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Prevalence, bacterial etiology and antibiotic susceptibility pattern of bovine mastitis in Meerut

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Abstract
Increasing antimicrobial resistance has become a big challenge worldwide. Mastitis is the most common disease for antibiotic use in dairy herds and thus, antimicrobial resistance of mastitis pathogens has received recent attention. The objective of the current study was to assess the status of bovine mastitis in and around Meerut region, bacterial pathogen involved and to determine the susceptibility of different antibiotics. A total of 85 milk samples were presented to Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Meerut from April, 2016 to June, 2017 with a history of swelling in udder, loss of milk, flakes formation in milk, RBC in milk and watery milk. The milk samples were cultured on brain heart infusion agar, MacConkey's agar, eosin methylene blue agar and sabrourad dextrose agar. The isolated organisms through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to a twelve number of antibiotics. The major prevalent pathogens isolated were *Staphylococcus Spp.* (42.55%), *E. coli* (21.28%), *Streptococcus* spp. (6.38%), *Proteus* spp. (8.51%), *Candida* spp. (2.88%) and mixed infection (18.26%). The present results revealed that gentamicin (65.96%) was the most effective antibiotic followed by enrofloxacin (63.83%), cefotaxime+clavulanic acid (52.13%), amoxicillin+sulbactum (42.55%), ciprofloxacin (41.49%), colistin (41.49%), chloramphenicol (39.36%) and ampicillin+sulbactum (38.29%). Least effective drugs were oxytetracycline (22.34%), streptomycin (25.53%) whereas maximum resistance drug were found amoxyclave (8.51%), and ampicillin/cloxacillin (8.51%).

Keywords: Mastitis, antibiotics, disk diffusion

Introduction
Acquired antimicrobial resistance in bacteria has been a growing concern worldwide [1]. The era of overuse and misuse has lead to the evolution of resistant forms of previously harmless bacteria. Mastitis is one of the most costly and important disease of dairy industry and antimicrobials are important part of therapy [2]. Mastitis is a multi-etiological and complex disease, which is defined as inflammation of parenchyma of mammary glands and represents one of the most difficult veterinary diseases to control [3]. Mastitis adversely affects animal health, quality of milk and the economics of milk production, affecting every country, including developed ones and causes huge financial losses [4]. One important reason for treatment failure is assumed to indiscriminate use of antibacterials without testing *in-vitro* sensitivity of causal organisms [5]. Bovine mastitis is classified as: contagious mastitis, generally caused by contagious bacteria presiding on the skin of the teat and inside the udder, transmitted from one cow to another during milking (e.g., *Staphylococcus aureus* or *Streptococcus agalactiae*) and environmental mastitis, caused by environmental pathogens normally found in shed surroundings such as bedding, manure, soil, and feed (e.g., *Escherichia coli*, *Streptococcus uberis*, *Klebsiella* sp.) [6]. It has been estimated that the mastitis alone can cause approximately 70% of all avoidable losses incurred during milk production. In India, annual economic loss to dairy industry due to subclinical mastitis and clinical mastitis is estimated to be Rs. 7165.5 crores [7]. The occurrence of disease is an outcome of interplay between three major factors: infectious agents, host resistance, and environmental factors [8]. Multi-drug resistant bacteria are a persistent problem in modern health care, food safety and animal health. There is a need for new antimicrobials to replace over used conventional antibiotics [9]. Therefore, a bacteriological diagnosis, prevalence study in the herd and proper selection of antibiotic based on antibiotic sensitivity are critical for rational and effective control of mastitis.

Keeping these points in view the present study was undertaken to identify the major pathogens associated with mastitis and to select a suitable antibiotic for treatment.

Materials and Methods

Sampling

During April 2016- June 2017 a total of 85 milk samples from bovine mastitis cases were collected from different dairy farms located in and around Meerut and from cases that were presented to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Meerut. 5 to 10 ml of milk samples from the infected cattle were collected aseptically in sterile vials. Before sample collection the udder was thoroughly washed with the potassium permanganate solution (1:1000) and wiped with clean cloth to allow dry and the teats were mopped with 70% ethyl alcohol. Relevant information about the farm, breed and history of individual animal were recorded.

Bacteriological culture

The media and chemicals were obtained from Hi-media, Mumbai (India) and prepared in the laboratory as per the standard procedures [10]. Milk samples were cultured microbiologically by plating on brain heart infusion agar (BHI), macConkeys lactose agar (MLA), sabrouard dextrose agar (SDA). Plates were incubated 48 h at 37◦C and bacterial growth recorded at both 24 and 48 h of incubation [11]. The typical colonies were sub-cultured in a selective broth and subjected to various tests viz., Gram reaction, oxidase, catalase, IMViC, motility and growth on TSI slant for biochemical characteristics as per the method of Quinn *et al*. [12].

Susceptibility testing

Sensitivity of isolates was determined by the disc diffusion method as described by Bauer *et al*. [13] was employed and the interpretation was made as per the zone size interpretation chart provided by the manufacturer of discs. All the bacterial isolated were analysed for twelve different antimicrobials discs (M/s Hi Media Laboratories Ltd., Mumbai, India) namely – amoxyclav (30 mcg), amoxicillin+sulbactum (30/15 mcg), ampicillin+cloxacillin (10 mcg), ampicillin+sulbactum (10/10 mcg), cefotaxime+clavulanic acid (30/10 mcg), ciprofloxacin (5 mcg), chloramphenicol (30 mcg), colistin (10 mcg), enrofloxacin (10 mcg), gentamicin (10 mcg), oxytetracycline (30 mcg) and streptomycin (10 mcg).

Results

Isolates and infection pattern

A total of 85 milk samples were processed, 71 (83.52%) samples showed bacterial as well as fungal growth and 14 (16.47%) were negative for any bacterial as well as fungal growth after incubation of 3 days for bacteria and 5 days for fungal growth at the standard temperature. A total of 94 bacterial isolates including 3 yeast were recovered from 71 milk samples obtained from mastitic cases. The study indicated that the major prevalent pathogen associated with bovine mastitis in and around Meerut was *Staphylococcus* spp. (42.55%) followed with *E. coli* (21.28%). Other commonly isolated pathogens were *Streptococcus* spp. (6.38%), *Proteus* spp. (8.51%) mixed infection (Gram positive and gram negative bacilli) (18.09%) and *Candida* spp. (3.19%). Table 1 shows prevalence of mastitic agents in bovine in and around Meerut.

Table 1: Prevalence of bacterial isolates against mastitic milk (n=94)

S. N.	Isolates	No. of positive samples	Percentage (%)
1	*Staphylococcus* spp.	40	42.55
2	*Streptococcus* spp.	06	6.38
3	*E. coli*	20	21.28
4	*Proteus* spp.	08	8.51
5	Mixed infection (Gram Positive & Gram negative bacilli)	17	18.09
6	*Candida* spp.	03	3.19

Antimicrobial susceptibility

A total of 94 isolates were subjected to *in-vitro* susceptibility testing (Table 2). The results revealed that gentamicin to be most effective drug (65.96%) followed by enrofloxacin (63.83%), cefotaxime+clavulanic acid (52.13%). Other moderate effective drugs were amoxicillin+sulbactum (42.55%), ciprofloxacin (41.49%), colistin (41.49%), chloramphenicol (39.36%), ampicillin+sulbactum (38.29%). Least effective drugs were oxytetracycline (22.34%), streptomycin (25.53%) whereas maximum resistance drug were found amoxyclave (8.51%), and ampicillin/cloxacillin (8.51%).

Table 2: Sensitivity pattern of antibiotics (n=94)

Antibiotics	No. of sensitive samples	Per cent (%)
Amoxyclav	08	8.51
Amoxycillin/sulbactum	40	42.55
Ampicillin/cloxacillin	08	8.51
Ampicillin/sulbactum	36	38.29
Cefotaxime/clavulanic acid	49	52.13
Ciprofloxacin	39	41.49
Chloramphenicol	37	39.36
Colistin	39	41.49
Enrofloxacin	60	63.83
Gentamicin	62	65.96
Oxytetracycline	21	22.34
Streptomycin	24	25.53

Discussion

Bovine mastitis is the expensive disease affecting lactating cattles and therefore, antimicrobial resistance of mastitis pathogens has received a lot of interest in the past few years. In the present study mastitic pathogens were isolated from 71 (83.52%) milk samples in and around Meerut region. The failure of 14 (16.47%) milk samples to grow *in-vitro* may be because of premedication of the animals with antibiotics, non-bacterial cause and the type of media which do not support the growth of whole range of bacteria associated with mastitis. The study revealed that *Staphylococcus spp.* was the major pathogen causing mastitis. In line with this finding, Verma *et al.* [14]; Workneh *et al.* [15]; Nessru *et al.* [16] and Vaarst and Envoldsen [17] presented similar data on the primary role of *S. aureus* in bovine mastitis followed by *E. coli.* These results are almost in the concurrence of previous study conducted in the region in 2010, which revealed *S. aureus* as a major pathogen in the cases of mastitis in Mathura and its surroundings. The incidence of *S. aureus* was 37.03% and 31.70% in cattle and buffaloes, respectively [18]. The finding of Sudhakar *et al*. [19] also reported higher prevalence rate of *Staphylococcus aureus* and *E. coli* in and around Udgir. Various studies have been conducted in different parts of country to assess the prevalence status of bacterial pathogens in mastitis of dairy animals. Similar to the present

findings, Sumathi *et al*. [20] also reported the higher proportion of *E. coli* and *Staphylococcus aureus* in clinical mastitis cases of dairy cattle in and around Bangalore. The high prevalence of staphylococci has been reported by several workers in India and abroad (Das & Joseph [21]; Sharma & Sindhu [22]; Bhanot *et al*. [23]; Hawari and Dabas [24]; Tenhagen *et al*. [25]; Nickerson [26] and Zutic *et al*. [27] also indicate the highest prevalence of *Staphylococci* followed by other mastitogenic bacteria. Hawari azmi and fowzi [24] revealed that *Staphylococcus aureus* (40.60%) and coliform (26.10%) were the chief aetiological agents responsible for clinical mastitis. They reported the incidence of *Proteus* spp. (1.40%), *Pseudomonas* spp. (4.30%), mixed (7.30%) and other (5.80%) in clinical mastitis which were similar results reported by Verma *et al*. [14]. Distribution of pathogens in mastitis changes over time, therefore, bacteriological examination at herd level must be taken regularly to monitor udder health. The higher incidence of *Staphylococci* indicates unhygienic milking practices as this pathogen is mainly spread during milking via milker's hands. The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of *Staphylococcus aureus* whereas prevalence of *E. coli* is an indication of poor hygienic practices in dairy as these organisms originate from the cow's environment and infect the udder through the teat canal. Contamination of end of the teat is a major predisposing factor in development of environmental mastitis [28].

Early treatment of mastitis with effective antibiotics significantly limits the severity of mastitis, economic loss and development of antimicrobial resistance. Antibiotic sensitivity test is done and based on Zone of inhibiton. The antibiogram profile of different bacterial isolates indicated that gentamicin was the most effective drug followed by enrofloxacin, ampicillin+sulbactum and cefotaxime+clauvanic acid against mastitis causing bacteria in the study (Table-2). Almost similar antibiogram pattern was reported by Verma *et al*. [14] and Iqbal *et al*. [29] who described gentamicin, enrofloxacin and norfloxacin as most effective drugs amongst the 12 antibiotics tested *in vitro*. The results are also in line with the work of Sumathi [20] and Sudhakar *et al*. [19] who reported higher efficacy of gentamicin, enrofloxacin and ciprofloxacin in the area of study.

In this study gentamicin proved to be the highly sensitive to mastitic agents which supported by the study conducted by Verma *et al*. [14]. Few workers found highest sensitivity of mastitic agents to gentamycin, enrofloxacin [30, 31] and chloramphenicol [32] and least sensitive to ampicillin and cloxacillin which supports this study. The mastitic bacteria showed less sensitive to these commonly used antibiotics oxytetracycline, amoxyclave, and ampicillin+cloxacillin due to the frequent use of these antibiotics. Indiscriminate and frequently use of these antibiotic in animals could be the reason for their ineffectiveness against mastitic bacteria.

Mastitis was primarily a concern of dairy farmers and dairy processors. However, because of worries about antimicrobial residues, antimicrobial resistance, milk quality and animal welfare, mastitis has also become a concern to consumers and society. Mastitis is difficult disease to control because many different bacteria are capable of infecting the udder and producing the disease. Data presented should therefore provide a good estimate of antimicrobial susceptibility of udder pathogens encountered in mastitis in the field. Moreover, due to lack of prophylactic agents, chemotherapy continues to play a major role in therapeutic management of the disease. For success of the treatment the antibiotic sensitivity test play a major role.

Conclusion

Livestock production makes an important contribution to economic development, rural livelihood. Mastitis is a heavy economic burden in dairy sector. Apart from the risk therapy, bacteria gradually become resistant to routinely used antibiotics. So regular screening of samples is necessary to choose an effective antibiotic to treat and control multi-drug resistance.

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