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Original Article

Molecular Identification and Antimicrobial Susceptibility Profiling of Bacterial Isolates from Foot Rot Lesions in Bargur Cattle from Anthiyur, Tamil Nadu, India

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Abstract

Foot rot is a contagious, debilitating polymicrobial infection that compromises bovine health and productivity. This investigation delineates the molecular etiology of foot rot in Bargur cattle, an indigenous breed endemic to the Bargur Hills of Anthiyur, Tamil Nadu, India, and establishes antibiotic susceptibility profiles. Swab samples from interdigital lesions of foot rot-affected cattle underwent culture-dependent isolation, with five representative colonies identified through 16S rRNA gene amplification and Sanger sequencing. The predominant pathogens comprised *Escherichia coli* (BCFR1), *Shigella sonnei* (BCFR7), and *Staphylococcus chromogenes* (BCFR8). The results indicate that *E. coli* and *S. sonnei* played principal pathogenic roles in lesion progression. Notably, the isolation of *Shigella sonnei* from bovine foot rot wounds represents a novel finding, expanding the known pathogen spectrum and highlighting potential zoonotic risks. Kirby-Bauer disk diffusion assays revealed that the isolates were predominantly susceptible to gentamicin, ciprofloxacin, and enrofloxacin. These findings substantiate the need for molecular diagnostics for precise pathogen identification and support susceptibility-guided antimicrobial interventions. This study provides foundational etiological and antibiotic sensitivity data to support evidence-based health management of Bargur cattle.

Keywords: *16S rRNA Sequencing, Antimicrobial Resistance, Bargur Cattle, Bovine Foot Rot, Molecular Epidemiology*

Introduction

Foot rot is a communicable disease affecting the interdigital area of ruminants such as cattle, goats, and sheep (Raadsma & Egerton, 2013). The disease exhibits a mortality rate of 40% and a morbidity rate ranging from 8% to 100% (Sağlıyan, 2003). It affects the hoof and surrounding area, causing lameness and swelling in one or more feet of multiple animals in a herd simultaneously (Maas, 2009). The lameness induced by foot rot often results in decreased appetite and poor performance. Delayed treatment or the absence of treatment may lead to chronic conditions, which can be fatal (Greenough, 2007). Noninfectious lesions, such as screw claw, sole hemorrhage, ulcers, fissures, and false soles,

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often develop as secondary consequences of foot rot (Walker *et al.*, 2023). Infectious foot conditions, including interdigital phlegmon, papillomatous digital dermatitis, and heel erosions, can lead to septic processes (Newcomer & Chamorro, 2016). The disease-causing microorganisms spread through contact with mud, feces, and contaminated roughage in pastures (Raadsma & Egerton, 2013).Foot rot is commonly caused by soil-borne bacteria such as *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*, which enter the interdigital area through wounded skin. Additionally, some soil-borne bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Corynebacterium pyogenes*, and *Porphyromonas levii*, have also been found to be associated with foot rot infection (Lincoln, 2009; Raadsma & Egerton, 2013). The inflammatory process begins in the interdigital region and skin, causing hair loss, tenderness, redness, serous exudate, and necrosis (Sağlıyan, 2003). In advanced cases, the inflammation extends to the abaxial wall of the capsulae ungulae (Stewart & Claxton, 1993). *Dichelobacternodosus* and *Fusobacterium necrophorum*, the primary pathogens responsible for bovine foot rot, initiate infection by colonizing the interdigital skin (Pyakurel *et al.*, 2025), where they secrete tissue-damaging proteases that contribute to the progression from initial damage to an exacerbated stage. This progression is further supported by the production of leukotoxins and other virulence factors, which intensify tissue destruction, impair host defenses, and create an oxygen-deprived microenvironment that further supports bacterial proliferation (Yaman *et al.*, 2017; Farooq *et al.*, 2018; Fesseha *et al.*, 2021). Other bacteria, including *Porphyromonas levii*, *Prevotella intermedia*, *Trueperella pyogenes*, *Staphylococcus aureus*, and *Escherichia coli*, can act synergistically with *F. necrophorum* (Currin *et al.*, 2009). Although animals of all ages are susceptible to foot rot infection, it is more prevalent in cattle that are weaning or older, and prevalence rates can reach 20%–30% in intensive dairy operations (Kontturi *et al.*, 2017; Zhao *et al.*, 2026). The morbidity level may range from one or two animals per herd to large-scale epidemics (Griffin, 1998; Cortes *et al.*, 2021). A recent study clearly showed that foot rot in dairy cows is associated with significant alterations in microbial communities across the hoof, vagina, and milk, indicating that this condition may have broader implications for cow health and management practices (Zhao *et al.*, 2026). Foot rot infection can cause significant economic losses to farmers by reducing milk production in dairy cattle and weight gain in cattle of all ages (Kausche & Robb, 2003). Severe or untreated foot rot can lead to myiasis (McCarter, 2019).

Myiasis is a parasitic infection that occurs when flies lay their eggs and the emerging larvae feed on living tissues and complete their life cycle within the host (Dutto *et al.*, 2013). Most myiasis-causing flies are members of the order Diptera (Olea *et al.*, 2014). In true myiasis, flies purposefully lay their eggs in or on tissues (Sunny *et al.*, 2016). Myiasis symptoms can vary, ranging from obligatory to facultative. In facultative myiasis, flies purposefully use wounds or degenerative necrotic conditions as sites to incubate their larvae. In obligatory myiasis, maggots are required to feed on living tissues (Menaka *et al.*, 2021). Bargur cattle are mainly used for numerous agricultural operations in addition to milk production, such as plowing. These cattle are traditionally reared in herds using a penning system constructed of bamboo near the forest boundary (Pundir *et al.*, 2009). Overcrowding, inadequate sanitation in the rearing environment, foot injuries sustained during grazing, and environmental conditions make these cattle more susceptible to ectoparasites, especially flies (Yaswanthkumar *et al.*, 2024), as well as foot rot disease. This study mainly focuses on the isolation and molecular identification of bacterial pathogens associated with foot rot in Bargur cattle and determines their sensitivity to various commonly used antibiotics.

Materials and Methods

Study Area

The Bargur Hills, situated at latitude 11.805° and longitude 77.534° in Anthiyur Taluk, Erode District, form part of the Eastern Ghats, with elevations averaging 1,000 m above mean sea level (MSL). The region experiences a temperate climate, with temperatures ranging from 15°C to 32°C and annual rainfall of approximately 350 cm, primarily from the southwest and northeast monsoons. The Bargur Hills comprise 34 hamlets, including Bargur, Thamarakarai, the highest elevation at 1,078 m MSL,

and Bejiletti, the lowest at 988 m MSL. A total of 34,043 ha of the Bargur cattle breeding tract predominantly falls under the reserve forest category and was recently designated as Periyar Wildlife Sanctuary by the Tamil Nadu Forest Department. Rain-fed agriculture dominates the region, with major crops including ragi, maize, dry paddy, and tapioca. Indigenous Bargur cattle are maintained under the traditional zero-input “Patti” system and exhibit semi-wild behavior. This unique agro-ecological interface underscores the ecological and cultural significance of Bargur cattle conservation. Samples were collected from foot-rot-affected Bargur cattle in Thamarakarai village during June 2024 (Figure 1).

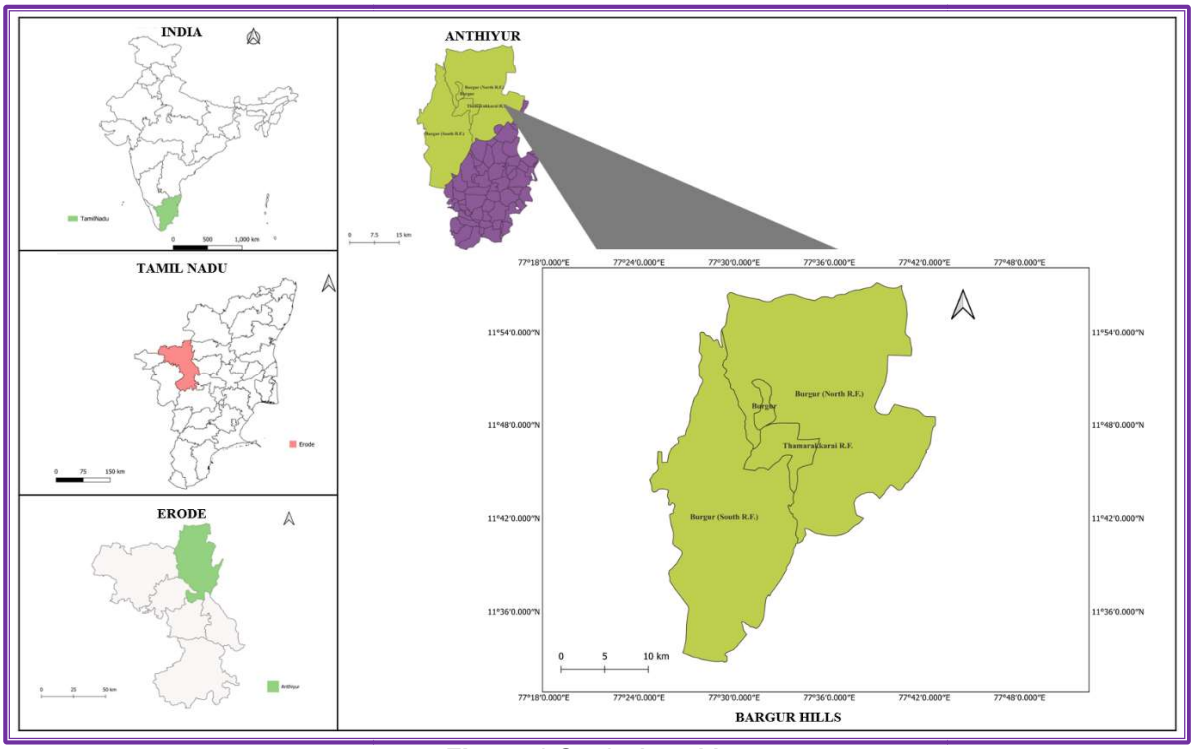

Figure 1:*Study Area Map*

Sample Collection

Open lesion stage Healing stage

Figure 2: *Foot Rot Wound*

A total of ten foot-rot-infected Bargur cattle were selected for the study (Figure 2). Sterile plastic swab sticks with tubes were used to collect the swabs from the affected cattle. The swab sticks were then transported to the laboratory for further analysis to identify the bacteria associated with foot rot infection.

a) Experimental Design

The experiment was conducted in two distinct phases. The initial phase involved culture-based morphological and molecular identifications. The subsequent phase included investigation of the isolated bacteria's response to commercially available antibiotic discs (Akter *et al.*, 2020).

b) Bacterial Culturing

The received bovine sample swab was inoculated into Luria Bertani agar and blood agar (for the identification of hemolysis) via simple streaking and incubated at 370C for 24 hrs. The colonial characteristics of the colonies were noted and the cellular morphologies of the obtained bacteria were identified by differential gram staining (Abdalhamed *et al.*, 2018).

c) DNA Isolation and PCR Amplification

DNA isolation was performed using the standard phenol-chloroform method, and the 16s rRNA gene was amplified in a reaction volume. The PCR products were treated with ExoSAP-IT PCR product clean-up reagent, and sequencing PCR was performed using ABI PRISM Big Dye terminator ready reaction mix. Cycle-extension products were purified by ethanol/EDTA/sodium acetate precipitation. The study was conducted using an Applied Biosystems ABI 3730XL Thermofisher. The sequences were quality-checked and trimmed using the software Sequencer V4.10.1.

d) Bioinformatics Analysis

The sequences were quality-checked and trimmed using the software Sequencer V4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Trimmed sequences were searched in NCBI using the BLASTn tool, and the identity of the sample was confirmed based on percentage similarity and query coverage of the nearest neighbors. The bacteria isolated from the milk of mastitis affected cows were taken to analyze evolutionary divergence between sequences using the maximum composite likelihood model and to analyze the evolutionary relationships of taxa using the Neighbor-Joining method (Yalcin *et al.*, 2024).

e) Antibiotic Sensitivity Test

Antibiotic sensitivity tests of the samples named BCFR1 (BCR1), BCFR 7 (BCRY) and BCFR8 (BCRW) were performed by agar disc diffusion assay against the antibiotics Gentamicin, Ciprofloxacin and Enrofloxacin. Muller-Hinton agar plates were prepared and used for the assay. The plates were inoculated with bacteria using sterile swabs and antibiotic discs were placed aseptically onto each of the bacterial plates and incubated at 370C for 24 hrs. The antibiotics diffused into the agar medium and inhibited the growth of the tested microbial strains. Antibiotic properties are expressed as the diameter of the clearing zone around each well (Abdalhamed *et al.*, 2018).

(Note: the internal laboratory codes for BCRF as BCR1, BCFR 7 as BCRY and BCFR 8 as BCRW)

Results

a) Morphological Characterization of the Isolates

Bacterial growth was identified based on the turbidity present on Luria-Bertani agar and blood agar after incubation for 24 hours at 370 C. On Luria-Bertani agar, eight varieties of colonies (BCFR1, BCFR2, BCFR3, BCFR4, BCFR5, BCFR6, BCFR7 and BCFR8) were obtained and on blood agar, three varieties of colonies (BCFR9, BCFR10 and BCFR11) were obtained. Colony morphological characteristics such as size, shape, margin, elevation, color, opacity, and texture identified are shown in Table 1. Based on the growth, appearance, and color, five colonies, namely, BCFR1, BCFR4,

BCFR5, BCR7 and BCFR8, were selected for Gram staining. The colonies BCFR 1, 4, 5, and 7 were found to be Gram-negative, and BCRF8 was Gram-positive bacteria.

Table 1: *Colony Morphology*

Medium	Strain Code	Size	Shape	Margin	Elevation	Colour	Opacity	Texture
In Luria Bertani agar	BCFR1	Medium	Irregular	Undulate	Umbonate	White	Opaque	Smooth
	BCFR2	Small	Circular	Entire	Raised	White	Opaque	Smooth
	BCFR3	Medium	Circular	Entire	Raised	Orange	Opaque	Smooth
	BCFR4	Pinpoint	Circular	Entire	Flat	White	Opaque	Smooth
	BCFR5	Pinpoint	Circular	Entire	Raised	White	Opaque	Smooth
	BCFR6	Small	Circular	Entire	Raised	Orange	Opaque	Smooth
	BCFR7	Small	Circular	Entire	Flat	White	Opaque	Smooth
	BCFR8	Small	Circular	Entire	Flat	Yellow	Opaque	Smooth
In Blood agar	BCFR9	Medium	Circular	Entire	Flat	Grey (no lysis)	Opaque	Smooth
	BCFR10	Pinpoint	Circular	Entire	Flat	Grey (no lysis)	Opaque	Smooth
	BCFR11	small	Circular	Entire	Flat	Grey (no lysis)	Opaque	Smooth

b) Molecular Identification of the Isolates

The colonies were selected for gene sequencing and comparison. The amplified sequences with sequence length and accession number were given in Table 2. The isolates were compared with sequences from GenBank. The strains BCRF1, 4 and 5 showed 100% similarity with *Escherichia coli;* the strain BCFR 7 showed 100% similarity with *Shigella sonnei* and the strain BCFR 8 showed 99.86% similarity with *Staphylococcus chromogenes* sequences from GenBank. The evolutionary history was inferred using the Neighbour-Joining method (Figure 3) and the evolutionary distances were computed using the Maximum Composite Likelihood method.

Table 2: *Sequence Length of Isolated Strains and Its NCBI Accession Number*

S.No	Strain Code	Sequence Length (bp)	Identity (%)	Nearest Neighbour	Accession Number
1	BCFR1	752	100	*Escherichia coli*	PQ288860
2	BCFR4	811	100	*Escherichia coli*	PQ288864
3	BCFR5	666	100	*Escherichia coli*	PQ288863
4	BCFR7	770	100	*Shigella sonnei*	PQ288861
5	BCRF8	719	99.86	*Staphylococcus chromogenes*	PQ288862

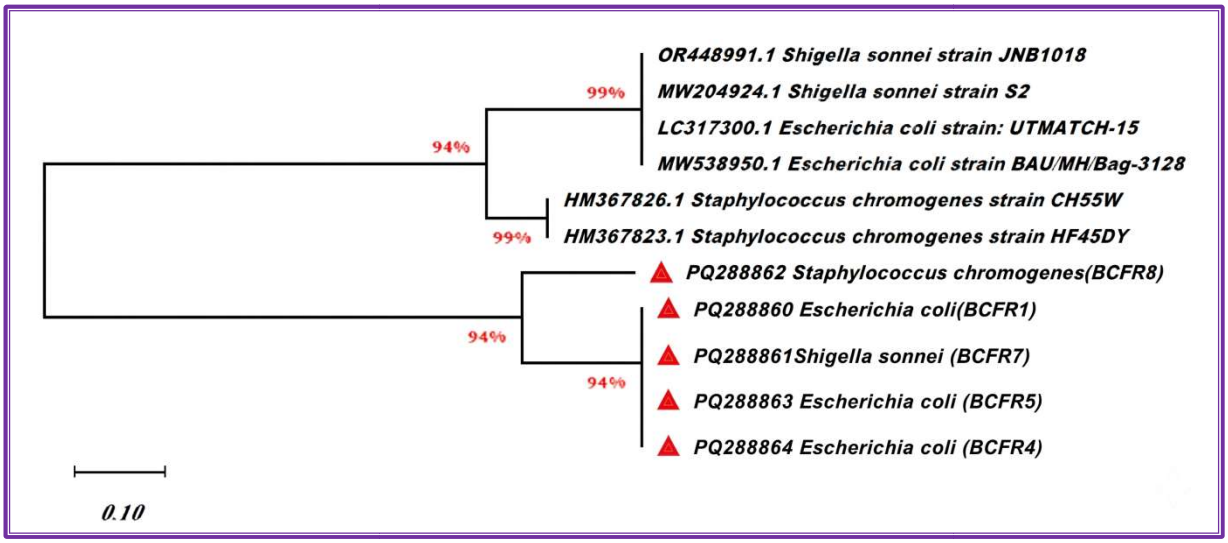

Figure 3: *Evolutionary Tree by Using Neighbor-Joining Method*

c) *Antibiotic Sensitivity Test*

The antibiotic susceptibility profiles of the bacterial isolates, assessed by disc diffusion method, revealed varying zones of inhibition (Table 3). For *Escherichia coli* (BCFR1), Gentamicin produced a zone of 22.00 ± 1.00 mm (S.E = 0.58 mm; 95 % CI: 19.52 - 24.48 mm), Ciprofloxacin yielded 25.00 ± 1.00 mm (S.E = 0.58 mm; 95 % CI: 22.52–27.48 mm) and Enrofloxacin resulted in 26.33 ± 0.58 mm (S.E = 0.33 mm; 95 % CI: 24.90 - 27.77 mm) (Figure 4). In *Shigella sonnei* (BCFR7), Gentamicin showed 23.33 ± 0.58 mm (S.E = 0.33 mm; 95 % CI: 21.90 - 24.77 mm), Ciprofloxacin exhibited 31.00 ± 1.00 mm (S.E = 0.58 mm; 95 % CI: 28.52 - 33.48 mm) and Enrofloxacin gave 32.33 ± 1.15 mm (S.E = 0.67 mm; 95 % CI: 29.46 - 35.20 mm) (Figure 5). For *Staphylococcus chromogenes* (BCFR8), Gentamicin had 19.33 ± 0.58 mm (SE = 0.33 mm; 95 % CI: 17.90 - 20.77 mm), Ciprofloxacin displayed 38.00 ± 1.00 mm (S.E = 0.58 mm; 95 % CI: 35.52–40.48 mm) and Enrofloxacin measured 33.00 ± 1.00 mm (S.E = 0.58 mm; 95 % CI: 30.52–35.48 mm) (Figure 6). Fluoroquinolones (Ciprofloxacin and Enrofloxacin) consistently produced larger zones across all strains compared to gentamicin.

Table 3: *Clearing Zone (In Mm) of Bacterial Growth Shown by the Agar Well Diffusion Method Against the Following Antibiotics*

Strain	Antibiotics	Zone of Inhibition Mean (S.D ± S.E) mm	Lower	Upper	Interpretation
Escherichia coli (BCFR1)	Gentamicin	22.00 (1.00 ± 0.58)	19.52	24.48	S
	Ciprofloxacin	25.00 (1.00 ± 0.58)	22.52	27.48	MS
	Enrofloxacin	26.33 (0.58 ± 0.33)	24.90	27.77	S
Shigella sonnei (BCFR7)	Gentamicin	23.33 (0.58 ± 0.33)	21.90	24.77	S
	Ciprofloxacin	31.00 (1.00 ± 0.58)	28.52	33.48	S
	Enrofloxacin	32.33 (1.15 ± 0.67)	29.46	35.20	S
Staphylococcus chromogenes (BCFR8)	Gentamicin	19.33 (0.58 ± 0.33)	17.90	20.77	S
	Ciprofloxacin	38.00 (1.00 ± 0.58)	35.52	40.48	S
	Enrofloxacin	33.00 (1.00 ± 0.58)	30.52	35.48	S

S - Sensitive, MS - Moderately Sensitive

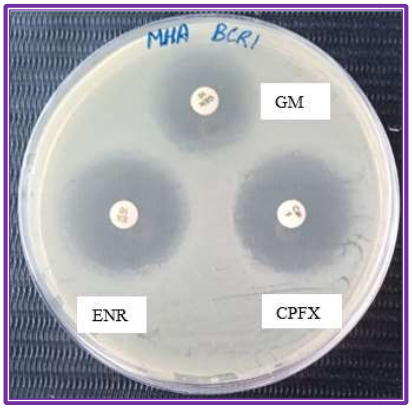

Figure 4: *Zone of Inhibition of ABST of BCFR1 (BCR1) against Gentamicin, Ciprofloxacin and Enrofloxacin*

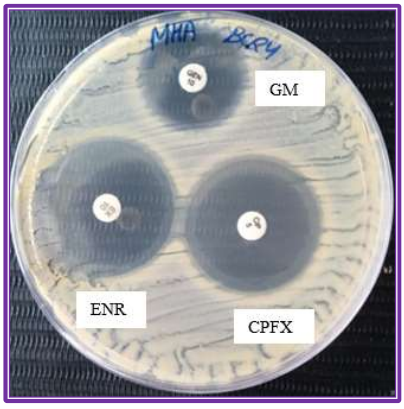

Figure 5: *Zone of Inhibition of ABST of BCFR7 (BCRY) against Gentamicin, Ciprofloxacin and Enrofloxacin*

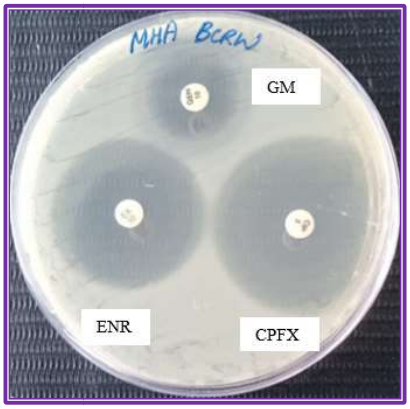

Figure 6: *Zone of Inhibition of ABST of BCFR8 (BCRW) against Gentamicin, Ciprofloxacin and Enrofloxacin*

Discussion

The bacteria such as *Escherichia coli*, *Staphylococcus* sp., *Streptococcus* sp. and *Klebsiella pneumoniae* were commonly associated with wound infection (Sani *et al.*, 2012) and a few opportunistic pathogens were predominantly found in the environment (Olayinka *et al.*, 2004). The bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Klebsiella pneumoniae,

Enterococcus faecalis and *Acinetobacter baumannii* and certain *Treponema* species were most commonly found in the infected wound (Puca *et al.*, 2021; Mekková*et al.,* 2025).The severity of the infection caused by pathogens was dependent on the number and virulence of the infecting microorganisms and the nature of host resistance (Gelasakis *et al.*, 2019). Previous studies have provided information on the primary causative agent of foot rot, such as *Fusobacterium necrophorum* and *Porphyromonas levii* (Yiğitarslan *et al.*, 2023; Zhang *et al.*, 2025). The samples for our study were obtained from an intensive cattle-rearing system. In our investigations, several opportunistic pathogens like gram-negative bacterial strains, such as *E. coli* BCFR1 and *Shigella sonnei* BCFR7, and the gram-positive bacterial strain *Staphylococcus chromogenes* BCFR8 from the infected wounds of cattle were isolated and identified. *E. coli* is one of several bacteria that can be isolated from foot rot wounds, along with *Staphylococcus aureus*, *Porphyromonas levii* and *Truperella pyogenes* (Walk er *et al.*, 2023). The *Staphylococcus chromogenes* can be isolated from the wounds of cattle, particularly from the skin of the udder and teat, and from milk (Huebner *et al.*, 2021). These bacteria cause mastitis (Dego & Vidlund, 2024) and were predominantly present in the environment as various strains (Fry *et al.*, 2014).Among these three isolates, *Shigella sonnei* was primarily found in contaminated faeces and water, causing bacillary dysentery, which results in shigellosis (Chen *et al.*, 2001). This was not previously reported in foot rot infections and the isolation of *Shigella sonnei* represents a novel finding that expands the pathogen spectrum and highlights potential zoonotic risks (Shad & Shad, 2020)**.** Shigellosis is highly contagious, and although humans also serve as the natural reservoir, it can affect both humans and non-human primates (CDC, 2024). The multi-antibiotic resistant *Shigella sonnei* has been isolated from cow's milk in Egypt (Elkenany*et al.*, 2022). The molecular investigation of the different isolates pertains to three genera: *Escherichia*, *Staphylococcus* and *Shigella*. Based on the phylogenetic analysis, the three isolates were hypothesized to be different strains that may have been transferred from contaminated soil in the rearing environment. According to a previous antibacterial study, *E. coli* strains isolated from wound infections were susceptible to gentamicin but resistant to ciprofloxacin (Alharbi *et al.*, 2019). The *E. coli* isolated from bovine mastitis demonstrated a susceptibility rate of 83.3% to gentamicin and 71.8% to ciprofloxacin (Xu *et al.*, 2022). Enrofloxacin exhibits significant antibacterial effects on *E. coli* with a minimum inhibitory concentration (Li *et al.*, 2019). The recent study shows that *Escherichia coli* as a predominant opportunistic and pathogenic bacteria on dairy farms, with notable antibiotic resistance profiles and some of the *E. coli* being multidrug-resistant (Zubareva *et al.*, 2025).All strains of *Staphylococcus chromogenes* are typically susceptible to gentamicin, ciprofloxacin, and enrofloxacin (Devriese*et al.*, 2002), and *Shigella sonnei* was 100% sensitive to gentamicin (Madhavan *et al.*, 2018), 90% sensitive to ciprofloxacin (Lappe *et al.*, 2015) and 96% sensitive to other antibiotics (Williams & Berkley, 2018). The superior zones of inhibition exhibited by fluoroquinolones Ciprofloxacin and Enrofloxacin compared to Gentamicin across *Escherichia coli* (BCFR1), *Shigella sonnei* (BCFR7) and *Staphylococcus chromogenes* (BCFR8) isolates reflect fundamental differences in their mechanisms of action and physicochemical properties (Lungu *et al.*, 2022). Fluoroquinolones inhibit DNA gyrase and topoisomerase IV, disrupting DNA supercoiling and replication to achieve bactericidal activity even against non-growing cells common in agar diffusion assays (Sood *et al.*, 2018). Beyond pathogen identification and antibiotic sensitivity, recent immunological investigations have revealed that dairy cows with *Fusobacterium necrophorum* induced foot rot exhibit significantly increased percentages of NK cells, NKT cells, CD4+CD8− helper T cells, and CD4+CD8+ double-positive T cells compared to healthy controls, while WC1+ γδ T cells remain unchanged (Yang *et al.*, 2025).

Limitations

This study was limited by its geographic scope and reliance on phylogenetic analysis rather than whole-genome sequencing.

Future Scope

Future research should expand to diverse Bargur cattle-rearing systems, integrating longitudinal immunological monitoring with genomic and metagenomic tools. Incorporating microbiome dynamics, spatial epidemiology, and climate-linked modeling will strengthen predictive disease management.

Community-based surveillance and antimicrobial stewardship will ensure sustainable herd health and long-term conservation of this vulnerable, endemic Bargur cattle breed.

Conclusion

The findings of the present study indicate that foot rot infection results from polymicrobial interactions involving *Escherichia coli*, *Shigella sonnei*, and *Staphylococcus chromogenes*, all of which demonstrated increased sensitivity to commonly available antibiotics. Although these bacteria are typically commensal, their abundance in contaminated environments enabled them to act as opportunistic pathogens. The isolation of *Shigella sonnei* from bovine foot rot wounds represents a novel observation, expanding the spectrum of pathogens associated with this condition and highlighting potential zoonotic risks. The rearing system of Bargur cattle was open and free-ranging, suggesting that specific areas within grazing lands may serve as reservoirs for pathogens. These results underscore the importance of screening grazing areas and implementing hygienic practices to reduce infection risks, while future studies should incorporate genomic approaches and epidemiological surveys to better understand pathogen transmission and resistance dynamics.

Declaration

The authors declare that the work described in this manuscript is original, has not been published previously and is not under consideration for publication elsewhere. All data were generated, analyzed and interpreted by the authors in accordance with ethical research standards.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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