ISOLATION, IDENTIFICATION AND ANTIBIOTICS TREATMENT OF SOME BACTERIAL STRAINS CAUSED BOVINE MASTITIS IN LIBYA

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ABSTRACT

Bovine mastitis is the major problem for milk producers throughout the world and responsible for substantial losses of revenue annually. Antibiotic therapy is an important tool in the scheme of mastitis control. A total of 181 milk samples from the farms were collected aseptically from suspected cows for mastitis. Seven bacterial strains (*Staphylococcus aureus, S. epidermidis, Streptococcus agalactiae, Escherichia coli , Pseudomonas aeruginosa, Klebsiella spp*. and *Proteus spp*.) were isolated and tested for antibacterial activity of the drugs. *Staphylococcus aureus* isolates were the most predominant in dairy farms. The results declared that *Staphylococcus epidermidis* isolates were more sensitive to gentamycin (81.2%). Penicillin , erythromycin and neomycin are effective against *S. epidermidis*, while nitrofuratoin , penicillin , ampicillin and neomycin are effective against *S. agalactiae* isolates. On the other hand *P. aeruginosa* isolates were observed sensitive manner to gentamycin, pencillin , ampicillin and streptomycin whereas they showed resistant effects to erythromycin. However *Escherichia coli* isolates were exhibited inhibitory effects to nitrofuratoin, while they represented a negative response to pencillin and erythromycin. Also *Klebsiella spp*. and *Proteus spp*. isolates were showed sensitive results to gentamycin whereas resistant effects were occurred to pencillin. Remarkable antibacterial activities against *S.aureus , S.epidermidis , S. agalictiae , E. coli, P. aeruginosa and Proteus spp.* strains were shown for gentamycin and /or nitrofuratoin. The results indicated that the increasing prevalence of multidrug resistant strains with reduce susceptibility to antibiotics adds urgency to the search for new mastitis fighting strategies. Hence, there is a need to investigate the antibacterial properties of drugs that have not been done.

INTRODUCTION

Mastitis is an endemic disease that is considered to be one of the most frequent and costly diseases in the dairy industry. Moreover, mastitis affects milk quality directly in the technical characteristics and the hygienic quality of the milk, and indirectly through the intrinsic milk quality (Hogeveen,2002).

Mastitis in dairy cattle is the persistent, inflammatory reaction of the udder tissue. It is also the most costly to the dairy industry. Milk from cows suffering from mastitis has an increased somatic cell count. This disease can be identified by abnormalities in the udder such as swelling , heat , redness, hardness or pain. Other indications of mastitis may be abnormalities in milk such as a watery appearance, flakes , clots , or pus. There are two types of mastitis : clinical and sub-clinical mastitis.

Mastitis is considered as one of the major cause of antibiotics use in dairy animals. The use of antibiotics in dairy cattle industry for the treatment and prevention of mastitis in addition to its significant economic cost (Kossaibati and Esslemont, 1997) because very often untargeted

in many cases promotes the selection of resistant bacteria. Thus, antibiotic resistance may be a cause of treatment failure it can also result in treatment failure in other conditions, transfer of resistance genes.

Treatment failure in mastitis is due also to indiscriminate use of antibiotics without testing in vitro sensitivity. This practice increases economic losses, thus, it is essential to identify and quantify pathogens and their resistance to assess the adequacy of the therapeutic arsenal avoid further complications, adopt and maintain efficiency on a large scale and finally for implementation of effective control of mastitis.

Milk is a highly perishable foodstuff, and hence subjected to microbial contaminations ; as milk is collected under different climates, by different handling practices , its microbiological quality is very variable, and the level of contaminations is reflected both in number and types of microorganisms in the samples. Milk from cows with sub-clinical mastitis accidentally mixed into bulk milk enters food chain and poses a threat to human health . Milk and other dairy products are reported to be frequently infected with *Staphylococcus aureus* . Also *Streptococcus agalactiae* has been described as one of the most common agents to invasive infections (Abdel Hameed et al., 2006). Mastitis is difficult disease to control because many different bacteria are capable of infecting the udder and producing the disease. Microorganisms that most frequently cause mastitis can be divided into two categories: First one contagious pathogens , which spread from cow to cow primarily during the milking process and other one is environmental pathogens which are found throughout the habitat of dairy cows, current mastitis control programs, which were advised in the 1060 s are based on hygiene and include teat distinction , antibiotic therapy and culling of chronically infected cows. Acceptance and applications of these measures throughout the world has led to considerable progress in controlling mastitis caused by contagious mastitis pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus*. However, as prevalence of these contagious mastitis pathogens was reduced , the proportion of intramammary infections caused by environmental pathogens such as Escherichia coli has increased markedly (Oliver and Mitchell, 1983). Higher incidence rate of E.coli mastitis may be due to poor hygienic conditions of the farm and animal environment as *E.coli* infects the udder via teat canal from the environment. This study is aimed to evaluate the prevalence of mastitis in cows Tawargah dairy farms, Mysrata , Libya in order to determine the susceptibility of isolated bacteria to ten antimicrobial agents that were commonly used in dairy cows.

MATERIALS AND METHODS

Collection of milk samples

The specimen for the present research work comprised of milk samples obtained from cows suspected for mastitis from Tawargah dairy farms in Mysrata , Libya. A total of 181 milk samples from the farms were collected aseptically from suspected cows for mastitis. After proper sanitization of teat orifice with 70 % ethyl alcohol , 10-20 ml of milk samples from the teats were collected aseptically after discarding first few streams, in sterile polyethylene screw caped wide mouth vials (Sharma, 2010). The milk samples were kept in an ice box and carried to the Laboratory of Microbiology Department, where the milk samples were kept at 4 – 8 C in refrigerator for further laboratory investigation.

Testing of milk samples for mastitis

All the milk samples were subjected to the California Mastitis Test (CMT) according to Schneider et al. (2004).

Isolation and Identification of bacteria

milk samples which were positive to mastitis test were subjected to isolation and identification of bacteria on the basis of morphological , cultural and biochemical characteristics according to method described by Coleman et al., 1986. Ten different antibiotics (vis. Nitrofuratoin, neomycin, oxytetracyclin, streptomycin, chloramphenicol, ampicillin, erythromycin, kanamycin, pencillin and gentamycin) were used against bacterial strains identified from cow mastitis milk samples from Tawargah regions in Libya. Susceptibility of individual bacterial isolates to commonly used antibiotics was determined by antibiotics Disc Diffusion Method (Bauer et al., 1966). The positive samples collected from the field were subjected to cultural examination using standard laboratory procedures of inoculating the samples in Nutrient agar , Blood agar, Mac Conkey agar.

The antibiotics discs (Hi –Media , Mumbai, India) were placed on the surface of an agar plate previously seeded with a standard amount of the organism to be tested. Bacteriological Techniques This section provides a brief description of bacteriological techniques employed for the study of antibacterial properties of selected antibiotics. The bacteriological techniques followed were those described by Cruickshank et al., 1975. Different type of media , solid , semi-solid , and fluid were used to identify the pathogenic bacteria were supplied by Oxoid and prepared according to Cheesbrough, 1984.

RESULTS AND DISCUSSION

In spite of the important role that antibiotics play in the control of bovine mastitis, there have only been a few studies of the antibiotic resistance of mastitis pathogens (Stephen et al 1983). Consequently, little information is available on the prevalent resistance patterns of the bacterial strains that serve as the source of new infections. Furthermore, little is known about the effects of various herd management practices on the incidence of resistance in these microorganisms. Antibiotic treatment has become the primary method for controlling mastitis. There are numerous clinical and economic disadvantages inherent in the treatment of mastitis in lactating cows, including the rapid dilution of the antimicrobial agent, the loss of saleable milk, and the possible presence of antibiotic residues in milk reaching the consumer (Gu_tfo et al.1977 and Smith et al 1981). For these reasons, infusion of antibiotics into the udders of nonlactating animals (referred to as dry treatment or dry cow therapy) has become an increasingly popular means of mastitis control.

Staphylococcus aureus isolates were the most predominant in dairy farms(53.04%) Table 1.They were recorded as quite sensitive to gentamycin (73%) and as a moderate sensitive to nitrofurantoin (41.6%) ,whereas were showed a weak activity against other antibiotics (Table 2). In clinical mastitis, antibiotic treatment failures are often attributed to multiple antibiotic resistance, particularly among staphylococci and gram-negative pathogens (Da Mson et al 1982 and Huber 1977). Presently, it is difficult to isolate mastitis pathogens that are completely sensitive to all commonly used antibiotics. Because of the important role of antibiotics in the control of mastitis, we felt that it would be advisable to examine the antibiotic resistance of bovine udder pathogens from dairy herds. These enteric Gram–negative microorganisms possess lipopolysaccharides (LPS) – so called endotoxins – in the outer layer of the cell wall, which in contact with the immune system lead to liberation of potent pro–inflammatory mediators (cytokines). Mammary glands of the domestic animals are extremely sensible to LPS (Munoz et al., 2006). The endotoxins induce to severe changes

in vascular permeability, and increase of somatic cells in mammary gland and milk, resulting in edema, depression, toxemia, and severe per acute or acute clinical signs of mastitis.

Some strains of *S. aureus* can live within cells such as macrophages and many of them produce beta-lactamase hence rendering them resistant to certain formulations of pencillin , in addition to other antibiotics that have been deemed more effective (such as certain aminoglycosides, cephalosporins and tetracyclines) (Haber et al., 1977).

Results of *Staphylococcus epidermidis* are shown in Table 2. They revealed that the gentamycin was a quite sensitive to this species (69.2%), while the other antibiotics were exhibited a weak activities, suggesting the production of beta- lactmase. This was in agreement with what was reported by Stephen 1983 who seated that the resistant of *S. aureus* against ampicillin and gentamycine was the most active . Most of antibiotics are only able to circulate in the body fluids surrounding cells and cannot penetrate within cells themselves , hence these staphylococci are protected from the majority of antibiotics (Haber et al., 1977).
On the other hand *Streptococcus agalactiae* strains were recorded a quite sensitive against nitrofuratoin , chloramphenicol , kanamycin and gentamycin, while a moderate activity was shown against oxytetracyclin and weak activity was recorded to neomycin, streptomycin, ampicillin, erythromycin and penicillin. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a bacterium capable of causing mastitis in dairy cows. This bacterium presents a difficult challenge, as it tends to protect itself from antibiotics and white blood cells in layers of slim. Nitrofuran (60%), neomycin (60%) , kanamycin (60%), chloramphenicol (40 %) were showed promising result against *Pseudomonas aeruginosa* while gentamycin was showed highly sensitive against *P. aeruginosa* (80%) .

E. coli infects the udder via the teat canal (Eberhart, 1984). An environment contaminated with feces is the main source of mastitis-causing E. coli bacteria (Linton et al., 1979). Environmental factors, herd management and use of antimicrobial agents may select bacterial strains and affect their virulence factors and antimicrobial resistance, causing the variation in the course of E. coli mastitis seen between countries and herds. Poor barn design and management of dairy cows may also provide the cows with predisposing factors for coliform mastitis (Ward et al., 2002;). As matter of fact, the results revealed that the Escherichia coli strains is the promising one with remarkable activity against antibiotics. The four antibiotics nitrofuratoin, neomycin, chloramphenicol and gentamycin, have the highest antibacterial effects with values equal to 83.3, 66.6, 62.5 and 54.1% , respectively.

In addition *Klebsiella spp*. strains were showed quite sensitive against nitrofuratoin , neomycin , kanamycin and gentamycin. Moreover other six antibiotics showed weak activity and inactive or resistant against penicillin. The majority of Klebsiella isolates are inherently resistant to ampicillin (Bengtson et al., 2009) and ampicillin is used to create semi-selective media for studies of environmental Klebsiella (Munoz et al., 2006). Some farmers use products with ampicillin for treatment of Klebsiella mastitis, even though this is not supported by label claims and is not expected to have a beneficial effect. Without bacteriological culture of milk samples, a farmer may not even know that (s)he is treating a Klebsiella. Culture and rational selection of antimicrobials are essential for successful treatment of Klebsiella mastitis. Unfortunately, culture and rational treatment selection are by no means a guarantee of treatment success. Proteus species are Gram negative bacteria that are found in the cow's environment in bedding, manure, feed and water. Generally, Proteus species is not a common mastitis pathogen. If this organism is isolated it is generally a contaminant. However, rare infections can occur between milking when teat ends are in

direct contact with contaminated surfaces. However, it has been known to cause mastitis outbreaks. Mastitis due to Proteus spp. is often chronic, difficult to cure, and unresponsive to antibiotics. Erythromycin and gentamycin were showed activity equal to 50, 72.2% against Proteus spp., while moderate activity was shown by chloramphenicol (44.4%). Also weak activity was represented by nitrofuratoin , neomycin , oxytetracyclin, streptomycin , ampicillin and kanamycin, while a negative results were obtained by penicillin .The results indicated that the drugs had a differential antibacterial effects which were in agreement with what was reported by Cho et al., (1986), Dudko (2003) and Ali et al., (1990).

Table (1): Percentage of distribution and frequency of isolation of different bacterial species from mastitis milk samples.

No.	Bacterial species	No. of isolates	%
1	*Staphylococcus aureus*	96	53.04
2	*S. epidermidis*	13	7.18
3	*Streptococcus agalactia*	12	6.63
4	*Pseudomonas aeruginosa*	5	2.76
5	*Escherichia coli*	24	13.26
6	*Klebsiella ssp.*	13	7.18
7	*Proteus ssp.*	18	9.94

Table (2): Antibacterial drug susceptibility of bacterial strains from mastitis milk samples.

Antibiotics used	Sensitivity (Degree %)						
	S. aureus	S.epedermidis	S.agalactia	P.aeruginosa	E. coli	Klebsiella spp.	Proteus spp.
Nitrofuratoin (15 mg/disc)	41.6	38.4	66.6	60	83.3	61.5	16.1
Neomycin (30 mg/disc)	9.3	15.3	25	60	66.6	54	16.6
Oxytetracyclin (10mg/disc)	31.2	23	41.6	40	8.3	23	11.11
Streptomycin (5mg/disc)	20.8	23	33.3	20	37.5	15.3	27.7
Chloramphenicol (5mg/disc)	26	30.7	50	40	62.5	38.4	44.4
Ampicillin (10mg/disc)	26	23	25	20	33.3	23	22.2
Erythromycin (25mg/disc)	36.4	15.3	33.3	0	0	30.7	50
Kanamycin (10mg/disc)	15.6	30.7	58.3	60	20.8	38.4	16.6
Penicillin (10mg/disc)	5.2	15.3	25	20	0	0	0
Gentamycin (10 mg/disc)	73	69.2	58.3	80	54.1	77	72.2

100-80 Highly sensitive; 79-50 Quite; 49 – 40 moderate; Less than 40 weak; 0 = Resistant.

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