JAVeST
Journal Of Applied Veterinary Science and Technology

Journal of Applied Veterinary Science and Technology

http://e-journal.unair.ac.id/javest

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Research Reports

Isolation and Antimicrobial Susceptibility Profile of Citrobacter Species from Small Ruminants and Water Sources

Isolasi dan Profil Kepekaan Antimikroba Spesies Citrobacter dari Ruminansia Kecil dan Sumber Air

Enenche Francis Ejeh*[1], Yusuf Madaki Lekko[2], Fatima Adamu Lawan[1], Juliana James Ndahi[3], Adama Musa Abdullahi[3]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri-Nigeria
[2]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri-Nigeria
[3]Veterinary Teaching Hospital, University of Maiduguri, Maiduguri-Nigeria

ABSTRACT

Background: Citrobacter species are gram negative, enteric bacilli that are mostly found in water, soil, food, and intestine of animals and humans causing wide range of infections. **Purpose:** It will provide critical insights that can improve the diagnosis, treatment, and management of infections caused by Citrobacter species, thereby enhancing the overall health of small ruminants. **Method:** A cross-sectional study using stratified random sampling method was used. A total of 120 samples were analyzed, consisting of 116 from small ruminants and 4 from water. Samples were cultured on selective media and Mac Conkey Agar. Antimicrobial sensitivity testing was done for Citrobacter species using Kirby-Bauer disc diffusion method and are classify as susceptible, intermediate, or resistant for each antimicrobial agent. **Results:** A total of 23 isolates (19.2%) were identified as Citrobacter species, while 97 isolates (80.8%) belonged to other members of the Enterobacteriaceae family. Citrobacter species showed a 100% prevalence in water samples, compared to 16.4% in small ruminants. Statistical analysis demonstrated a significant association between Citrobacter occurrence and factors including sample source ($p = 0.000$), age ($p = 0.000$), and breed ($p = 0.002$). Antimicrobial susceptibility testing revealed that *Citrobacter portucalensis* isolates exhibited complete resistance (100%) to cefotaxime and ceftriaxone, with similarly high resistance levels to amoxicillin/clavulanic acid (94.7%), pefloxacin (84.2%), cephalexin (84.2%), and trimethoprim/sulfamethoxazole (89.5%). Ofloxacin showed the greatest level of susceptibility (73.7%), although resistance was still observed in 21.0% of the isolates. **Conclusion:** The detection of Citrobacter species in both animals and water underscores the need for effective therapeutic choices and environmental control to reduce antimicrobial resistance.

ARTICLE INFO

Received: 23 September 2025
Revised: 27 October 2025
Accepted: 31 October 2025
Online: 31 October 2025

***Correspondence:**
Enenche Francis Ejeh
E-mail: enechefrancis@yahoo.com

Keywords: Antimicrobial Sensitivity; Citrobacter; Prevalence; Resistance

ABSTRAK

Latar Belakang: Spesies Citrobacter merupakan basil enterik gram-negatif yang umumnya ditemukan di air, tanah, makanan, serta di dalam usus hewan dan manusia, dan dapat menyebabkan berbagai jenis infeksi. **Tujuan:** memberikan wawasan penting yang dapat meningkatkan diagnosis, pengobatan, dan manajemen infeksi yang disebabkan oleh spesies Citrobacter, sehingga dapat meningkatkan kesehatan ruminansia kecil secara keseluruhan. **Metode:** Penelitian potong lintang dilakukan dengan menggunakan metode pengambilan sampel acak bertingkat (stratified random sampling). Sebanyak 120 sampel dianalisis, terdiri atas 116 sampel dari ruminansia kecil dan 4 sampel dari sumber air. Sampel dikultur pada media selektif dan agar MacConkey. Uji kepekaan antimikroba terhadap spesies Citrobacter dilakukan dengan metode difusi cakram Kirby-Bauer, dan setiap isolat diklasifikasikan sebagai sensitif, intermediet, atau resisten terhadap masing-masing agen antimikroba. **Hasil:** Sebanyak 23 isolat (19,2%) teridentifikasi sebagai spesies Citrobacter, sedangkan 97 isolat lainnya (80,8%) termasuk dalam famili Enterobacteriaceae lainnya. Spesies Citrobacter menunjukkan prevalensi 100% pada sampel air, dibandingkan dengan 16,4% pada ruminansia kecil. Analisis statistik menunjukkan adanya hubungan yang signifikan antara keberadaan Citrobacter dengan faktor-faktor seperti sumber sampel ($p = 0{,}000$), umur ($p = 0{,}000$), dan bangsa hewan ($p = 0{,}002$). Uji kepekaan antimikroba mengungkapkan bahwa isolat *Citrobacter portucalensis* menunjukkan resistensi penuh (100%) terhadap cefotaxime dan ceftriaxone, serta tingkat resistensi yang tinggi terhadap amoksisilin/asam klavulanat (94,7%), pefloksasin (84,2%), cephalexin (84,2%), dan trimetoprim/sulfametoksazol (89,5%). Ofloxacin menunjukkan tingkat kepekaan tertinggi (73,7%), meskipun resistensi masih ditemukan pada 21,0% isolat. **Kesimpulan:** Deteksi spesies Citrobacter pada hewan dan sumber air menegaskan perlunya pilihan terapi yang efektif serta pengendalian lingkungan untuk menekan peningkatan resistensi antimikroba.

Cite This Article:
Ejeh, E.F., Lekko, Y.F., Lawan, F.A., Ndahi, J.J., and Abdullahi, A.M., 2025. ***Isolation and Antimicrobial Susceptibility Profile of Citrobacter Species from Small Ruminants and Water Sources.*** Journal of Applied Veterinary Science and Technology.6(2):143-147 .https://doi.org/10.20473/javest.V6.I2.2025.143-147

Kata kunci: Citrobacter; Prevalensi; Resistensi; Sensitifitas Antibakteri

INTRODUCTION

Citrobacter is a genus of Gram-negative, facultative anaerobic bacteria in the family Enterobacteriaceae. These bacteria are typically found in the intestines of animals and humans, as well as in soil and water. While often classified as opportunistic pathogens, they are known to cause infections under certain conditions. Species like *Citrobacter freundii* and *Citrobacter koseri* have been implicated in infections in small ruminants, especially in neonatal animals under stress or with compromised immunity (Jabeen *et al.,* 2023). Sheep and goats, crucial for the agricultural economy, can be affected by Citrobacter species, especially in neonatal stages. Though these infections are not as frequently reported as other bacterial diseases in small ruminants, Citrobacter species have been known to cause gastrointestinal infections, septicemia, and even pneumonia in lambs and kids (Quinn *et al.,* 2011). These infections tend to occur under suboptimal management practices, poor hygiene, or inadequate colostrum intake, which compromises the immune defense of newborn animals (Awad and Al-saadi, 2022; Azad *et al.,* 2024; Constable *et al.,* 2016; Hawaldar and Sadhna, 2019; Jabeen *et al.,* 2023; Sekhi and Al-Samarraae, 2022).

In small ruminants, Citrobacter infections can lead to various clinical presentations, including: Diarrhea: One of the most common symptoms, Citrobacter can cause severe enteritis in lambs and kids, leading to dehydration and, in severe cases, death (Jabeen *et al.,* 2023). Pneumonia: Respiratory infections occur when the bacteria invade the lungs, often in stressed or immunocompromised animals. This can lead to significant respiratory distress (Quinn *et al.,* 2011). Septicemia: In neonates, especially those deprived of adequate colostrum, Citrobacter can invade the bloodstream, leading to systemic infection and potential septicemia, which is often fatal if not treated early (Awad and Al-saadi, 2022; Azad *et al.,* 2024; Constable *et al.,* 2016; Hawaldar and Sadhna, 2019; Jabeen *et al.,*2023; Sekhi and Al-Samarraae, 2022). Meningitis: Though rare, *Citrobacter koseri* has been implicated in cases of neonatal meningitis in small ruminants, similarly to how it affects human neonates (Bonasoni *et al.,* 2022; Jabeen *et al.,* 2023). The virulence of Citrobacter is mainly due to its ability to produce endotoxins, which trigger inflammation and tissue damage. In neonatal animals, once the bacteria breach the gastrointestinal or respiratory barriers, they can disseminate through the bloodstream, causing systemic infections such as septicemia or even meningitis (Bonasoni *et al.,*, 2022; Jabeen *et al.,* 2023). The objective is to isolate species of Citrobacter and determine antimicrobial susceptibility profile of Citrobacter isolated from small ruminant's water sources.

MATERIAL and METHOD

Study Area

The University of Maiduguri campus is located between latitudes 11°49'7.68"and 11°47'49.92" North and longitudes 13°11'12.48" and 13°12'43.2" East, with an average elevation of approximately 350 meters above sea level (Figure 1). It is situated along Bama Road in the Jere Local Government Area (LGA) of Maiduguri. The campus comprises staff and students and includes residential quarters, hostel accommodations, religious centers, staff schools, clubs, and sporting facilities (Vashala et al., 2019; Yelwa, 2012).

Research Design and Sampling

The research employed a cross-sectional study design to assess the isolation and antimicrobial susceptibility profile of Citrobacter species in soil, water and small ruminants in University of Maiduguri. Stratified random sampling was employed to select goats, sheep, water, and soil samples within the study area.

Ethical Considerations

Approval was obtained from the University of Maiduguri Ethical Review Committee. Informed consent was soughted from animal owners before sample collection. Proper handling and disposal of biological samples was ensured to minimize risks to human and animal health.

Sample Collection and Transportation

Samples from sheep and goats: Rectal swabs were collected from 70 sheep and 80 goats. Water sample were collected directly from the source, holding the bottle underwater to avoid surface contaminants. The following data were collected from the animals: sex, age, body condition and presence or absence of disease conditions. All samples, including rectal swabs, water, and soil, were transported to the laboratory for further analysis.

Culture and Sub Culturing

Upon arrival to the laboratory the samples were cultured on selective media and incubated under optimal conditions to isolate Citrobacter. Mac Conkey Agar was later used to inhibits the growth of Gram-positive bacteria due to bile salts and crystal violet to obtain pure cultures. The cultures were incubated at 37^{0}C for 24 hours. Pure cultures obtained were stored for antimicrobial susceptibility testing.

Biochemical Tests

Several biochemical test were performed on colonies from pure cultures of isolates. This test is Triple Sugar Iron (TSI) Agar, Indole Test, Methyl Red (MR) Test, Citrate Utilization Test, Urease Test, Motility Test, Voges-Proskauer Test (VPT), Catalase Test, Hydrogen Sulfide Test oxidase test to determine the presence of lactose fermenting Citrobacter. The colonial, morphology, gram stain, and biochemical test was performed base on standard bacteriological procedures (Cheesbrough, 2006). Antibiotic susceptibility testing (AST) for Citrobacter species is performed to determine which antibiotics are effective for treating infections caused by Citrobacter.

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing was done for Citrobacter species using Kirby-Bauer disc diffusion method (Clinical and Laboratory Standards Institute, 2020). Initially, Mueller-Hinton agar plates and Mueller-Hinton broth were prepared according to manufacturer's instructions, and the bacterial isolate was inoculated into the Mueller-Hinton broth poured in cryoviles and was allowed to grow for 1hour 30minutes. 200 μm of the Mueller-Hinton broth was used to inoculate the Mueller-Hinton agar plate using a sterile swab, allowed to dry for 5-10 minutes. Hi-Profil Gram-Negative Discs containing various antimicrobial agents were then placed onto the agar surface. The plate was incubated at 37°C for 24 hours. Following incubation, the zone of inhibition (mm) around each disc was measured using 15cm meter rule and compared to established breakpoints (Clinical and Laboratory Standards Institute, 2020), to classify the bacterial isolate as susceptible, intermediate, or resistant to each antimicrobial agent. The results were reported as susceptible, intermediate, or resistant for each antimicrobial agent, along with the zone sizes.

RESULTS

Citrobacter Species Isolated from Small Ruminants and Water Sources

Prevalence of Citrobacter species was 96.7% out of 116 samples from small ruminant. Water samples showed the highest rate of Citrobacter species (100%) compared to small ruminants (16.4%). This suggests that water may serve as a significant reservoir for Citrobacter species in the environment (Table 1). Citrobacter isolation is significantly higher in ruminants aged 4 years (66.7%), compared to other age groups (Table 2). Citrobacter isolation was highest in the Uda breed (25.0%) and the Yankasa breed (22.2%), It was lower in Balami (14.3%), Crossbreed (15.4%), and Sahel (14.3%) (Table 3). The small ruminants were categorized into: Caprine (goats), 78 (67.2%), and Ovine (sheep), 38 (32.8%). Citrobacter isolation rates are very similar for both species: Caprine: 16.7% of samples, Ovine: 15.8% of samples, this indicates that Citrobacter species affect goats and sheep at comparable rates (Table 4). Thin ruminants have the highest prevalence of Citrobacter species (35.3%), suggesting they may be more susceptible (Table 1). Male ruminants have a higher prevalence of Citrobacter species (22.4%) compared to female ruminants (11.9%) (Table 6).

Percentage Distribution and Antimicrobial Susceptibility Profile of Citrobacter species

Figure 2 illustrates the percentage distribution of Citrobacter species isolated from small ruminants. This chart highlights the relative prevalence of different Citrobacter species, providing insights into their distribution within the studied population, with each species represented by a segment proportional to its occurrence. Figure 3 represents the antimicrobial susceptibility profile of Citrobacter gilenii isolated from small ruminants and water samples. The chart provides detailed data on the response of the isolate to various antimicrobial agents, categorizing them as susceptible, intermediate or resistant. This information highlights the effectiveness of specific antimicrobials against the isolate and underscores potential challenges in managing infections caused by *Citrobacter gilenii*. Antimicrobials Tested: The following antimicrobial agents were tested for susceptibility against *Citrobacter portucalensis*: CTZ (Cefotaxime), CPX (Ciprofloxacin), PEF (Pefloxacin), OFX (Ofloxacin), AU (Amoxicillin/Clavulanic acid), CEP (Cephalexin), TRX (Trimethoprim/Sulfamethoxazole), CEF (Ceftriaxone). CTZ (Cefotaxime): 0% susceptible, 0% intermediate, 100% resistant. CPX (Ciprofloxacin): 26.3% susceptible, 10.5%

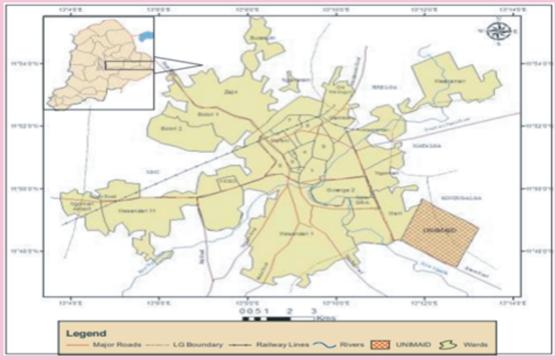

Figure 1. Map of study area.

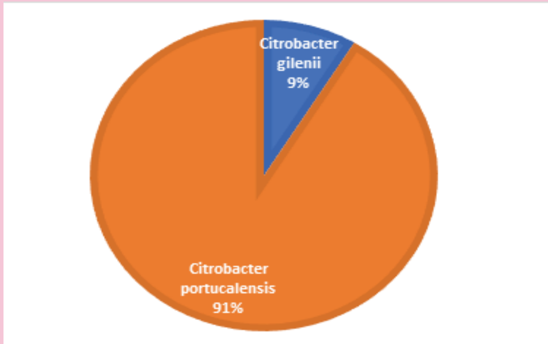

Figure 2. Percentage distribution of Citrobacter species isolated from small ruminants.

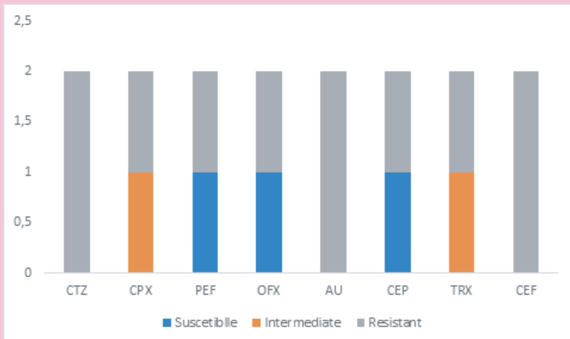

Figure 3. Antimicrobial Susceptibility profile of *Citrobacter gilenii* isolated from small ruminants and water sources

intermediate, 63.2% resistant. PEF (Pefloxacin): 15.8% susceptible, 0% intermediate, 84.2% resistant. OFX (Ofloxacin): 73.7% susceptible, 5.3% intermediate, 21.0% resistant. AU (Amoxicillin/Clavulanic acid): 0% susceptible, 5.3% intermediate, 94.7% resistant. CEP (Cephalexin): 15.8% susceptible, 0% intermediate, 84.2% resistant. TRX (Trimethoprim/Sulfamethoxazole): 5.3% susceptible, 5.3% intermediate, 89.5% resistant. CEF (Ceftriaxone): 0% susceptible, 0% intermediate, 100% resistant. Citrobacter portucalensis shows high resistance to most of the tested antimicrobials, with 100% resistance to CTZ (Cefotaxime) and CEF

Table 1. Distribution of Citrobacter Species

Sample	No sample (%)	Citrobacter species	Other Enterobacteriaece (%)	X^2 (P-Value)
Small ruminants	116 (96.7)	19 (16.4)	97(83.6)	17.451(0.000)
Water	4(3.3)	4 (100)	0(0.0)	
Total	120 (100)	23 (19.2)	97 (80.8)	

Table 2. Age Distribution of Citrobacter Isolated from Small Ruminants

Age	No age (%)	Citrobacter species	Other enterobacteriaceae	X^2(P-Value)
Less than 1	24(20.7)	39(12.5)	21(87.5)	24.888(0.000)
1-2 years	64(55.2)	6(9.4)	58(90.6)	
3 and above	16(13.8)	2(12.5)	14(87.5)	
4 years	12(10.3)	8(66.7)	4(33.3)	
Total	116(100)	19(16.4)	97(83.6)	

Table 3. Breed Distribution of Citrobacter Isolated from Small Ruminants

Breed	No breed (%)	Citrobacter species	Other Enterobacteriaceae	X^2(P-Value)
Balami	21(17.5)	3(14.3)	18(85.7)	18.458(0.002)
Crossbreed	13(10.8)	2(15.4)	11(84.6)	
Sahel	56(46.7)	8(14.3)	48(85.7)	
Uda	8(6.7)	2(25.0)	6(75.0)	
Yankasa	18(15.0)	4(22.2)	14(77.8)	

Table 4. Species Distribution of Citrobacter Isolated from Small Ruminants

Species	No species (%)	Citrobacter species	Other Enterobacteriaceae	X^2(P-Value)
Caprine	78(67.2)	13(16.7)	65(83.3)	0.014(0.905)
Ovine	38(32.8)	6(15.8)	32(84.2)	
Total	116(100)	19(16.4)	97(83.6)	

Table 5. Distribution of Citrobacter Isolated from Small Ruminants Based on Body Condition Score

Body Condition	No body condition(%)	Citrobacter species	Other enterobacteriaceae	X^2(P-Value)
Emaciated	26(22.4)	6(23.1)	20(76.9)	7.752(0.051)
Thin	17(14.7)	6(35.3)	11(63.7)	
Ideal	32(27.6)	3(9.4)	29(90.6)	
Fat	41(35.3)	4(9.8)	37(90.2)	
Total	116(100)	19(16.4)	97(83.6)	

Table 6. Sex Distribution of Citrobacter Isolated from Small Ruminants

Sex	No sex (%)	Citrobacter species	Other Enterobacteriaceae	X^2(P-Value)
Female	67(57.8)	89(11.9)	59(88.1)	2.282(0.131)
Male	49(42.2)	11(22.4)	38(77.6)	
Total	116(100)	19(16.4)	97(83.6)	

Table 7. Antimicrobial Susceptibility Profile of *Citrobacter portucalensis*

Antimicrobials	Susceptible (%)	Intermediate (%)	Resistant (%)
CTZ	0 (0.0)	0 (0.0)	19 (100.0)
CPX	5 (26.3)	2 (10.5)	12 (63.2)
PEF	3 (15.8)	0 (0.0)	16 (4.2)
OFX	14 (73.7)	1 (5.3)	4 (21.0)
AU	0 (0.0)	1 (5.3)	18 (94.7)
CEP	3 (15.8)	0 (0.0)	16 (84.2)
TRX	1 (5.3)	1 (5.3)	17 (89.5)
CEF	0 (0.0)	0 (0.0)	19 (100.0)

Note: CTZ-Cefotaxime, CPX- Ciprofloxacin, PEF – Pefloxacin, OFX-Ofloxacin, AU-Amoxicillin/Clavulanic acid, CEP-Cephalexin, TRX - Trimethoprim/Sulfamethoxazole, CEF- Ceftriaxone.

(Ceftriaxone). High resistance is also observed for PEF (Pefloxacin), CEP (Cephalexin), and TRX (Trimethoprim/-Sulfamethoxazole), with 84.2% and 89.5% resistance, respectively. AU (Amoxicillin/Clavulanic acid) also has a very high resistance rate at 94.7%. Only OFX (Ofloxacin) has a relatively higher susceptibility rate (73.7%), but resistance is still notable at 21.0%. Intermediate Responses: CPX (Ciprofloxacin) shows some moderate effectiveness with 26.3% susceptibility, but still a high level of 63.2% resistance (Table 7).

DISCUSSION

The overall prevalence of Citrobacter species was (19.2%), this reflects a notable presence in small ruminants and water, with a strikingly high prevalence in water samples (100%) compared to small ruminants (16.4%). This reinforces the role of water as a critical reservoir for zoonotic pathogens. Similar studies, such as Meradji *et al.,* (2025) have demonstrated that water sources contaminated with animal feces often harbor Enterobacteriaceae, posing risks for both livestock and humans. Among small ruminants, the age-specific prevalence highlights older ruminants, particularly 4-year-olds (66.7%), as more susceptible. This aligns with Ndegwa *et al.,* (2020), who attributed higher pathogen loads in older animals to prolonged environmental exposure and immune system challenges. The Uda (25%) and Yankasa (22.2%) breeds had higher prevalence of *Citrobacter spp* compared to other breeds, such as Balami and Sahel. This may be due to genetic differences or variations in exposure to contaminated environments, as suggested by Olorunleke *et al.,* (2021). The comparable prevalence in caprine (16.7%) and ovine (15.8%) species indicates that Citrobacter affects goats and sheep at similar rates, consistent with the findings of Ganesan *et al.,* (2024), who reported negligible interspecies differences in pathogen colonization. Thin ruminants exhibited the highest prevalence (35.3%), suggesting that nutritional status and overall health condition significantly influence Citrobacter colonization in small ruminants. Emaciated animals (23.1%) also showed a higher prevalence, which may reflect compromised immunity. Although the statistical analysis (p=0.051) was not significant, these trends warrant attention in management practices. Male ruminants (22.4%) showed higher prevalence than females (11.9%). Though, the difference was not statistically significant (p=0.131). Livestock studies, such as those by Iwu-Jaja *et al.,* (2021), have suggested that behavioral factors, such as grazing habits, may expose males to higher contamination risks.

The study revealed alarming resistance patterns in Citrobacter portucalensis, with 100% resistance to cefotaxime (CTZ) and ceftriaxone (CEF), both critical third-generation cephalosporins. This trend is consistent with global reports on Enterobacteriaceae resistance, as highlighted by Salam eet *al.,* (2023). Amoxicillin/clavulanic acid (AU), cephalexin (CEP), and trimethoprim/sulfamethoxazole (TRX) also exhibited high resistance rates (94.7%, 84.2%, and 89.5%, respectively). Such resistance compromises the efficacy of commonly used veterinary antibiotics and highlights the urgent need for antimicrobial stewardship. Interestingly, ofloxacin (OFX) demonstrated the highest susceptibility (73.7%), suggesting it may remain a viable treatment option. Kocsis *et al.,* (2021) similarly identified fluoroquinolones as effective alternatives for resistant Enterobacteriaceae. The high prevalence of Citrobacter in water sources underscores the need for improved water sanitation practices. Pathogens from water can easily contaminate livestock and agricultural products, creating pathways for zoonotic transmission. Meradji *et al.,* (2025) emphasized that such contamination can contribute to outbreaks of gastrointestinal illnesses in humans, particularly in community where the livestock and human share the same water sources. The antimicrobial resistance patterns observed in this study have direct implications for human health. Resistant strains of Citrobacter may transfer resistance genes to other pathogens, complicating treatment options for infections in both humans and animals.

CONCLUSION

In small ruminants, variations in Citrobacter prevalence were observed across age groups, breeds, and body conditions. Higher prevalence of Citrobacter species was observed with water, Uda, and Yankasa breed. Therefore, highlighting the potential role of water as a significant environmental reservoir. Antimicrobial susceptibility testing revealed alarming levels of resistance in Citrobacter portucalensis isolates. The isolates were resistant to cefotaxime, ceftriaxone, amoxicillin/clavulanic acid, pefloxacin, cephalexin, and trimethoprim/sulfamethoxazole but demonstrated a higher susceptibility to Ofloxacin.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the staff of the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri, for their valuable technical support.

CONFLICT of INTEREST

All authors declare that they have no conflict of interest regarding the content and publication of this manuscript.

FUNDING INFORMATION

No funding was received for this research work

ETHICAL APPROVAL

Ethical approval was not necessary for this work. Informed consent was soughted from animal owners before sample collection.

AUTHORS' CONTRIBUTIONS

The study was conceptualized and designed by EFE and FAL. Data collection was carried out by MA, and data analysis and interpretation were performed by EFE and JJN. Manuscript drafting, critical review, and final proofreading were conducted by EFE and YML.

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