

Phenotypic analysis of female reproductive tract disease of cattles

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Abstract

Aim: To identify the organisms causing endometritis in cattle and to determine their in vitro sensitivity to various antibiotics

Materials and methods: Sixty uterine secretion samples, of cattles were collected in Bikaner district of Rajasthan from clinical cases of endometritis. Both aerobic and anaerobic bacteria were isolated from endometritis showed the characteristic colony, were gram stained and confirmed by standard biochemical tests. The invitro antibiotic sensitivity test with different antibiotic discs were carried out.

Results and Discussion: Out of 60 samples, 50 contained different bacterial strains and 10 were bacteriologically sterile. Different bacterial species isolated from these samples were, *Escherichia coli* (36.66%), *S.aureus*(30%), *Klebsiella spp* (13.33%), *Pseudomonas aeruginosa* (6.66%), *Streptococcus spp* (3.33%). The in vitro antibiotic sensitivity test indicated that the highest number of isolates (64%) were sensitive to Ceftriaxone, followed by Gentamicin, Enrofloxacin and Chlortetracycline(32%).Chloramphenicol showed sensitivity in minimum number (8%) of isolates.

Conclusion : This study revealed that the bacteria *E.coli* and *S.aureus* are more commonly isolated in endometritis in cattles and the drug Ceftriaxone is highly effective.

Keywords: antibiotic sensitivity, bacteriology, cattles, metritis, endometritis

Introduction

Reproduction is essential for the propagation of life. Reproductive health performance in mammals is mainly influenced by various postpartum uterine diseases such as metritis, endometritis and mastitis. Postpartum uterine disease is a global problem with higher prevalence in animals. Metritis, pyometra, endometritis, RFM and some other non specific infections of the uterus are the most important factors causing infertility in the dairy cattle. Most important causes of subfertility in dairy cows are reported to be metritis and endometritis[1]. Metritis involves the endometrium, the underlying glandular tissues and the muscular layer[2,3]. While, endometritis, involves only the endometrium with the underlying glandular tissues [4], without any systemic signs [5]. Bacterial isolation has been reported from uterine secretions of cattles suffering from endometritis [14]. A wide variety of bacteria has been isolated from uteri of postpartum cows. Most common pathogens of endometritis are *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Prevotella species* [15,16]. Besides *Actinomyces pyogenes*, some Gram-negative anaerobic bacteria like *Fusobacterium*

necrophorum and *Bacteroides spp* may play an important role in endometritis [12].

Objectives of this study were, to find out the organisms causing endometritis in cattle by phenotypic analysis

Materials and Methods

Sixty uterine secretion samples, of cattles were collected in Bikaner district of Rajasthan from clinical cases of endometritis. Immediately after sample collection, sterile nutrient broth was added to each sample and incubated at 37°C for overnight. These samples were streaked on blood agar, Mac Conkey agar and Nutrient agar. The inoculated media were incubated, both under aerobic and anaerobic conditions, at 37°C and examined after every 12 hours post inoculation for the presence of any growth. Growth characteristics of the bacterial isolates were recorded. By subculturing the cultures were purified and were refrigerated for further studies. Both aerobic and anaerobic bacteria isolated from endometritis showed the characteristic colony, were gram stained and confirmed by standard biochemical tests such as catalase test, oxidase test, indole test, methyl-red test, voges-proskauer test, citrate utilization test and sugar fermentation tests as described by Barrow and Feltham [18]. Each isolate was characterized on the basis of staining behaviour, size, motility, cultural and biochemical tests. All the isolates were tested for in-vitro antibiotic sensitivity as per Muneer et.al. [19] as follows. All the isolated colonies were inoculated in nutrient broth and then incubated for over-night. The sterile cotton swab was dipped in the bacterial suspension and then rolled over the surface of the Muller-Hinton agar medium and covered evenly with the bacterial suspension. Eight different antibiotic discs were placed over the surface of the agar plate. For this purpose, separate antibiotic discs (Himedia, Mumbai) containing Gentamicin 10 mg, Chloramphenicol 30 mg, Enrofloxacin 5 mg, Ceftriaxone

30 mg, Amoxycilin 30 mg, Tetracycline 30 mg, and Ciprofloxacin 5 mg per discs were employed.

Results

Various species of bacteria isolated from uterine secretion samples of cattles and there in vitro antibiotic sensitivity to different antibiotics are presented here. Out of the total 60 uterine samples, 50 contained different bacterial strains while 10 were found bacteriologically sterile. Bacterial species isolated from these samples were, *Escherichia coli* (36.66%), *S.aureus* (30%), *Klebsiella spp* (13.33%), *Pseudomonas aeruginosa* (6.66%) and *Streptococcus spp* (3.33%). In the present study, 16.66% samples did not show any microbial growth. Since these samples were taken from animals showing clinical signs of endometritis, they cannot be bacteria-free. It is possible that the cattles were treated with antibiotic before sampling.

Discussion

The present study revealed the bacteria *E.coli* followed by *S.aureus* are most commonly responsible for endometritis in cattles. Udhayavel et al [24] in 2012, isolated *Escherichia coli*, *Klebsiella spp*, *Proteus spp*, *Pseudomonas aeruginosa*, *Clostridium spp*. Bhat and Bhattacharyya [20] isolated. *Staphylococcus spp*, *E. coli*, *Bacillus spp*, *Corynebacterium spp*, *Pseudomonas spp*, *Proteus spp*, *Klebsiella spp* and *Streptococcus spp* from metritis affected crossbred cows of Kashmir valley. Similarly, Costa et. al. [21] isolated *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella spp*, *Proteus spp*, in more number from uterine discharge and washings of cows suffering from metritis. Shweta [22] also reported the isolation of these organisms from uterine discharge and washings of cows suffering from endometritis. But, Bonnett et. al. [23] reported that endometrial biopsies in Holstein Friesian cows were highly positive for *Actinomyces pyogenes* followed by *Escherichia coli* and

Streptococci bacteria. The results of in vitro antibiogram test in the present study indicated that the maximum isolates were sensitive to Ceftriaxone, then Gentamicin, Enrofloxacin and Chlortetracycline. Chloramphenicol showed the lowest results in terms of invitro antibiotic sensitivity. However, Muneer et.al. [19] and Bhat and Bhattacharyya [20] reported that oxytetracycline is the best antibiotic for the treatment of metritis in cows. These results shows that Ceftriaxone, as well as, Gentamicin, Enrofloxacin, Chlortetracycline are equally effective to treat endometritis in cattles. The use of proper anti-biogram is necessary, in the context of variation factors concerning the frequency of different bacterial species, and their susceptibility to different antimicrobial products. Although non concordances were found between in vitro and in vivo susceptibility, caused by in vitro instability of organisms, different requirements to the culture environment, drug overdosing or technical errors, the antibiogram could be useful to the practitioner in choosing the most efficient antibacterial products. However, therapeutic trials using these antibiotics should have been carried out to find out the most effective drug.

Conclusion

This study revealed that the endometritis in cattle is mainly caused by *E.coli* and *S.aureus* and the drug Ceftriaxone is highly effective.

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