptococcus agalactiae*, *Streptococcus* spp. and *Pseudomonas* spp. (Table 1).

Table 7. Bacterial isolates of milk from Magadu Dairy Farm

Bacteria species	Friesian		Holstein		Total	
	Number of isolates	Percent	Number of isolates	Percent	Number of isolates	Percent
Staphylococcus spp	11	35.5	8	32	19	33.9
Streptococcus spp	7	22.6	6	24	13	23.2
Enterobacter spp	4	12.9	4	16	8	14.3
Staphylococcus aureus	3	9.7	3	12	6	10.7
Pseudomonas spp	4	12.9	2	8	6	10.6
Streptococcus agalactiae	2	6.5	2	8	4	7.1
Total number of isolates	31	100	25	100	56	100

Antimicrobial susceptibility results

The results on antimicrobial susceptibility to antibiotics are shown in Table 2 and the

interpretations in Table 3. It was found that majority of the bacteria were susceptible to Kanamycin and Norfloxacin. Most of the bacteria were resistant to Tetracycline and Streptomycin.

Table 8. Antimicrobial susceptibility test for the isolates

Bacteria species	Zone of inhibition in different antibiotic (mm)					
	K	COX	TE	AX	NOR	S
Staphylococcus spp	24	26	10	25	17	0
Streptococcus spp	18	15	16	13	13	0
Enterobacter spp	18	21	22	21	19	15
Staphylococcus aureus	19	22	17	22	14	13
Pseudomonas spp	20	25	20	23	18	8
Streptococcus agalactiae	19	19	0	25	20	0

*Kanamycin (K), Cloxacillin (COX), Tetracycline (TE), Amoxilline (X), Norfloxacin (NOR), Streptomycin (S) and Neomycin (NE).

Table 9. Inhibition zone interpretation standards for different antibiotics

Antibiotic disc	Resistant	Intermediate	Susceptible
	≤13 mm	14-17 mm	≥18 mm
kanamycin	13	14-17	18
Cloxacillin	14	15-17	18
Tetracycline	14	15-18	19
Amoxilline	21	22-27	28
Norfloxacin	15	16-17	18
Streptomycin	14	15-20	21

DISCUSSION

This study revealed that the majority of the animals at the Magadu Dairy Farm had mastitis since the isolated bacteria have a potential of causing mastitis in cows. The bacterial pathogens isolated in the current study were predominantly coagulase-negative *Staphylococcus* spp (Table 1). The preponderance of *Staphylococcus* spp was about 33.9% in the study animals, which agrees with previously reported studies (Mdegela *et al.*, 2009). The dominance of this group of pathogens is possibly the result of poor milking hygiene. For example, in this study it was observed that during milking one towel was used to wipe the tit for all the animals. This practice could facilitate spread of infectious agents. According to Blowey and Edmonson (2010), coagulase negative staphylococci commonly colonize the teat end and teat canal only, and are difficult to associate with clinical mastitis. Under some circumstances, however, they may maintain subclinical mastitis (Mdegela *et al.*, 2009).

It was also found that the isolated bacteria were resistant to many antibiotics. This is a serious problem since such antibiotics can be used in treatment of mastitis in animals. Similarly, some of the isolated bacteria have a potential of causing diseases in humans. Therefore, such bacteria whenever are also involved in causing diseases in humans cannot be treated by using the antibiotics which the bacteria showed resistance. The possible reason for the high level of bacterial resistant could be due to indiscriminate uses of antibiotics. Studies show that Penicillin, penicillin-streptomycin, ampicillin and oxytetracycline are the frequently used antibiotics in cattle (Midenge, 2010; Kanyeka, 2014). Reasons for development of resistant bacteria to antibiotic could be inappropriate use of the antibiotics in cattle, wrong dosage and routes of administration, arbitrary drug combinations and the acquisition of mobile genetic characteristics (Katakweba *et al.*, 2012; Sharma *et al.*, 2012; Yakubu *et al.*, 2012). Other studies have reported a number of resistant bacteria to commonly used

antibiotics in livestock production in Tanzania (Mdegela *et al*., 2004; Nonga and Muhairwa, 2009; Katakweba, 2014). Elsewhere, Yakubu *et al.* (2012) reported high resistance rates in bacteria isolates to different kinds of antibiotics used in animals in Nigeria.

This study has given some preliminary data of common bacterial pathogens in cow milk at Magadu dairy farm, SUA Morogoro. It has also highlighted on antimicrobial resistance shown by the isolated bacteria and has shown that most of the bacteria were resistant to Tetracycline and Streptomycin. It is important that principles of hygiene should be followed in cattle so that to minimize the magnitude of udder infection.

ACKNOWLEDGMENTS

This study was funded by the Tanzania Higher Education Students' Loan Board. We are grateful to the support provided by the Magadu farm authority and the laboratory technicians at TVLA, Dar es Salaam.

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