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PREVALENCE OF COMMON MASTITOGENS AND THEIR ANTIBIOTIC SUSCEPTIBILITY IN TEHSIL BUREWALA, PAKISTAN

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The study was conducted on lactating cattle and buffaloes in tehsil Burewala, district Vehari, Pakistan. The purpose of study was to isolate different types of microorganisms associated with mastitis and to see the effectiveness of different antibiotics against these isolates. Relative prevalence of *Staphylococcus aureus* was the highest (50% and 53.85% in cattle and buffaloes respectively) followed by *Streptococcus agalactiae* (27.78% and 23.07%), *Escherichia coli* (16.67% and 15.38%), *Streptococcus dysagalactiae* (5.55% and 3.85%), and *Corynebacterium bovis* (0% and 3.85%) in cattle and buffaloes respectively). On the whole, the isolates were sensitive in descending order to Enrofloxacin, Chloramphenicol, Gentamycin, Oxytetracycline, and Amoxicillin.
Keywords: Mastitis, cattle, buffaloes, antibiotics, Enrofloxacin, Chloramphenicol

INTRODUCTION

The term mastitis, regardless of the cause, refers to the inflammation of the mammary glands, and is characterized by physical, chemical and usually biological changes in milk and pathological changes in glandular tissue. It is considered most important and a costly disease of dairy animals (Lightener *et al., 1988).* Field surveys of major livestock diseases in Pakistan have indicated that mastitis is one of the most important health hazards in the country (Cady *et al.*, 1983; Ajmal, 1990).

Mastitis occurs in two forms i.e. subclinical and clinical. Subclinical form has been found to be 3 to 40 times more common than clinical mastitis (Schultz *et al.*, 1978). Mastitis is caused by interaction of various factors associated with the host, pathogens and the environment, so nature and duration of the disease varies accordingly. Infectious agents like bacteria, viruses, fungi and algae are mostly the primary causes of the disease. Among these the most important are bacteria, which can be divided into two classes: major pathogens (*Staphylococcus aureus,* Streptococci, *Corynebacterium pyogenes* and Coliform) and minor pathogens (coagulase negative Staphylococci and *Corynebacterium bovis*) (Hinckley *et al.*, 1987). For every clinical case of mastitis in cows, there are 15-40 undetected cases of subclinical mastitis and each subclinical case of mastitis reduces milk production to the tune of 63 to 77 litres per cow per year (Anonymous, 1988). It is frequently said that every dairy buffalo and cow develops mastitis before she dies (Muhammad, 2008).

The overall picture of mastitis in the world has been documented to be comparable to the figure of about 40% morbidity in dairy cows and buffaloes and a quarter infection rate of about 25% (Blood and Radostits, 1989). The incidence rate of mastitis in Pakistan has been reported as 20-23% in buffaloes and cattle (Rasool *et al.*, 1985).

Despite annual production of over 34 million tonnes of fresh milk by 22 million head of cattle and nearly 23 million head of buffaloes (Anonymous, 2008), Pakistan is facing an acute shortage of milk supplies in major urban cities. Besides poor genetic potential, management and nutrition, sub-optimal health of milch animals particularly of milk producing organ (udder) i.e., mastitis is among the leading factors responsible for the shortfall of milk supply in Pakistan.

All immunization procedures developed against the disease have remained unsuccessful due to its multiple etiological agents. The misuse of antibiotics at low levels over longer periods may cause the therapeutic failure and the development of drug resistance. Therefore, regular studies on antibiotic sensitivity of bacterial isolates are mandatory for effective and economical treatment of the disease (Sanchez *et al.*, 1988).

Mastitis can more efficiently be controlled and treated if we know the prevalence of the pathogens causing mastitis and antibiotic susceptibility of the pathogens for different antibiotics. The present study has, therefore, been designed to: 1) determine the prevalence of common mastitogens, and 2) study the effectiveness of different antibiotics against these isolates.

MATERIALS AND MEHTODS

The study was carried out on lactating cattle and buffaloes in tehsil Burewala, district Vehari, Pakistan. Thirty milk samples (11 from cattle and 19 from

buffaloes) positive for clinical mastitis were collected and brought to the laboratory of Department of Veterinary Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan for isolation and biocharacterization of prevalent mastitis pathogens.

Primary isolation of the bacteria

Procedures described by NMC (1990) were followed for culturing the milk samples and identification of mastitis pathogens. The mastitic milk samples were gently shaken 8 times to get a uniform dispersion of the pathogens. Using a platinum-rhodium loop, 0.01 ml of milk sample was streaked onto blood agar plates. Cultured plates were incubated at 37 °C for 48 hours. The cultural and morphological characteristics of primary growth were studied by examination of colony characteristics and preparation of smears from different colonies. These smears were stained with Gram's staining method and examined under the microscope.

Identification and purification of bacteria

Primary growths were purified by frequent sub-culturing on selective and differential media. The selective and differential media used were MacConkey's agar (for enterobacteriaceae), Staph-110 (for staphylococcal species), crystal violet blood agar (for streptococcal species) and blood agar (for haemolytic species) and where relevant, the tests for sugar fermentation, indole, methyl red, catalase, coagulase, and hydrogen sulphide tests were used. Each of the isolates was identified on the basis of cultural and morphological characteristics, motility, haemolytic and biochemical properties as described by Wilson and Miles (1964) and Collee *et. al.* (1989).

Antibiogram studies

All the bacteria isolated through microbiological procedures were subjected to antimicrobial susceptibility testing by disc diffusion method (Anonymous, 2004). The sensitivity against amoxicillin, enrofloxacin, gentamycine, chloramphenicol and oxytetracycline was determined on Muellar-Hinton agar as described by National Committee for Clinical Laboratory Standards.

RESULTS AND DISCUSSION

Relative prevalence of Isolates

Of 30 milk samples (11 from cattle and 19 from buffaloes) collected from clinically mastitic animals, all yielded bacterial isolates of different species. A total of 44 isolates were obtained. Relative prevalence of *Staphylococcus aureus* was the highest (50% and 53.85% in cattle and buffaloes, respectively) followed by *Streptococcus agalactiae, Escherichia coli, Streptococcus dysagalactiae* and *Corynebacterium bovis.* About 27.78% isolates were identified as *Streptococcus agalactiae,* 16.67% *Escherichia coli,* 5.55% *Streptococcus dysagalactiae,* in cattle, whereas in buffaloes 23.07% *Strep. agalactiae,* 15.38% *E. coli,* 3.85% *Strep. dysagalactiae* and 3.85% *Corynebacterium bovis* were reported in the present study. The findings of present study are in complete accordance with the findings of Shireen (1984) who reported 55.26%, 18.42%, 15.78% and 3% prevalence of *Staph. aureus, Strep. agalactiae, E. coli* and Corynebacterium spp. in cattle. Razzaq (1998) reported 53% prevalence of *Staph. aureus* in buffaloes, which is in line with the findings of present study. Khan (2002) recorded 23% prevalence of *Strep. agalactiae* in buffaloes which also is supported by the results of present study. Memon *et al.* (1999) also reported a higher prevalence of *Staph. aureus* followed by streptococci and *E. coli,* supporting the findings of present study.

Antibiotic susceptibility

All the 44 isolates were tested against 5 common antibiotics. The results obtained showed that the average sensitivity of the isolates was in descending order to Enrofloxacin, Chloramphenicol, Gentamycin, Oxytetracycline and Amoxicillin. Enrofloxacin was found to be the most effective and amoxicillin least effective against various mastitis pathogens in this study. Sensitivity of different mastitis pathogens to different antibiotics is shown in Table 1.

Table 1. In vitro antibiotic sensitivity results of bacterial isolates

Bacterial isolate	% of sensitive isolates against				
	Amoxicillin	Enrofloxacin	Gentamycin	Chloramphenicol	Oxytetracycline
Staphylococcus aureus	39	78	96	86	69
Streptococcus agalactiae	36	82	91	82	54
Escherichia coli	57	100	85	71	71
Streptococcus dysagalactiae	0	100	50	100	50
Corynebacterium bovis	100	100	00	00	00

Costa *et al.* (2000) also found high sensitivity of *Staphylococcus aureus* to gentamycin (80%), which is congruent with the findings of present study. The findings of present study are in accordance with those of Dhakal *et al.* (2007) who found that enrofloxacin had the highest average sensitivity (91%) for all types of bacteria and the effectiveness of gentamycin and chloramphenicol was 87% and 82% respectively. Less effectiveness of amoxicillin to all the isolates may be due to the resistance produced in the bacteria due to extensive use of this antibiotic in cattle and buffaloes.

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