

Microbiological Profile and Antimicrobial Susceptibility of Bacteria Associated with Clinical Vaginitis in Dairy Cows

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Abstract

Introduction: Bovine vaginitis is an important reproductive disorder in dairy cattle that can negatively affect fertility, herd productivity, and economic performance. Bacterial infections are among the primary causes of this condition, and the emergence of antimicrobial resistance among opportunistic pathogens has complicated treatment strategies. Therefore, identifying the causative bacteria and determining their antimicrobial susceptibility patterns is essential for effective therapeutic management.

Materials and Methods: In this study, vaginal discharge samples were collected from 8 dairy cows showing clinical signs of vaginitis. Samples were subjected to standard bacteriological culture and identification procedures. Antimicrobial susceptibility testing (AST) was carried out using the Kirby–Bauer disk diffusion assay with 16 routinely prescribed antibiotics.

Results: Bacterial growth was detected in 62.5% of the samples, while three samples remained culture-negative. *Streptococcus* spp. were identified as the predominant etiologic agents, isolated from 62.5% of the cows, whereas *Enterobacter aerogenes* was detected in 12.5% of the cases. Antimicrobial susceptibility testing demonstrated considerable heterogeneity among the *Streptococcus* isolates. All isolates were fully susceptible to amoxicillin, amikacin, enrofloxacin, florfenicol, and ceftiofur. However, variable resistance patterns were observed for penicillin,

gentamicin, streptomycin, and erythromycin. Interestingly, earlier isolates showed intrinsic resistance to sulfonamides (trimethoprim-sulfamethoxazole and Soltrim), while a newly isolated multidrug-resistant strain exhibited susceptibility, resulting in an overall resistance rate of approximately 80%.

Conclusion: The presence of heterogeneous antimicrobial susceptibility patterns and the coexistence of strains with different resistance profiles within the same herd indicate the circulation of multidrug-resistant opportunistic pathogens. These findings highlight that empirical antibiotic therapy may lead to ineffective treatment and emphasize the necessity of routine antimicrobial susceptibility testing for proper clinical management of bovine reproductive infections.

Keywords: Bovine vaginitis, Dairy cattle, *Streptococcus* spp., *Enterobacter aerogenes*, resistance

1-Introduction

Reproductive tract infections, including vaginitis, endometritis, and metritis, are major sources of economic loss in the global dairy industry. As a consequence, conception rates decline, calving intervals lengthen, and culling becomes more frequent. [1, 2]. While the postpartum uterus and vagina are naturally exposed to a variety of environmental and fecal microorganisms, clinical disease occurs when pathogenic loads overwhelm the local mucosal immunity, often exacerbated by periparturient stress or tissue trauma [3, 4].

Among the diverse microbiome of the bovine reproductive tract, opportunistic environmental bacteria, particularly *Streptococcus* spp. and members of the *Enterobacteriaceae* family, are frequently implicated in inflammatory conditions [5]. The routine use of broad-spectrum antimicrobials in dairy farming has inadvertently accelerated the selection pressure for resistant bacterial strains. The emergence of antimicrobial resistance (AMR) in bovine reproductive pathogens is not only a veterinary challenge but also a critical public health concern under the “One Health” perspective [8]. This study investigates the bacterial agents responsible for clinical vaginitis in a dairy herd and maps their specific antimicrobial susceptibility to guide evidence-based therapeutic interventions.

2-Materials and Methods

2-1-Sample Collection

Vaginal swabs were obtained from 8 dairy cows diagnosed with clinical vaginitis, characterized by abnormal purulent or mucopurulent vaginal discharges. Samples were collected using sterile guarded swabs to prevent contamination from the vulva and perineum. Swabs were placed immediately into Amies transport medium, stored at 4°C, and delivered to the laboratory within 2 h.

2-2-Bacteriological Examination and Isolation

Upon arrival, the samples were directly streaked onto Blood Agar (containing 5% sheep blood) and MacConkey Agar plates. The plates were incubated aerobically at 37°C for 24–48 hours. Bacterial identification was systematically performed based on colony morphology, hemolytic patterns, Gram staining, and standard biochemical assays, including catalase, oxidase, and coagulase tests [10].

2-3-Antimicrobial Susceptibility Testing

The susceptibility of the isolated strains to antimicrobial agents was assessed by the Kirby–Bauer disk diffusion technique on Mueller–Hinton agar following Clinical and Laboratory Standards Institute (CLSI) guidelines. [11]. Bacterial suspensions were adjusted to a 0.5 McFarland standard. A comprehensive panel of 16 antimicrobial disks was utilized: penicillin, amoxicillin, ampicillin, gentamicin, amikacin, enrofloxacin, erythromycin, streptomycin, florfenicol, ceftiofur, tetracycline, neomycin, lincospectin, colistin, trimethoprim-sulfamethoxazole, and Soltrim. The inhibition zones were measured after 18–24 hours of incubation at 37°C and interpreted as susceptible (S), intermediate (I), or resistant (R).

3-Results

3-1. Bacterial Isolation

A total of eight clinical vaginal samples were bacteriologically examined. Positive bacterial growth was detected in 5 samples (62.5%), whereas 3 samples (37.5%) showed no bacterial growth. Among the recovered isolates, *Streptococcus spp.* was identified as the predominant bacterial pathogen and was isolated from all positive cases (n=5). In addition, *Enterobacter aerogenes* was recovered from one sample, suggesting occasional involvement of Gram-negative bacteria in clinical vaginitis of dairy cows. The bacteriological profile of the examined samples is presented in Table 1.

75 Table 1. Bacteriological profile of vaginal samples from dairy cows with clinical vaginitis

Bacteriological finding	Number of samples (n=8)	Percentage (%)
Positive bacterial culture	5	62.5
Negative bacterial culture	3	37.5
<i>Streptococcus spp.</i>	5	62.5
<i>Enterobacter aerogenes</i>	1	12.5

76

77 3-2. Antimicrobial Susceptibility Patterns

78 Antimicrobial susceptibility testing of the *Streptococcus spp.* isolates (n=5) demonstrated considerable heterogeneity
 79 in resistance and susceptibility profiles. All isolates showed complete susceptibility to amoxicillin, amikacin,
 80 enrofloxacin, florfenicol, and ceftiofur, indicating high efficacy of these antimicrobial agents against the recovered
 81 strains. However, resistance patterns varied among several antibiotics. Four isolates (80%) were susceptible to
 82 penicillin, gentamicin, streptomycin, and erythromycin, whereas one isolate exhibited complete resistance to these
 83 agents. This multidrug-resistant isolate also demonstrated resistance to ampicillin and lincospectin.

84 In contrast, resistance to sulfonamide-containing drugs showed an opposite trend. While four isolates (80%) were
 85 resistant to trimethoprim-sulfamethoxazole and Soltrim, the multidrug-resistant isolate remained susceptible to these
 86 drugs. Additionally, this isolate was susceptible to tetracycline and neomycin and showed intermediate susceptibility
 87 to colistin. The detailed antimicrobial susceptibility pattern of the isolates is summarized in Table 2.

88 Table 2. Antimicrobial susceptibility pattern of *Streptococcus spp.* isolates (n=5)

Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Amoxicillin	5 (100)	0	0
Amikacin	5 (100)	0	0

Enrofloxacin	5 (100)	0	0
Florfenicol	5 (100)	0	0
Ceftiofur	5 (100)	0	0
Penicillin	4 (80)	0	1 (20)
Gentamicin	4 (80)	0	1 (20)
Streptomycin	4 (80)	0	1 (20)
Erythromycin	4 (80)	0	1 (20)
Ampicillin	4 (80)	0	1 (20)
Lincospectin	4 (80)	0	1 (20)
Trimethoprim-sulfamethoxazole	1 (20)	0	4 (80)
Soltrim	1 (20)	0	4 (80)
Tetracycline	1 (20)	0	4 (80)
Neomycin	1 (20)	0	4 (80)
Colistin	0	1 (20)	4 (80)

4-Discussion

The current investigation highlights the predominance of Streptococcus species as the principal bacterial pathogens associated with clinical bovine vaginitis, emphasizing their important role as opportunistic environmental agents in reproductive tract infections. These findings are consistent with previous studies that describe the widespread distribution and ecological adaptability of environmental streptococci in dairy farm environments, where they can readily colonize different substrates and infect susceptible animals [1,2,4,21,22,24]. In intensive dairy systems, these bacteria are commonly found in bedding materials, soil, manure-contaminated surfaces, milking areas, and water sources, continuously exposing the genital tract of dairy cows to potential infection under favorable conditions [10,18].

98 Their environmental persistence, together with their ability to adhere to epithelial tissues and form microbial
99 communities, enhances their capacity to establish infection when host defenses are weakened.

100 The postpartum period is considered a particularly critical stage of immunological vulnerability in dairy cows. During
101 this period, animals undergo major physiological and metabolic changes related to calving and the onset of lactation.
102 Oxidative stress, hormonal fluctuations, negative energy balance, and temporary impairment of leukocyte function
103 collectively weaken innate immune defenses and reduce the reproductive tract's ability to eliminate invading
104 pathogens [2,3,18,21,26]. In addition, trauma to the vaginal and uterine epithelium during parturition disrupts the
105 protective mucosal barrier, creating favorable conditions for bacterial invasion. Such epithelial damage facilitates
106 bacterial adhesion, colonization, and biofilm formation, all of which are essential steps in the development and
107 persistence of infections such as vaginitis and endometritis [1,3,27]. Biofilm formation is particularly important
108 because it enhances bacterial resistance to both host immune responses and antimicrobial agents, increasing the
109 likelihood of chronic or recurrent infections.

110 Recent advances in metagenomic and microbiome research have considerably improved our understanding of the
111 bovine reproductive tract ecosystem. Earlier assumptions that the uterus and vagina were largely sterile have now
112 been replaced by evidence showing that these organs harbor dynamic microbial communities that undergo substantial
113 changes during the postpartum period. High-throughput sequencing studies have demonstrated significant postpartum
114 microbial dysbiosis characterized by reduced microbial diversity and overgrowth of opportunistic pathogenic bacteria,
115 especially streptococci and other facultative anaerobes [4,5,21,23,24,25]. Such dysbiotic changes have been associated
116 with reproductive disorders, delayed uterine involution, reduced fertility, and prolonged calving intervals, illustrating
117 the complex interaction between host physiology, environmental exposure, and microbial ecology.

118 The antimicrobial susceptibility profiles obtained in this study revealed considerable variability among *Streptococcus*
119 isolates. Although most isolates remained susceptible to several commonly used veterinary antimicrobials, a notable
120 proportion exhibited multidrug resistance (MDR), including resistance to beta-lactams, aminoglycosides, macrolides,
121 and lincosamides. This resistance pattern likely reflects the selective pressure resulting from the frequent and
122 sometimes empirical use of antibiotics in dairy herd management. Repeated antimicrobial exposure promotes the
123 emergence and horizontal transfer of resistance determinants through mobile genetic elements such as plasmids,

transposons, and integrons [8,9,12,15,20]. These elements facilitate the rapid dissemination of resistance genes among bacterial populations and contribute to the increasing burden of antimicrobial resistance within farm environments.

The molecular mechanisms responsible for antimicrobial resistance in streptococci are diverse and complex. They include modification of antibiotic target sites, enzymatic inactivation of antimicrobial agents, and activation of efflux pumps that reduce intracellular antibiotic concentrations [12,20,30]. Together, these mechanisms compromise therapeutic efficacy and make infection management increasingly challenging in dairy herds. In addition, the persistence of resistant strains in environmental reservoirs such as manure and bedding materials may contribute to recurrent infections and ongoing transmission within herds.

Despite the detection of MDR strains, all isolates in the present study remained susceptible to amoxicillin, amikacin, enrofloxacin, florfenicol, and ceftiofur. These findings suggest that these antimicrobials may still represent effective therapeutic options for bovine vaginitis when treatment is guided by antimicrobial susceptibility testing. However, dependence on a limited number of effective drugs highlights the urgent need for responsible antimicrobial stewardship in veterinary medicine. Rational antibiotic use, supported by culture-based diagnosis and susceptibility testing, is essential to preserve drug efficacy, limit further resistance development, and promote sustainable livestock production [13,28,29].

An interesting observation in this study was the paradoxical susceptibility of MDR isolates to sulfonamide combinations, whereas some non-MDR isolates showed resistance. This finding may indicate genetic heterogeneity among bacterial populations, possibly influenced by differences in antimicrobial exposure, evolutionary adaptation, or farm management practices. Such variability emphasizes the need for more detailed molecular epidemiological studies to better understand resistance gene distribution, clonal relationships among isolates, and the contribution of mobile genetic elements to resistance dissemination [12,20,25,30].

In addition to streptococcal isolates, the detection of *Enterobacter aerogenes* in one clinical case suggests the opportunistic involvement of Gram-negative bacteria in bovine reproductive tract infections. Members of the Enterobacteriaceae family are frequently associated with fecal contamination and environmental exposure, and their presence in the reproductive tract often reflects inadequate hygiene and biosecurity measures on dairy farms. *E. aerogenes* possesses several virulence factors that support colonization and infection, including adhesins, endotoxin production, and the capacity to acquire antimicrobial resistance determinants [10,14,18]. The presence of such

151 opportunistic pathogens raises concerns not only for animal health and productivity but also for potential zoonotic
152 transmission.

153 From a One Health perspective, the occurrence of multidrug-resistant Enterobacteriaceae in livestock environments
154 represents an important public health concern. These bacteria may serve as reservoirs of resistance genes that can be
155 transmitted to human pathogens through direct animal contact, environmental contamination, or the food chain
156 [9,14,28]. Therefore, improving farm hygiene, manure management, and biosecurity practices is essential to reduce
157 bacterial dissemination and minimize the risk of cross-species transmission.

158 Overall, the findings of the present study emphasize the multifactorial nature of bovine reproductive tract infections
159 and highlight the importance of integrated herd health management strategies. Effective control requires continuous
160 microbiological monitoring, evidence-based antimicrobial therapy guided by susceptibility testing, and strict
161 environmental and management hygiene practices aimed at reducing pathogen load within dairy farm environments
162 [8,15,18,21,29]. Furthermore, improved bedding management, proper calving hygiene, and careful postpartum
163 monitoring may significantly reduce the incidence of opportunistic infections during early lactation.

164 Future studies should utilize advanced genomic, transcriptomic, and metagenomic technologies to further clarify
165 pathogen–host interactions, microbial community dynamics, and resistance gene dissemination in dairy production
166 systems. These approaches may help identify novel biomarkers for early diagnosis, improve understanding of
167 microbial dysbiosis, and support the development of innovative preventive and therapeutic strategies tailored to bovine
168 reproductive health [20,21,23,26,30]. Ultimately, combining advanced molecular diagnostics with effective herd
169 management and antimicrobial stewardship will be essential for protecting animal welfare and ensuring the long-term
170 sustainability of the dairy industry.

171 5-Conclusion

172 The findings of this study delineate a shifting paradigm in the antimicrobial resistance (AMR) profiles of pathogens
173 associated with bovine vaginitis. The coexistence of highly susceptible isolates alongside emerging multidrug-
174 resistant (MDR) phenotypes specifically those circumventing traditional beta-lactam and aminoglycoside therapies
175 highlights a precarious gap in current clinical management. Our data suggest that the historical reliance on empirical,
176 symptom-based protocols is increasingly untenable and may inadvertently serve as a catalyst for resistance escalation.

Consequently, the transition toward evidence-based medicine through the systematic integration of Antimicrobial Susceptibility Testing (AST) at both the individual and herd levels is paramount. Such diagnostic precision is not merely a clinical recommendation but a fundamental requirement for optimizing therapeutic efficacy and ensuring the long-term sustainability of the veterinary antimicrobial arsenal.

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