

STUDIES ON BACTERIAL ISOLATES ASSOCIATED WITH REPRODUCTIVE PROBLEMS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PROFILE IN BOVINES OF HARYANA

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ABSTRACT

A study for three years was carried out to know the bacterial association with reproductive problems among bovines of Haryana. The vaginal swabs or discharges were collected from bovines exhibiting reproductive issues such as repeat breeding, endometritis and retained placenta to assess the presence of pathogenic bacteria. The bacterial isolates were phenotypically identified based on their colony morphology, haemolytic pattern, biochemical tests and Gram staining pattern. A total of 857 bacterial isolates were obtained from the vaginal samples collected from 416 bovines. The major pathogenic bacterial isolates were found as *Staphylococcus* (34.89%), *Streptococcus* (30.46%) and *E. coli* (24.04%). All the isolates were analyzed for their antimicrobial susceptibility pattern. Among 26 antibiotics used in this study, the greatest resistance level was observed towards carbenicillin (93.38%). However, the isolates exhibited maximum sensitivity for gentamicin (58.31%) followed by ciprofloxacin (43.98%). The reduced sensitivity of the isolates to frequently used antibiotics for both human and animal use suggests that there is a worrisome level of antibiotic resistance across the study area. There is utmost need to focus on the judicious use of antibiotics in bovines of the States.

Keywords: AMR, Endometritis, *E. coli*, *Staphylococcus*, *Streptococcus*, Vaginal swab

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Infertility is a critical issue in the dairy sector, contributing to significant economic losses (Patel *et al.*, 2019). The reproductive issues such as repeat breeding, endometritis and retained placenta are often associated with microbial infections that can disrupt uterine and ovarian functions (Nguyen *et al.*, 2025). These infections can significantly impact fertility and overall reproductive health in bovines. Bacterial infections of the reproductive tract play a crucial role in repeat breeding among bovines. Common bacterial pathogens identified in such cases include *Staphylococcus*, *Escherichia coli*, *Klebsiella*, *Trueperella pyogenes* and *Pseudomonas*. The presence of these bacteria can lead to inflammation of the endometrium leading to endometritis characterized by mucopurulent or purulent vaginal discharge (Neelam *et al.*, 2018). This condition is often observed postpartum and can be diagnosed through clinical examination and bacteriological culture of vaginal swabs. The regular monitoring and prompt diagnosis of reproductive issues in bovines are essential for effective management and to mitigate the risks associated with reproductive tract infections (Nguyen *et al.*, 2025).

The bacterial infections of the uterus and vagina are typically treated with antibiotics. The choice of antibiotic regimen for treating reproductive tract infections in dairy animals is influenced by several factors, including the type of infection, its severity, the causative bacteria and the animal's overall health status (Moraes *et al.*, 2024). Antimicrobial resistance (AMR) in bovine reproductive

infections arises primarily due to inappropriate antibiotic use and selective pressure in the farm environment. Due to the complexity of reproductive tract infections and the potential for antibiotic resistance, it is crucial to conduct the isolation of the causative agent along with antibiogram studies for an accurate diagnosis and appropriate treatment plan. Addressing AMR in bovine reproductive infections requires a multifaceted approach, including the responsible use of antibiotics in animal husbandry, improved management practices for prevention and control of infections and enhanced research and surveillance systems to ensure sustainable dairy production (Manoj *et al.*, 2024; Mekibib *et al.*, 2024). Therefore, this study was carried out to analyze the bacterial association with reproductive problems among bovines of Haryana and adjoining States.

MATERIALS AND METHODS

Sample Collection

This retrospective study analyzed the data for three consecutive years from 1 July, 2021 to 30 June, 2024. The vaginal swab/discharge received at the College Central Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana for the bacteriological examination were used for this study. These samples were routinely submitted to the laboratory for the cultural examination and antibiotic sensitivity testing either by the dairy farmers or veterinarians of the Haryana and adjoining States. The details of each case were recorded from the owner of the animal. The bovines involved in this study were exhibiting reproductive issues such as repeat

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breeding, endometritis or retained placenta.

Isolation of the bacteria

A total of 416 vaginal swab/discharge samples were subjected to bacteriological analysis as per the standard method (Quinn *et al.*, 2011). The bacterial isolation of both Gram positive as well as Gram negative organisms were performed separately using sheep blood agar (SBA) and Mac Conkey's Lactose agar (MLA) plates, simultaneously under aseptic conditions. These plates were incubated aerobically at 37° C. The plates were checked for bacterial growth after 16-18 hours of incubation. If no growth were observed, then the plates were reincubated upto 48 hours. The colonies developed on the SBA and MLA was identified presumptively, based on their morphological and phenotypical features such as colony size and shape, haemolytic pattern, colour development, biochemical tests and Gram staining.

In vitro antimicrobials sensitivity testing

The bacterial isolates obtained were tested for their antibiotic susceptibility pattern using the Kirby-Bauer disc diffusion method according to CLSI guidelines (2020). A total of 26 different antibiotics from nine classes viz. aminoglycosides, penicillins, cephalosporins, lincomycins, macrolides, phenicol, quinolones, sulphonamides, tetracyclines were used, which include the following antibiotics, amoxicillin (10 µg), ampicillin (10 µg), amoxyclav (10 µg), amikacin (30 µg), chloramphenicol (30 µg), carbenicillin (100 µg), clindamycin (2 µg), ciprofloxacin (10 µg), cephalixin (10 µg), co-trimoxazole (25 µg), cloxacillin (30 µg), cefoperazone (75 g), ceftriaxone (30 µg), cephotaxime (30 µg), erythromycin (15 µg), enrofloxacin (10 µg), gentamicin (10 µg), kanamycin (30 µg), levofloxacin (5 µg), moxifloxacin (5 µg), neomycin (30 µg), oxytetracycline (30 µg), penicillin (10 units), streptomycin (10 µg) and tobramycin (30 µg). The isolates were classified as resistant, intermediate or sensitive towards the tested antibiotics based on the zone of inhibition developed as per manufacturer's quality control instructions (HiMedia, Mumbai, India).

RESULTS AND DISCUSSION

The major reproductive health problems affecting dairy bovines in India include anoestrus, repeat breeding, retention of foetal membranes, dystocia, abortion, uterine and vaginal prolapse as well as various uterine infections such as endometritis and pyometra (Khan *et al.*, 2016, Ashoo *et al.*, 2020). Bacterial isolation from uterine secretions of bovines with endometritis has identified a diverse array of microorganisms. Bacterial endometritis

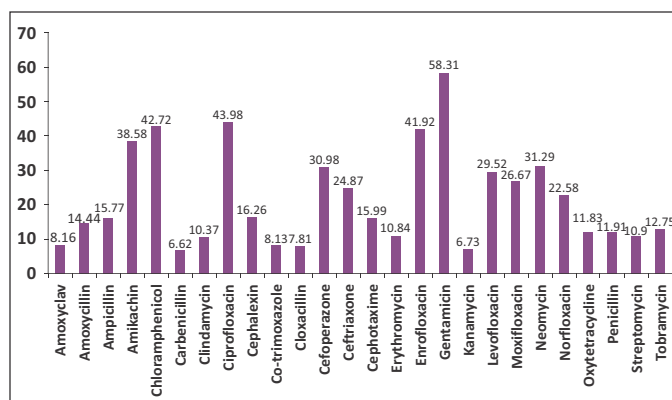


Fig.1. In vitro antibiotic sensitivity pattern of bacterial pathogens isolated

cause infertility in animals as it impedes uterine involution, delays the onset of first estrus, increases the number of services per conception and extends the interval to calving (Gani *et al.*, 2008, Neelam *et al.*, 2018). Endometritis due to bacterial pathogens has been identified as a leading cause of conception failure in a significant proportion of bovines in India (Udhayavel *et al.*, 2013). Out of 416 vaginal swab samples analyzed, 380 samples (91.35%) were found as culture positive for microorganisms and a total of 857 pathogenic bacterial isolates were obtained from bovines involved in this study. Among these, 67.91% of isolates were identified as Gram positive, while 30.81% were found as Gram negative. In our study, Gram positive bacterial pathogens were more prevalent as compared to Gram negative bacterial isolates. Very few fungal isolates of *Candida* spp. (1.28%) were also recovered during this study.

In this study, the major Gram positive bacterial pathogen associated with reproductive problems in bovines were found as *Staphylococcus* species with an average occurrence rate of 34.89%. The *Streptococcus* species were also found as significant contributors to reproductive tract infections, comprising 30.46% of the total isolates in this study. *Trueperella pyogenes* (2.33%) and *Bacillus* spp. (0.2%) were also recovered from bovines under this study. Among Gram negative bacteria, *Escherichia coli* were found as the most predominant bacteria (24.04%) followed by *Klebsiella* spp. (5.02%), *Pseudomonas* spp. (1.05%) and *Proteus* spp. (0.7%) from the bovines of study area (Table 1). These results are similar to the findings from infertility cases of bovines reported by other studies (Gani *et al.*, 2008, Bhat and Bhattacharyya, 2012, Patel *et al.*, 2019). However, some studies have identified *Escherichia coli* as the most prevalent bacterial pathogen in reproductive infections in bovines (Udhayavel *et al.*, 2013, Pohl *et al.*, 2018, Mekibib

Table 1. Bacterial isolates obtained from vaginal swabs/secretions of bovines

Organisms isolated	Major mixed infections
<i>Staphylococcus</i> spp. (299), <i>Streptococcus</i> spp. (261), <i>E. coli</i> (206), <i>Klebsiella</i> spp. (43), <i>T. pyogenes</i> (20), <i>Pseudomonas</i> spp. (09), <i>Proteus</i> spp. (06), <i>Bacillus</i> spp. (02), <i>Candida</i> spp. (11)	<i>Staphylococcus</i> spp. + <i>Streptococcus</i> spp. (91), <i>Staphylococcus</i> spp. + <i>E. coli</i> (36), <i>Staphylococcus</i> spp. + <i>Streptococcus</i> spp. + <i>E. coli</i> (81), <i>Streptococcus</i> spp. + <i>E. coli</i> (21), <i>Staphylococcus</i> spp. + <i>Klebsiella</i> spp. (08), <i>Staphylococcus</i> spp. + <i>Streptococcus</i> spp. + <i>Klebsiella</i> spp. + <i>E. coli</i> (07), <i>Staphylococcus</i> spp. + <i>Streptococcus</i> spp. + <i>Klebsiella</i> spp. (06), <i>Staphylococcus</i> spp. + <i>T. pyogenes</i> (06)
	Number in the parenthesis indicates number of isolates

et al., 2024). The variation in the percentage of different organisms observed may be attributed to differences in the animals' health status, the prevalence rate of organisms in a specific area and individual susceptibility among the animals (Kusum *et al.*, 2022). Some studies do not consistently identify a specific bacterium or group of bacteria as the primary cause of uterine infections in bovines, instead they recognize mixed bacterial contamination as the underlying cause (Mekibib *et al.*, 2024). In our study as well, the majority of infections were found to be of mixed nature (Table 1).

All the isolates obtained were subjected to *in vitro* antibiotic sensitivity testing. The sensitivity patterns of isolates were given in Fig. 1. Our investigation into the antibiotic susceptibility profiles of bacteria isolated from bovines revealed that the infections were of a multidrug-resistant nature, exhibiting varying levels of resistance to two or more of the tested class of antibiotics. Among 26 antibiotics used in this study, the greatest resistance level was observed towards penicillin group of antibiotics especially to carbenicillin (93.38%). However, these isolates had shown maximum sensitivity for aminoglycosides (gentamicin, 58.31%) and fluoroquinolones (ciprofloxacin, 43.98%) class of antibiotics. Mekibib *et al.* (2024) also identified gentamicin as the most effective antimicrobial agent in their study. Bacterial infections responsible for reproductive diseases in bovines generally localize within the tubular genital tract. According to Pyorala *et al.* (2014), recent research consistently indicates that routine parenteral antibiotic treatment for all bovines with reproductive disorders is not justified. The efficacy of antimicrobial agents may differ significantly depending on geographical variations, prevailing bacterial resistance patterns and the health status of individual animals (Reygaert, 2018).

CONCLUSIONS

The study identified multiple bacterial species associated with reproductive problems in bovines of Haryana State. Gram-positive organisms such as *Staphylococcus* and *Streptococcus* spp. were the most

frequently isolated pathogens in the present study. Varying degrees of resistance to many of the tested antibiotics were also observed, however gentamicin proved to be effective against most of the isolates. These findings underscore the importance of responsible use of antibiotics to prevent the emergence of resistance. Promoting awareness among dairy farmers about proper herd health management and the adverse effects of indiscriminate antibiotic use is essential. In summary, AMR poses a significant threat to global health, necessitating urgent and coordinated actions across human, animal and environmental health sectors. By adopting One Health approach we can mitigate the risks associated with AMR and safeguard the effectiveness of antimicrobial treatments for animals as well as public health.

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