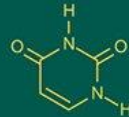


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
 ISSN Online: 2617-4707  
 NAAS Rating (2026): 5.29  
 IJABR 2026; 10(6): 814-818  
[www.biochemjournal.com](http://www.biochemjournal.com)  
 Received: 02-04-2026  
 Accepted: 06-05-2026

**Elaiyaraja Govindaraj**  
 Assistant Professor, Department  
 of Veterinary Microbiology,  
 Veterinary College and Research  
 Institute, TANUVAS, Salem,  
 Tamil Nadu, India

**Shanmugasundaram Udhayavel**  
 Assistant Professor, Department  
 of Veterinary Microbiology,  
 Veterinary College and Research  
 Institute, TANUVAS, Salem,  
 Tamil Nadu, India

**E Venkatesakaumar**  
 Professor and Head, Department  
 of Veterinary Medicine,  
 Veterinary College and Research  
 Institute, TANUVAS, Salem,  
 Tamil Nadu, India

**PA Enbavelan**  
 Assistant Professor, Department  
 of Veterinary Medicine,  
 Veterinary College and Research  
 Institute, TANUVAS, Salem,  
 Tamil Nadu, India

**E Kalaiselvan**  
 Assistant Professor, Department  
 of Veterinary Surgery and  
 Radiology, Veterinary college  
 and Research Institute,  
 TANUVAS, Salem, Tamil Nadu,  
 India

**S Jaisree**  
 Associate Professor and Head,  
 Department of Veterinary  
 Microbiology, Veterinary College  
 and Research Institute,  
 TANUVAS, Salem, Tamil Nadu,  
 India

**K Brindha**  
 Professor, Department of Animal  
 Biotechnology, Madras  
 Veterinary College, Chennai,  
 Tamil Nadu, India

**Corresponding Author:**  
**Elaiyaraja Govindaraj**  
 Assistant Professor, Department  
 of Veterinary Microbiology,  
 Veterinary College and Research  
 Institute, TANUVAS, Salem,  
 Tamil Nadu, India

## Antimicrobial susceptibility and multidrug resistance patterns of bacterial isolates associated with otitis in cattle

**Elaiyaraja Govindaraj, Shanmugasundaram Udhayavel, E Venkatesakaumar, PA Enbavelan, E Kalaiselvan, S Jaisree and K Brindha**

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6j.8759>

### Abstract

Otitis is an important health problem in cattle, causing discomfort, reduced productivity, and economic losses. Bacterial infections are among the major etiological agents of bovine otitis; however, increasing antimicrobial resistance has complicated therapeutic management. Knowledge of local antimicrobial susceptibility patterns is essential for effective treatment and antimicrobial stewardship. The present study was undertaken to identify bacterial pathogens associated with otitis in cattle and to determine their antimicrobial susceptibility patterns against commonly used antibiotics. Ear swab samples were collected aseptically from cattle clinically diagnosed with otitis. Samples were cultured on appropriate bacteriological media, and bacterial isolates were identified using colony morphology, Gram staining, and standard biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The susceptibility of isolates to selected antimicrobial agents commonly used in veterinary practice was evaluated. A total of 75 bacterial isolates were recovered from 75 otitis cases. The predominant isolates included *Staphylococcus* spp., *Pseudomonas* spp., *Escherichia coli*, *Actinomyces* spp., *Bacillus* spp., *Klebsiella*, *Streptococcus* spp. and *Rhizopus* spp. Gentamicin showed the highest susceptibility (95%), followed by ciprofloxacin (87%) and amikacin (82%). High resistance was observed against penicillin G (95%), ampicillin (83%), and amoxicillin (80%). The MAR index was 0.38 and MDR prevalence was 30.66%. The study highlights the occurrence of antimicrobial resistance among bacterial pathogens associated with bovine otitis. Regular surveillance of antimicrobial susceptibility patterns is necessary to guide evidence-based therapy and promote prudent antimicrobial use in livestock.

**Keywords:** Bovine otitis, antimicrobial resistance, MDR; MAR index, cattle, Kirby-Bauer test

### 1. Introduction

Otitis, characterized by inflammation of the external, middle, or inner ear, is an important clinical condition affecting cattle worldwide. The disease can result from bacterial, fungal, parasitic, or traumatic causes and may lead to pain, irritation, head shaking, ear discharge, reduced feed intake, and decreased productivity. In severe cases, chronic infection can compromise animal welfare and cause substantial economic losses to livestock producers (Yeruham *et al.*, 1999) [13].

Bacterial pathogens play a significant role in the development and progression of otitis in cattle. Commonly reported organisms include *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Escherichia coli*, and other opportunistic bacteria inhabiting the external ear canal. Environmental conditions, poor hygiene, concurrent parasitic infestations, and compromised immunity may predispose animals to bacterial ear infections (Duarte *et al.*, 2004) [6].

Antimicrobial therapy remains the primary treatment strategy for bacterial otitis. However, indiscriminate and prolonged use of antibiotics in veterinary practice has contributed to the emergence of antimicrobial-resistant bacterial strains. Antimicrobial resistance poses a serious challenge to successful treatment outcomes and represents a growing concern within the One Health framework due to the potential transmission of resistant bacteria and

resistance genes among animals, humans, and the environment.

Determination of antimicrobial susceptibility profiles of bacterial isolates from otitis cases is essential for selecting effective therapeutic agents and minimizing the development of resistance. Although several studies have reported antimicrobial resistance patterns in mastitis and other bovine infections, information regarding bacterial otitis in cattle remains limited in many regions (Lewis *et al.*, 2024)<sup>[10]</sup>.

Therefore, the present study was undertaken to isolate and identify bacterial pathogens associated with otitis in cattle and to evaluate their antimicrobial susceptibility patterns against commonly used veterinary antibiotics.

## 2. Materials and Methods

### 2.1 Study area and animals

Samples were collected from large animals presented to the Veterinary Clinical Complex, Veterinary College and Research Institute (VCRI), Salem, Tamil Nadu, originating from the Thalaivasal and Chinnasalem regions of Salem district. The study was conducted over a period extending from January 2023 to May 2026. Cattle presented with clinical signs suggestive of otitis, including ear discharge, head shaking, ear scratching, pain on palpation, foul odor, and inflammation of the ear canal, were included in the study.

### 2.2 Sample Collection

75 Ear swab samples were collected aseptically from affected cattle using sterile cotton swabs. Prior to sample collection, the external ear surface was cleaned with sterile saline to remove superficial contaminants. Swabs were inserted carefully into the ear canal and rotated gently to collect exudate and debris. Samples were immediately transported to the department of veterinary microbiology laboratory under refrigerated conditions for bacteriological examination.

### 2.3 Isolation and Identification of Bacteria

Samples were inoculated onto Nutrient Agar, Blood Agar, MacConkey agar and SDA agar and incubated aerobically at 37 °C for 24-48 h. Individual colonies were sub-cultured to obtain pure isolates. Bacterial identification was performed based on Colony morphology, Gram staining characteristics, Hemolytic pattern on blood agar and Standard biochemical tests, including catalase, oxidase, indole, methyl red, Voges-Proskauer, citrate utilization, urease, and triple sugar iron tests, as appropriate.

### 2.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar following CLSI guidelines. Pure bacterial cultures were adjusted to 0.5 McFarland turbidity standard and uniformly inoculated onto Mueller-Hinton agar plates. Commercial antibiotic discs were placed on the agar surface and plates were incubated at 37 °C for 18-24 h. The following antibiotic classes were tested: Aminoglycoside [Gentamicin (10 µg), Amikacin (30 µg)], Fluroquinolones [Enrofloxacin (10 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg)], Folate pathway inhibitor [Co-Trimoxazole (25 µg)], Beta lactam antibiotics [Amoxicillin-clavulanic acid (30 µg), Ampicillin (10 µg), Amoxicillin (25 µg) and Penicillin G (10 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg)] and Tetracycline [Oxytetracycline (30 µg)]. The zones of inhibition were

measured in millimeters and interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to CLSI breakpoints.

## 2.5 Data Analysis

The frequency of bacterial isolates and antimicrobial susceptibility profiles were expressed as percentages. Antibiotic sensitivity, intermediate susceptibility, and resistance rates were calculated for each bacterial species. Multidrug resistance was defined as resistance to three or more classes of antimicrobial agents. Statistical analysis was performed using GraphPad Prism statistical software version 5.01 (GraphPad, San Diego, California, USA). Differences in susceptibility patterns among the antibiotics were analyzed using Chi-square or Fisher's exact test, and a p-value of <0.05 was considered statistically significant.

## 2.6 Ethical Considerations

Sample collection procedures were performed in accordance with institutional animal ethics guidelines. Ear swab collection was non-invasive and carried out with minimal discomfort.

## 3. Results

A total of 75 ear swab samples were collected from cattle clinically diagnosed with otitis. From these samples, 75 bacterial isolates were recovered and subjected to antimicrobial susceptibility testing. The following predominant bacterial genera were identified *Staphylococcus* spp., *Pseudomonas* spp., *Escherichia coli*, *Actinomyces* spp., *Bacillus* spp., *Klebsiella* spp., and *Streptococcus* spp. In addition, *Rhizopus* spp. was detected among some cases, indicating possible mixed bacterial-fungal involvement (Figure 1).

The antimicrobial susceptibility pattern of the isolates revealed considerable variation among the antibiotics tested. Gentamicin exhibited the highest efficacy, with 95% of isolates showing susceptibility, followed by ciprofloxacin (87%) and amikacin (82%). Moderate susceptibility was observed for ceftriaxone (72%), enrofloxacin (71%), cefotaxime (57%), co-trimoxazole (55%), and levofloxacin (50%). High levels of resistance were observed against beta-lactam antibiotics. Penicillin G showed the highest resistance rate (95%), followed by ampicillin (83%), amoxicillin (80%), oxytetracycline (75%), and amoxicillin-clavulanic acid (70%). Intermediate susceptibility rates were generally low across the tested antibiotics (Table 1, Figure 2).

The highest resistance frequency was observed against Penicillin G (95.45%), followed by ampicillin (83.33%). Gentamicin showed the lowest resistance frequency (4.92%) and remained effective in treatment (Figure 3).

The Multiple Antibiotic Resistance (MAR) index was calculated as 0.38 (176 resistant reactions out of 466 antibiotic-isolate combinations tested), indicating exposure of bacterial populations to environments with substantial antimicrobial selection pressure. Multidrug resistance (MDR), defined as resistance to three or more antimicrobial classes, was observed in 23 of 75 isolates, resulting in an MDR prevalence of 30.66% (Table 2).

Statistical analysis demonstrated significant differences ( $p < 0.05$ ) in susceptibility patterns among the antimicrobial agents tested, indicating variable effectiveness of antibiotics against bacterial pathogens associated with bovine otitis (Table 3).

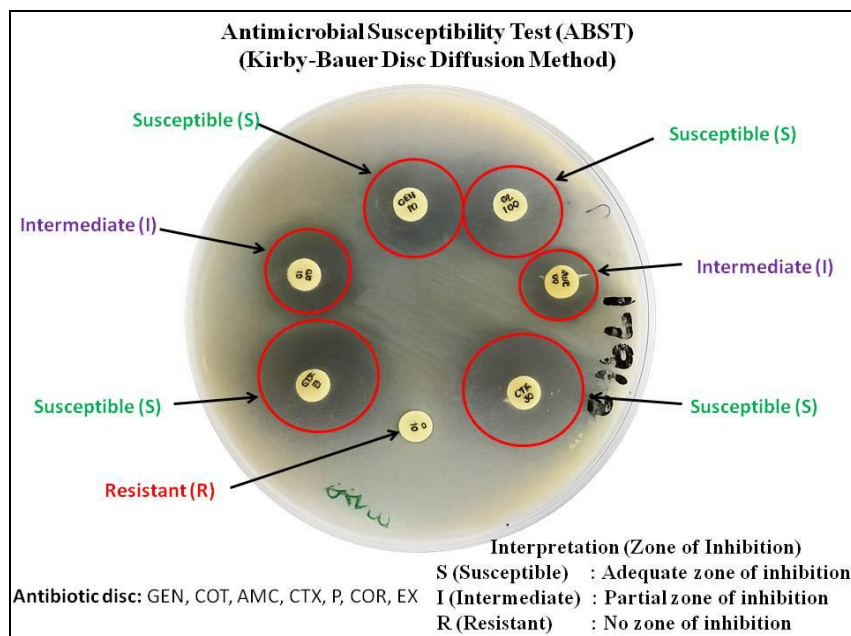


**Fig 1:** Antibiogram of the bacterial isolate showing zones of inhibition against different antimicrobial agents using the Kirby-Bauer disk diffusion assay on Mueller-Hinton agar

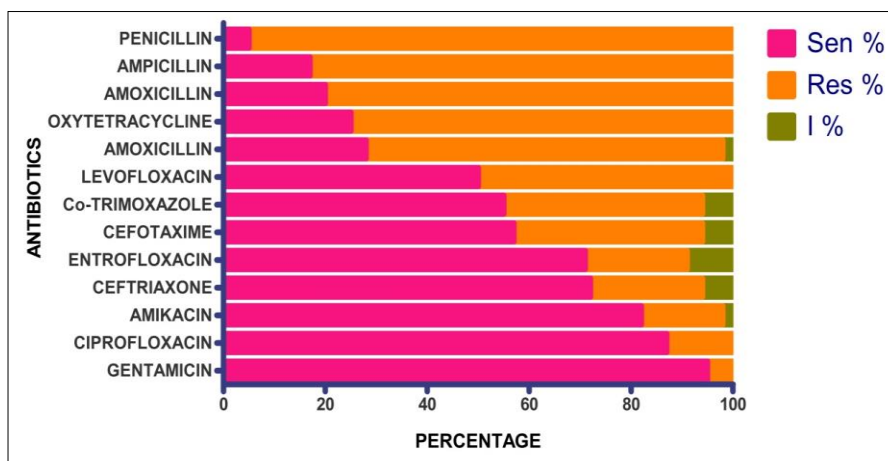


**Fig 2:** Diagram illustrating the percentage resistance of bacterial isolates to different antimicrobial agents as determined by the Kirby-Bauer disk diffusion method

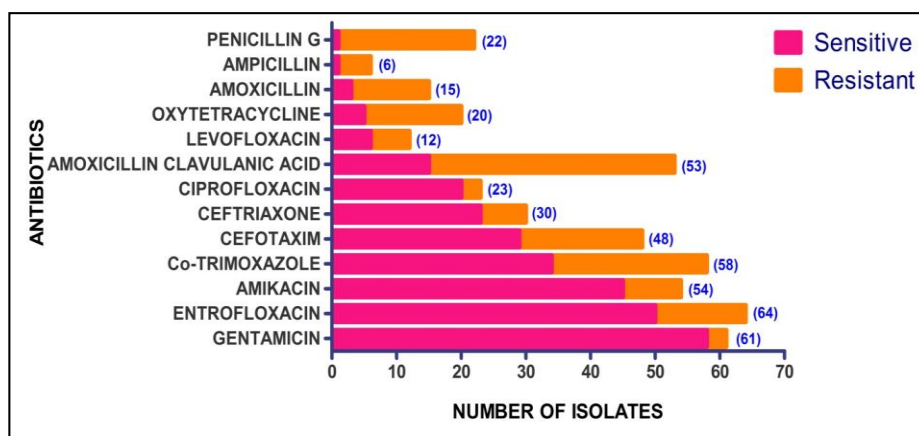


**Fig 3:** Diagram illustrating the resistance rate of bacterial isolates to different antimicrobial agents as determined by the Kirby-Bauer disk diffusion method. Numbers in bracket indicates total number of disc of particular antibiotic used in the current study.

#### 4. Discussion

The present study demonstrated that bovine otitis is associated with a diverse range of bacterial pathogens, including *Staphylococcus* spp., *Pseudomonas* spp., *Escherichia coli*, *Klebsiella* spp., *Streptococcus* spp., and

other opportunistic microorganisms. Similar bacterial profiles have been reported in otitis cases among cattle and other livestock species, where *Staphylococcus* spp. and *Pseudomonas* spp. are frequently identified as predominant etiological agents due to their ability to colonize moist

environments and damaged epithelial tissues (Duarte *et al.*, 2004<sup>[6]</sup>; Hariharan *et al.*, 2006<sup>[8]</sup>).

The high prevalence of Gram-positive cocci, particularly *Staphylococcus* spp., observed in the present investigation agrees with the findings of Chon *et al.*, (2017)<sup>[5]</sup>, who reported *Staphylococcus* species as major pathogens isolated from bovine ear infections. These organisms possess several virulence factors, including biofilm-forming ability, which may contribute to persistence and recurrent infections.

Gentamicin demonstrated the highest antimicrobial activity against the isolates recovered in this study. Similar findings have been reported by Bugden (2013)<sup>[3]</sup>, who observed excellent *in vitro* activity of aminoglycosides against bacterial pathogens associated with otitis. The high efficacy of gentamicin may be attributed to its bactericidal action and comparatively lower field-level resistance among bovine pathogens. The excellent activity of gentamicin and fluoroquinolones observed in the present study is consistent with findings reported by Pohl *et al.*, (2018)<sup>[12]</sup>.

Fluoroquinolones, particularly ciprofloxacin and enrofloxacin, also exhibited good antimicrobial activity. Comparable susceptibility patterns were reported by Giguère *et al.*, (2007)<sup>[7]</sup>, who found fluoroquinolones to be among the most effective therapeutic agents against Gram-negative bacterial isolates recovered from ear infections. Their broad-spectrum activity and excellent tissue penetration may explain their effectiveness.

The substantial resistance observed against penicillin, ampicillin, and amoxicillin is consistent with reports from several studies investigating antimicrobial resistance among livestock-associated bacteria. Aarestrup *et al.*, (2008)<sup>[1]</sup> reported widespread resistance to beta-lactam antibiotics among bacterial isolates from food-producing animals,

largely due to extensive and prolonged antibiotic use. Similar observations were documented by Catry *et al.*, (2016)<sup>[4]</sup>, who highlighted increasing resistance among veterinary bacterial pathogens to commonly used beta-lactam drugs.

High resistance to beta-lactam antibiotics may reflect prolonged and widespread field usage. Similar resistance patterns have been described in livestock-associated bacterial pathogens by Nedbalcova *et al.*, (2014)<sup>[11]</sup>.

The MAR index value of 0.38 recorded in the present study exceeded the threshold value of 0.20 generally considered indicative of high-risk antimicrobial exposure environments. According to Kruperman (1983)<sup>[9]</sup>, MAR indices greater than 0.20 suggest frequent exposure of bacterial populations to antimicrobial agents, leading to increased selection pressure and emergence of resistant strains.

Multidrug resistance was detected in 30.66% of isolates. This finding is of concern because MDR bacteria can compromise treatment success and increase the likelihood of therapeutic failure. Similar levels of multidrug resistance have been reported among bovine bacterial pathogens by Bandyopadhyay *et al.*, (2021)<sup>[2]</sup>, who emphasized the growing challenge posed by resistant microorganisms in food-producing animals.

The emergence of antimicrobial resistance among bovine otitis pathogens has important implications within the One Health framework. Resistant bacteria originating from livestock may serve as reservoirs of resistance genes capable of dissemination to other animals, humans, and the environment. Therefore, routine antimicrobial susceptibility testing before treatment, together with prudent antibiotic use, should be encouraged to preserve the efficacy of existing antimicrobial agents.

**Table 1:** Antimicrobial susceptibility profile of bacterial isolates recovered from bovine otitis tested by the Kirby-Bauer disk diffusion method

| Antibiotics                 | Anti Microbial susceptibility profile in numbers |    |   | Total no. of animals tested | Antimicrobial sensitivity results in percentage |       |      |
|-----------------------------|--------------------------------------------------|----|---|-----------------------------|-------------------------------------------------|-------|------|
|                             | S                                                | R  | I |                             | S%                                              | R%    | I%   |
| Gentamicin                  | 58                                               | 3  |   | 61                          | 95.08                                           | 4.92  | 0.00 |
| Ciprofloxacin               | 20                                               | 3  |   | 23                          | 86.96                                           | 13.04 | 0.00 |
| Amikacin                    | 45                                               | 9  | 1 | 55                          | 81.82                                           | 16.36 | 1.82 |
| Ceftriaxone                 | 23                                               | 7  | 2 | 32                          | 71.88                                           | 21.88 | 6.25 |
| Entrofloxacin               | 50                                               | 14 | 6 | 70                          | 71.43                                           | 20.00 | 8.57 |
| Cefotaxim                   | 29                                               | 19 | 3 | 51                          | 56.86                                           | 37.25 | 5.88 |
| Co-Trimoxazole              | 34                                               | 24 | 4 | 62                          | 54.84                                           | 38.71 | 6.45 |
| Levofloxacin                | 6                                                | 6  |   | 12                          | 50.00                                           | 50.00 | 0.00 |
| Amoxicillin Clavulanic Acid | 15                                               | 38 | 1 | 54                          | 27.78                                           | 70.37 | 1.85 |
| Oxytetracycline             | 5                                                | 15 |   | 20                          | 25.00                                           | 75.00 | 0.00 |
| Amoxicillin                 | 3                                                | 12 |   | 15                          | 20.00                                           | 80.00 | 0.00 |
| Ampicillin                  | 1                                                | 5  |   | 6                           | 16.67                                           | 83.33 | 0.00 |
| Penicillin G                | 1                                                | 21 |   | 22                          | 4.55                                            | 95.45 | 0.00 |

S-Sensitive; R-Resistant; I-Intermediate

**Table 2:** Multiple Antibiotic Resistance (MAR) index and Multidrug Resistance status of isolates

| Multiple Antibiotic Resistance Index (MAR)                     |                                                          | Multi Drug Resistant (MDR) |                     |
|----------------------------------------------------------------|----------------------------------------------------------|----------------------------|---------------------|
| MAR                                                            | Number of antibiotics resistant/Total antibiotics tested | MDR Positive               | 23                  |
| MAR                                                            | 176/466 = 0.38                                           | MDR Negative               | 52                  |
| MAR > 0.2 indicates high-risk antibiotic exposure environments |                                                          | Total isolate              | 75                  |
| MAR ≤ 0.2 indicates lower exposure pressure                    |                                                          | MDR%                       | 23/75 x100 = 30.66% |

**Table 3:** Differences in susceptibility patterns among antibiotics were analyzed using Chi-square test (p-value of <0.05)

| Comparison among | Chi-square value | df | P-value  | Results     |
|------------------|------------------|----|----------|-------------|
| Antibiotics      | 140.5            | 12 | < 0.0001 | Significant |

## 5. Conclusion

The present study revealed that bovine otitis is associated with a diverse bacterial population, predominantly comprising *Staphylococcus* spp., *Pseudomonas* spp., and *Escherichia coli*. Antimicrobial susceptibility testing demonstrated that gentamicin, ciprofloxacin, and amikacin were the most effective antibiotics against the isolates examined, whereas high levels of resistance were observed against penicillin, ampicillin, and amoxicillin. The detection of a high MAR index (0.38) and MDR prevalence (30.66%) highlights the growing concern of antimicrobial resistance among bovine otitis pathogens. These findings emphasize the necessity of routine culture and sensitivity testing before antimicrobial therapy and support the implementation of antimicrobial stewardship programs in livestock production systems. Continued surveillance of antimicrobial resistance patterns is essential for effective disease management and safeguarding public health.

## 6. Declaration Conflict of Interest

On behalf of all author declare that no conflict of interest with the publication.

## 7. Ethics Statement

The animals used in this study were in accordance with the ethics of the TANUVAS institutional animal ethics committee (IAEC).

## 8. Acknowledgement

I gratefully acknowledge and thankful to the TANUVAS University for funding and support during entire study period.

## References

1. Aarestrup FM, Wegener HC, Collignon P. Resistance in bacteria of the food chain: Epidemiology and control strategies. *Expert Rev Anti Infect Ther*. 2008;6(5):733-750.
2. Bandyopadhyay S, Bhattacharyya D, Samanta I. Characterization of multidrug-resistant biofilm-producing *Escherichia coli* and *Klebsiella pneumoniae* in healthy cattle and cattle with diarrhea. *Microb Drug Resist*. 2021;27(11):1457-1469.
3. Bugden DL. Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in Australia. *Aust Vet J*. 2013;91(1-2):43-46.
4. Catry B, Dewulf J, Maes D, Pardon B, Callens B, Vanrobaeys M, *et al*. Effect of antimicrobial consumption and production type on antibacterial resistance in the bovine respiratory and digestive tract. *PLoS One*. 2016;11(1):e0146488.
5. Chon J, Sung K, Khan S. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food-producing and companion animals and food products. In: *Frontiers in Staphylococcus aureus*. 2017.
6. Duarte ER, Hamdan JS. Otitis in cattle, an aetiological review. *J Vet Med B Infect Dis Vet Public Health*. 2004;51(1):1-7.
7. Giguère S, Sweeney RW, Bélanger M, *et al*. Comparative quantification of the *in vitro* activity of veterinary fluoroquinolones. *Vet Microbiol*. 2007;124(1-2):73-81.
8. Hariharan H, Coles M, Poole D, Lund L, Page R. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. *Can Vet J*. 2006;47(3):253-255.
9. Kruperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl Environ Microbiol*. 1983;46:165-170.
10. Lewis II, James S. Performance standards for antimicrobial susceptibility testing. 2024.
11. Nedbalcova K, Nechvatalova K, Pokludova L, Bures J, Kucerova Z, Koutecka L, *et al*. Resistance to selected beta-lactam antibiotics. *Vet Microbiol*. 2014;171(3-4):328-336.
12. Pohl A, Lübke-Becker A, Heuwieser W. Minimum inhibitory concentrations of frequently used antibiotics against *Escherichia coli* and *Trueperella pyogenes* isolated from uteri of postpartum dairy cows. *J Dairy Sci*. 2018;101(2):1355-1364.
13. Yeruham I, Elad D, Liberboim M. Clinical and microbiological study of an otitis media outbreak in calves in a dairy herd. *J Vet Med B*. 1999;46(3):145-150.