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Prevalence and Current Antibiogram Trend of Mastitic Agents in Udgir and its Visinity, Maharashtra State, India

Sudhakar P. Awandkar, Narendra V. Khode, Vikas M. Sardar and Mangesh S. Mendhe
Department of Microbiology,
College of Veterinary and Animal Sciences,
Maharashtra Animal and Fishery Sciences University,
Udgir-431 517, Maharashtra, India

Abstract: The study was carried out to investigate the current antibiogram status of clinical mastitis among bovines in and around Udgir. The prevalence of mastitis was assessed by the results of bacteriological evaluation of milk samples collected from clinical mastitis cases. A total of hundred clinical cases of mastitis were studied for isolation followed by antibiotic sensitivity test. The major prevalent pathogens isolated were *Staphylococcus aureus*, *Escherichia coli* and *Candida*. Antibiogram studies indicated that ciprofloxacine was the most effective antibiotic followed by gentamicin, enrofloxacine, chloramphenicol and cephotaxim. The least effective antibiotics include cloxacillin followed by ampicillin, streptomycine, oxytetracycline, amoxyciine, doxyciline and ceftriaxone. It was concluded that microbiological and antibiogram studies are necessary for treatment and control of bacterial mastitis.

Key words: Prevalence, etiology, clinical mastitis, antibiogram, antibacterial

INTRODUCTION

Mastitis is an inflammation of the mammary glands of dairy cows accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue. Bovine mastitis is most costly and has become extremely complex disease in India. Bovine mastitis is a major disease that affects the dairy industry. The disease causes significant losses to the dairy industry and affects milk hygienic and sanitary features. Mastitis is also of nutritional and technological significance in milk processing as valuable components like lactose, fat and casein are decreased, while undesirable components like ions and enzymes are increased (Anakalo *et al*., 2004).

Mastitis is produced by a variety of pathogenic microorganisms (Hawari Azmi and Fowzi, 2008). Although, stress and physical injuries may cause inflammation of mammary glands, infection by invading bacteria or other microorganisms (fungi, yeast and possibly viruses) is the primary cause of mastitis (Hawari Azmi and Fowzi, 2008). The mastitic milk carries bacterial toxins. These toxins are responsible for food poisoning outbreaks and toxigenic syndrome in humans. They may also contribute to the persistence of bacteria in bovine mammary glands and increased udder pathogenicity (Moon *et al*., 2006).

Bovine mastitis is associated with deteriorated milk quality, significant reduction in milk yield and increased costs of production. The economic losses from mastitis due to severe drop in milk production, potential health risks for other animals and human beings, increased cost of treatment and culling processes, are tremendous. In addition to economic losses to farmers, effective control of

Corresponding Author: Dr. Sudhakar P. Awandkar, Department of Microbiology,
College of Veterinary and Animal Sciences, Udgir-431 517, Maharashtra, India
Tel: +919881192650 Fax: +91-2385-257448

mastitis is also important from the consumer's and processor's point of view, because milk from affected animals may harbor organisms potentially pathogenic to humans and the processing of such milk may result in substandard fermented products (Dhakal *et al*., 2007). It has been estimated that the mastitis alone can cause approximately 70% of all avoidable losses incurred during milk production (Sumathi *et al*., 2008). The disease also results in partial or complete damage to udder tissues and decreases the productive life span of the animal.

Mastitis is considered as one of the major cause of antibiotics use in dairy animals. Over 135 different microorganisms have been isolated from bovine intramammary infections (Hawari Azmi and Fowzi, 2008). Bacteria are one of the major etiological agents of mastitis. Treatment failure in mastitis is due to indiscriminate use of antibiotics without testing *in vitro* sensitivity. This practice increases economic losses due to costly treatment and also results in development of resistance to antimicrobials. Antibiogram studies are essential to avoid further complications. Identification of mastitis causing pathogens and the results of the antibiotic resistance of the isolated bacteria are important prerequisites for implementation of effective control of mastitis. Such information is needed not only to treat and control mastitis but also to support public health concerns in developing countries (Dhakal *et al*., 2007). Considering these points in view the present study was undertaken to identify the mastitic agents and to study current antibiogram trend of mastitic bacteria in and around Udgir.

MATERIALS AND METHODS

Source of Milk Samples

Milk samples (100) were collected from cattle and buffaloes suffering from clinical mastitis from different locations in and around Udgir during January 2008 to December 2008. Relevant history of individual animal was recorded.

Isolation and Identification

The bacteria were isolated in pure culture on Nutrient Agar and the Candida were isolated on Corn Meal Agar (CMA). Further identification of bacteria was done on the basis of colony characters, staining (Grams) reaction, growth patterns on Blood Agar and Mac Conkey Agar (i.e., hemolysis and lactose fermentation, respectively) followed by motility, morphology, arrangements and biochemical tests (Indol, MR, VP, Citrate and Catalase). Candida was identified by colony characters on CMA and staining patterns.

Antimicrobial Susceptibility Testing

Minimal Inhibition Concentration (MIC) values of the bacterial organisms were analyzed for twelve different antimicrobials namely amoxicillin (30 mcg), ampicillin (25 mcg), ceftriaxone (30 mcg), cephotaxim (30 mcg), chloramphenicol (30 mcg), cloxacillin (10 mcg), ciprofloxacin (30 mcg), doxycycline (10 mcg), enrofloxacin (10 mcg), gentamycin (30 mcg), oxytetracycline (30 mcg) and streptomycin (25 mcg). The disc diffusion method as described previously (Bauer *et al*., 1966) was employed and the interpretation was made as per the interpretation chart provided by the manufacturer of discs depending on the diameter of zone of inhibition of bacterial growth.

RESULTS

The data analysis of this study showed that the age of all recorded cases ranged in between 5 to 8 years. Amongst hundred milk samples from clinical mastitis, 63% samples revealed bacterial growth and 7% samples revealed fungal (Candida) growth. No growth was evident in 30% samples.

Table 1 shows prevalence of mastitic agents in bovines in and around Udgir. The study indicated that the major prevalent pathogens associated with bovine mastitis in and around Udgir were

Table 1: Prevalence of mastitic agent from bovines in and around Udgir

Isolates	No. of positive samples	Percent	Overall percent
E. coli	28	40.00	28
S. aureus	24	34.29	24
Pasturella	1	1.43	1
Pseudomonas	1	1.43	1
Bacillus	2	2.86	2
Proteus	1	1.43	1
Mixed	6	8.57	6
Candida	7	10.00	7

Table 2: Percent sensitivity of bovine mastitic agents to different antibiotics

Antibiotics	No. of sensitive samples	Percent
Amoxycillin	6	9.52
Ampicillin	2	3.18
Ceftriaxone	9	14.29
Cephotaxim	15	23.81
Chloramphenecol	20	31.75
Ciprofloxacin	44	69.84
Cloxacillin	1	1.59
Doxycycline	6	9.52
Enrofloxacin	37	58.73
Gentamycine	43	68.25
Oxytetracycline	5	7.94
Streptomycine	3	4.76

Escherichia coli (40.00%) followed by *Staphylococcus aureus* (34.29%), other bacteria (15.71%) and *Candida* (10.00%) amongst isolated pathogens. The overall prevalence of *Escherichia coli* in bovine mastitis recorded during this study was 28.00% followed by *Staphylococcus aureus* (24.00%), other bacteria (11.00%) and *Candida* (7.00%). Other bacteria include *Pasturella* (1.43%), *Pseudomonas* (1.43%), *Bacillus* (2.87%), *Proteus* (1.43%) and mixed bacteria (8.57%).

The *in vitro* antibiogram studies were conducted on 63 samples which exhibited bacterial growth and antifungal therapy was advised for cases showing fungal mastitis. The *in vitro* antibiogram studies (Table 2) revealed ciprofloxacin to be the most effective drug (69.84%) followed by gentamycin (68.25%), enrofloxacin (58.73%), chloramphenicol (31.75%) and cephotaxim (23.81%). The sensitivity to cloxacillin observed was the lowest (1.59%) followed by ampicillin (3.18%), streptomycin (4.76%), oxytetracycline (7.94%), amoxicillin (9.52%), doxycycline (9.52%) and ceftriaxone (14.29%).

DISCUSSION

The age of all recorded cases ranged in between 5 to 8 years. Sharma *et al.* (2007) and Sharma and Prasad (2002) reported high prevalence of mastitis between 5 to 7 years and above 7 years of age. Increasing evidence of mastitis with advanced ages was recorded by Hawari Azmi and Fowzi (2008). They also noticed that the incidence of clinical and subclinical mastitis was increased with the sixth years old in some herds. Dhakal *et al.* (2007) found more incidence of mastitis in first and second calving which decreased with increased numbers of calving. The finding of the present study is in accordance with the observations of above workers with little variations which might be because of different geographical attributes and individual variation in susceptibility.

In this study the mastitic agents were isolated from 70% cases, while no growth was evident in 30%. The failure of pathogens to grow *in vitro* in high percentage of samples may be because of premedication of the animals with antibiotics, non-bacterial causes and the type of media that did not support the growth of whole range of bacteria associated with mastitis.

The study revealed *Escherichia coli* (40%) and *Staphylococcus aureus* (34.29%) as the major pathogens causing bovine mastitis. Sharma and Prasad (2002) recorded 54.50 per % incidence of

Staphylococcus in bovine mastitis, while Shrirame *et al.* (2002) recorded more prevalence of *Staphylococcus* (72.35%). The predominant bacterial isolates recovered by Sumathi *et al.* (2008) from bovine mastitis were *Staphylococcus aureus* and *Escherichia coli* which are in accordance with the present findings. Hawari Azmi and Fowzi (2008) revealed that *Staphylococcus aureus* (40.6%) and *Coliforms* (26.1%) were the chief aetiological agents responsible for clinical mastitis. They reported the incidence of *Proteus* sp. 1.4% *Pseudomonas* sp. 4.3%, mixed 7.3% and others 5.8% in clinical mastitis. These findings support the findings of present study.

The findings of present study showed the highest incidence of *Escherichia coli* (40.00%), which is presumably due to the fact that *Escherichia coli* is the commonest environmental contaminants, which is closely associated with hygiene. It becomes pathogenic whenever the hygienic conditions of the animal or environment become poor. Moreover, the existence of high concentration of *Escherichia coli* in milk also indicates the relatively poor quality of milk, related with substandard hygiene of farm management, milk collection and processing system. Staphylococci, Pseudomonas and mixed growth were the second after *Escherichia coli*. Their presence was also an indication of sub-standard dairy farming. Higher incidence of *Escherichia coli* mastitis may be due to poor hygienic conditions, as *Escherichia coli* originates from the cow's environment and infect the udder via the teat canal (Sumathi *et al*., 2008).

The hygiene at milking is of paramount important in control of these infections because the infections are spread during milking process (Harmon, 1993).

Gentamicin, enrofloxacin, ciprofloxacin and chloramphenicol are not commonly used for treatment of mastitis in the area of study resulting in higher efficacy of these drugs. Ciprofloxacin proved to be the drug of choice in this study. Few workers found highest sensitivity of mastitic agents to enrofloxacin, gentamycine (Dhakal *et al*., 2007; Kumar and Sharma, 2002) and chloramphenecol (Rao *et al*., 1989) and least sensitivity to ampicillin (Dhakal *et al*., 2007) and cloxacillin (Rao *et al*., 1989). Similar antibiogram patterns were reported by Sumathi *et al.* (2008) and Choudhri (2000), while Anakalo *et al.* (2004) reported *Staphylococcus aureus* and Coagulase Negative Staphylococcus (CNS) as the major mastitis-inducing pathogens suggestive of a possible development of resistance from prolonged and indiscriminate usage of beta-lactam antibiotics.

The results of Hawari Azmi and Fowzi, (2008) are not in agreement to the present findings. They found that the 64% isolates, isolated from mastitic milk were sensitive to Tetracycline and 52.8% to Ampicillin.

Amoxicillin, ampicillin, cloxacillin, streptomycin and oxytetracycline are commonly used antibiotics in bovine mastitis. The mastitic bacteria showed resistance to these commonly used antibiotics. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against mastitic bacteria. Since, streptomycin has been extensively used along with penicillin for treating mastitis; it may have led to the development of high resistance in bacteria against this antibiotic.

Moreover, due to lack of prophylactic agents, chemotherapy continues to play a major role in therapeutic management of the disease. For success of treatment the antibiotic sensitivity test play a major role. Recently higher antibiotics have been introduced in treatment of both sub-clinical and clinical mastitis (Sharma *et al*., 2007). It is believed that infections with *Staphylococcus aureus* respond poorly to therapy with antimicrobial agents, whether given parenterally or via the intramammary route (Moon *et al*., 2006). The emergence of antibacterial resistance among pathogens that affect animal health is of growing concern in veterinary medicine. Antimicrobial-resistant pathogens in animals have been incriminated as a potential health risk for humans from possible transmission as foodborne pathogens. Particularly, mastitis is the single greatest cause of antibacterial use on dairy farms (Moon *et al*., 2006).

Because the quality of the milk cannot be improved following extraction from the cow, production of high quality milk requires an efficient mastitis control program. Cows with a high prevalence of mastitis are incapable of producing high quality milk until the inflammation and infection

in the udder are brought under control. This has severe economic implications for the milk producer, as the milk is no longer marketable and other animals are easily infected. Treatment and decrease in milk volume also cause considerable losses (Anakalo *et al*., 2004). Therefore, establishing an antibiogram of pathogens is very important from the clinical and economic points of view.

The findings of this study showed the use of higher antibiotics as most effective antibiotic therapy for control of bovine mastitis in and around Udgir area. Since, this study reported overall 7.00% prevalence of fungal mastitis, during antibacterial treatment, the fungal cause shall also be taken into consideration. Further, antibiogram of mastitic milk is suggested before starting the treatment so as to get maximum efficacy of used antibiotics and to prevent the development of resistance to antibiotics due to indiscriminate and frequent use.

Systematic records regarding the epidemiology of bovine mastitis including status of infection, antibiogram studies and treatment patterns would provide useful management information to the producer, farmer and veterinarian. This has been evident from countries where records have been documented regularly internationally (Anakalo *et al*., 2004). Thus, there is a need to routinely investigate and record the epidemiology of bovine mastitis and antibiogram sensitivity of bacterial isolates in various parts of India.

REFERENCES

Anakalo, S., A. Gathoni, G. Tura and M. Milcah, 2004. Prevalence of bovine mastitis amongst small holder dairy herds in Kenya. Israel J. Vet. Med., 59: 1-2.

Bauer, A.M., W.M.M. Kirby, J.C. Sherris and M. Turk, 1966. Antibiotic susceptibility testing using a standard single disc method. Am. J. Clin. Pathol., 45: 493-496.

Choudhuri, P.C., 2000. Status paper on mastitis in Andhra Pradesh. Proceedings of Round Table Conference (IAAVR) on Mastitis. Feb. 18-19, Izzatnagar, pp: 84-92.

Dhakal, I.P., P. Dhakal, T. Koshihara and H. Nagahata, 2007. Epidemiological and bacteriological survey of buffalo mastitis in Nepal. J. Vet. Med. Sci., 69: 1241-1245.

Harmon, R.J., 1993. Symposium mastitis and genetic evaluation for somatic cell count. J. Dairy Sci., 77: 2103-2112.

Hawari Azmi, D. and A. Fawzi, 2008. Prevalence and distribution of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Jordan. Am. J. Anim. Vet. Sci., 3: 36-39.

Kumar, R. and A. Sharma, 2002. Prevalence, etiology and antibiogram of mastitis in cows and buffaloes in Hissar, Haryana. Ind. J. Anim. Sci., 72: 361-363.

Moon, J.S., A.R. Lee, H.M. Kang, E.S. Lee, Y.S. Joo, Y.H. Park, M.N. Kim and H.C. Koo, 2006. Antibiogram and coagulase diversity in staphylococcal enterotoxin-producing staphylococcus Aureus from bovine mastitis. J. Dairy Sci., 90: 1716-1724.

Rao, R., P.C. Choudhuri and M.S. Chetty, 1989. Incidence, aetiology and antibiogram of pathogens isolated from clinical cases of mastitis. Ind. J. Comp. Microbiol. Immunol. Infect. Dis., 10: 7-11.

Sharma, A.K. and B. Prasad, 2002. Microbial profile and antibiogram studies in mastitic dairy animals of Palampur of H.P. Proceedings of 10th Indian Society of Veterinary Medicine, Feb. 14-16, Bikaner, Rajasthan, India, pp: 93-93.

Sharma, H., S.K. Maiti and K.K. Sharma, 2007. Prevalence, etiology and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in durg, chhattisgarh state. Int. J. Dairy. Sci., 2: 145-151.(India).

Shrirame, K.R., S.S. Bhardwaj, D.R. Kalorey and S.D. Harne, 2002. Study of pthogenicity markers of *Staphylococci* isolated from subclinical mastitis. Proceedings of Round Table Conference on Mastitis, Feb. 4-5, Nagpur, India, pp: 18-22.

Sumathi, B.R., B.M. Veeregowda and R. Amitha Gomes, 2008. Prevalence and antimicrobial profile of bacterial isolates from clinical bovine mastitis. Vet. World, 1: 237-238.