Antimicrobial susceptibility profiling of milk samples from bovine clinical mastitis

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Abstract

Mastitis is one of the most important diseases of milch animals leading to heavy economic losses due to reduced milk yield, discard of milk because of compromised quality and premature culling of animals. Early and specific use of antimicrobials significantly limits the severity of the disease and in many cases prevent the appearance of any visible sign of infection. However, the indiscriminate use of antimicrobial is leading to development of resistance in microbes. Thus the regular screening for the selection of suitable and effective antimicrobial is need of the day. With this objective, in present study primary antimicrobial drugs susceptibility test was performed on fifty clinical mastitis milk samples of cattle and buffaloes against eight commonly used drugs and their combinations to know the status of drug susceptibility to select the most effective drug for an early treatment. Drug susceptibility test revealed resistance towards multiple antimicrobials. Overall, the highest resistance was observed against cefotaxime, ampicillin-sulbactam, cefixime and ceftriaxone while majority of the culture showed susceptibility to amikacin, chloramphenicol and gentamicin. The higher resistance against third generation cephalosporin and better susceptibility for amikacin, available only in injectable preparations is suggestive of indiscriminate use of oral therapy of cephalosporins as the first choice for treatment because of their broad spectrum of action and easy mode of administration. Therefore, the present study recommends regular use of antimicrobial susceptibility testing of mastitis bacterial pathogens to decide the appropriate antimicrobial drug for treatment and to limiting the indiscriminate use of antimicrobial drugs to avoid the development of resistance among microbes.

Keywords: Antimicrobial Susceptibility, Bovines, Mastitis, Mathura.

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Introduction

Bovine mastitis is an inflammatory condition of udder[(1)] of cattle and buffaloes which results in altered quality and decreased milk production[(2)]. In severe cases, mastitis may results in partial or complete damage to udder tissues leading to decrease productive lifespan of the animal and less occasionally fatalities[(3)]. *E. coli*, *Staphylococcus spp.*, *Streptococcus spp.*, *Pseudomonas spp*. and *Mycoplasma spp*. are the most common bacteria causing mastitis. Most of the clinical episode of mastitis can be effectively treated with the help of specific antimicrobial therapy and is commonly implemented for prevention and control of mastitis. However, the susceptibility to these antimicrobial may vary species to species, climate and geographical regions, stage of disease, previous use of antibacterial and many more reasons[(4)]. Over the years, over prescription and extensive use of antimicrobial have led for resistant bacteria at an alarmingly rapid rate which has worsened the clinical scenario and we are now facing one of the greatest medical challenges of our time[(5)]. The zoonotic importance of such drug resistant pathogens is also posing challenges to human health. Further, antimicrobial resistance in bacteria is one of the reasons for low cure rates, loss in the form of milk production and animal itself, particularly for mastitis[(6)]. Hence, monitoring antimicrobial susceptibility in both pathogenic and commensal bacteria in animals is recommended by OIE[(7)]. The present study was designed to examine antimicrobial resistance in bovine clinical mastitis with the aim to generate the data helpful for the field veterinarian in the early treatment and also to provide information on trends in resistance to aid in developing the antimicrobial usage policy.

Materials and Methods

Samples

A total of fifty milk samples from the cases of clinical mastitis in cattle and buffaloes from Mathura and adjoining areas (Agra, Aligarh, Hathras, Vrindavan, Barsana, Mainpuri, Palwal, Faridabad etc.) were received at Central Disease Diagnostic Laboratory (CDDL), Department of Veterinary Microbiology during 2014-15. All the cases were reported to Teaching Veterinary Clinical Complex (TVCC) with apparent clinical signs of mastitis and were referred for antimicrobial sensitivity test. These samples were comprised of milk from cross bred and indigenous cattle and buffaloes. All the samples were subjected to routine laboratory procedures according to method described

previously[8] for the bacterial growth in nutrient broth medium.

Antimicrobial Susceptibility Test

A loop full sample was inoculated from all the milk samples into nutrient broth for 4-6 hours. Then broth was centrifuged at 3000 rpm for 5 min to obtain the pellet of bacterial growth. The supernatant was discarded and pellet was suspended in Phosphate buffer saline (PBS, pH 7.2) and adjusted to 0.5 scale McFarland turbidity standard to carry out primary antibiotic susceptibility test by agar disc diffusion method on Mueller-Hinton agar plates as per the guidelines of Clinical and Laboratory Standards Institute (CLSI)[9].

To select the effective antimicrobial agent for successive treatment, all the cultures were tested for their sensitivity against eight different antimicrobials commonly used in veterinary practice. The antimicrobial discs obtained from Himedia (Mumbai) were used in the study included amikacin (30 µg), amoxyclav (10 µg), ampicillin-sulbactam (10/10 µg), cefexime (5 µg), cefotaxime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg) and gentamicin (10 µg). The zone of inhibitions were measured and interpreted as either susceptible or resistant to the exposed agent according to the zone diameter interpretative standards provided by the manufacturer. All the results were compiled and compared to ascertain the pattern of drug resistance in common bacterial pathogens of mastitis in Mathura and its surrounding.

Results

The antibiotic susceptibility tests of the cultures from mastitis milk revealed resistance to multiple antibiotics tested in the pathogens involved. The number of samples showing sensitivity or resistance was expressed in percentage. The cases from cattle milk samples showed resistance to more number of antimicrobial classes than the cultures from buffalo mastitis cases (Fig. 1). Among the thirty five samples of cattle origin 93.75% found resistant to cefotaxime, 88.24% to amphicillin-sulbactum, 85% to ceftriaxone, 83% to amoxyclav and 81% to cefepime. The susceptibility shown towards amikacin, chloramphenicol, gentamicin, ceftriaxone was 70%, 56.67% and 53%, respectively. In case of buffaloes samples, majority of the cultures (88.89%) showed sensitivity towards chloramphenicol followed by amikacin (81.82%), ceftriaxone (60%), gentamicin (50%) and resistance to ampicillin-sulbactam and cefotaxime with 71% and 60%, respectively. The overall antibiogram profile indicated amikacin, chloramphenicol and gentamicin to be the most effective antimicrobials against mastitis causing bacteria in the study.

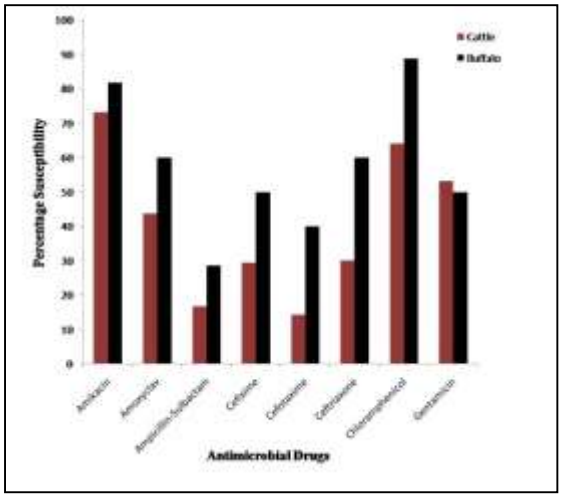

Fig. 1: Antimicrobial susceptibility profile for bovine mastitis samples

Discussion

One of the reasons for low production of milk is poor health condition of animals, particularly mastitis is the biggest problem in terms of economic loss in the country[10]. Most of the mastitis cases are of infectious etiology[11]. Over 135 microorganisms have been isolated from bovine intramammary infection with the major etiology of bacterial origin[12,13]. In recent time there are reports of multiple drug resistance in the common bacterial agents responsible for mastitis in bovines and that can effectively be cured by judicial use of antimicrobials. The early treatment significantly limits the severity of the disease and, in many cases, prevents the appearance of any visible signs of infection. The study conducted includes primary antimicrobial susceptibility test to select the effective drug for an early treatment of the animals. The test revealed resistance towards multiple traditional and newly introduced antibiotics. The multiple drug resistance among the cultures involved in mastitis has also been reported by several other researchers[14-16] and can be attributed to the injudicious use of antibiotics[17-20] or selection pressure of antimicrobials on pathogens or colonization of the mammary gland by resistant strains along with the negligence towards bacteriological examination of the mastitis suspected milk. Majority of the cultures showed resistance to the third generation cephalosporin's viz. cefotaxim, cefixime and ceftriaxone which is in concordance with most of the recent reports of drug resistance pattern in investigations conducted on mastitis pathogens[21,22]. This high emergence of resistance to cephalosporin's is not surprising as due to its broad spectrum of action, it has been adapted by most of the clinicians and all the quacks practicing in the field as the first line of treatment in recent years. Thus, the probable long-term indiscriminate use of these antibiotics in the region has led to the appearance of resistance in mastitogenic bacteria against third generation cephalosporin[23,24]. Susceptibility to

amikacin, chloramphenicol and gentamicin of the cultures under study was in close agreement with many workers[25-27]. Farooq et al.[28] during their study period have found that norfloxacin, gentamicin and chloramphenicol were the most effective drugs whereas Sumathi et al.[29] reported gentamicin to be most effective among the antimicrobials used in study. The susceptibility to qunilones can be attributed to their lesser use in treatment of mastitis now a day due to its higher dose and twice a day application resulting in higher efficacy of these drugs. Behavioral and physiological differences among cattle and buffaloes are quite evident, even then similar prevalence and antibiogram profiles in both the species is clear indicative of irrational use of antibiotics which is the major cause of bacterial resistance against various antibiotics.

Conclusion

Development of resistance among bacteria is a matter of great concern in the field of veterinary medicine especially when the organisms render an animal unproductive. Though species, region, manage mental practices and the preferences of antibiotics used in the studies leads to variation in the antibiograms patterns and inaccuracy in correlations of results from different studies carried out. Even then, the regular screening of cultures for susceptibility pattern will be useful for selecting an appropriate antibiotic and also to know the changing trends of antibiotic resistance for developing antibiotic usage policy and for limiting the indiscriminate use of powerful antibiotics like cephalosporin as initial treatment. The present study showed a high percentage of resistance against widely used third generation cephalosporin class of antibiotic and amikacin proved to be the drug of choice in majority of the cases. Considering the economic importance of mastitis and the rapid rate of emergence of resistance to multiple antibiotics in the pathogen involved, awareness is required among farmers and clinicians for reducing the unnecessary use of antimicrobial drugs. As mastitis is a major cause of antibiotic use in dairy animals, early diagnosis, establishment of correct *in-vitro* antibiogram along with proper food and hygiene status of animal is very much essential to control mastitis and to prevent the spread of resistant clones of bacteria to other susceptible animals.

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