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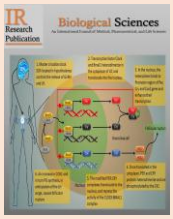

Screening of Extended Spectrum Beta Lactamase (ESBL) Producing *Citrobacter freundii* in Clinical and Animal Samples Isolated from Lagos, Nigeria

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ABSTRACT

The emergence in recent times of extended-spectrum beta-lactamases (ESBLs) producing pathogenic bacteria poses a serious antibiotic management problem. Citrobacter sp., though less commonly isolated are regarded as an emerging nosocomial multidrug-resistance (MDR) pathogen. The main objective of this study is to screen for ESBLs in *Citrobacter freundii* isolates. Over a period of two months, 50 clinical samples were collected from patients attending tertiary health institutions in Lagos state, and 45 animal samples were collected from cattle and poultry farms giving a total of 95 samples. The samples were cultured by standard procedures. Isolates were identified using standard methods. Antimicrobial susceptibility testing was performed on all isolates by Clinical and Laboratory Standards Institute (CLSI) guidelines. The isolates were further screened for ESBL production using double disk diffusion synergy techniques. 4 of the 48 Isolates were positive for *Citrobacter freundii* (8.33%). Other bacterial pathogens identified included *Citrobacter koseri* (14.58%), *Escherichia coli* (33.33%), *E. coli* O157 (10.42%), *Klebsiella oxytoca* (20.83%), Staphylococcus spp. (12.5%), and *Pseudomonas aeruginosa* (2.08%). Antimicrobial susceptibility testing revealed that high antibiotic resistance was observed in ceftazidime (100%), cefuroxime (100%), augmentin (100%), and low in ciprofloxacin (50%). Susceptibility was observed in gentamicin (100%), nitrofurantoin (100%), cefixime (75%) and ofloxacin (75%). None of the strains of *Citrobacter freundii* isolates showed ESBL production. Gentamicin and nitrofurantoin were found to be effective treatment option for *Citrobacter freundii*-associated infections. This study provides critical insights into antimicrobial resistance patterns, aiding in the development of better infection control strategies.

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Introduction

The increasing rate of antimicrobial resistance has become a worldwide problem predominantly caused by the family of the Enterobacteriaceae. One of the main contributors to nosocomial and community-acquired illnesses nowadays is Enterobacteriaceae. Hospital-acquired urinary tract infections (UTIs) represent a significant health risk since they are the most prevalent nosocomial infection, accounting for 35–40% of all infections [1]. Additionally, unchecked and careless use of antibiotics has led to an increase in the prevalence of antimicrobial resistance among urinary infections globally. Furthermore, certain bacteria that were discovered seldom have since become well-known infections linked to healthcare. An opportunistic infection that frequently appears in hospitalised patients' urine is *Citrobacter freundii*. This species can cause diarrhea, septicaemia, meningitis, respiratory and urinary tract infections.

Extended-spectrum cephalosporins are currently utilised to treat infections caused by Citrobacter sp., a bacteria that is becoming increasingly problematic due to its multidrug resistance [2]. Early in the 1980s, third-generation cephalosporins were brought into clinical use, which was hailed as a significant advance in the battle against antibiotic-resistant bacteria caused by beta-lactamases. The higher frequency of beta-lactamases in novel hosts prompted the development of these cephalosporins. Third-generation cephalosporins had the significant benefit of having fewer nephrotoxic effects than aminoglycosides and polymyxins, in addition to being effective against the majority of organisms that produce beta-lactamases [3]. In 1983, it was first shown that beta-lactamases encoded on plasmids might hydrolyse the extended-spectrum cephalosporins [4]. Thus, the term "extended-spectrum beta-lactamases" (ESBLs) was introduced to describe these novel enzymes.

"The ESBLs were the first instance in which beta-lactamase-mediated resistance to beta-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes," [5] wrote in the first significant review of ESBLs in 1989. With the exception of cephamycins and carbapenems, extended-spectrum beta-lactamases (ESBLs) inactivate practically all beta-lactam antibiotics, posing serious therapeutic challenges. After the first ESBL was discovered, TEM and sulfhydryl variable (SHV) derived ESBLs developed and spread around the world until the late 1900s. The majority of ESBL-producing Enterobacteriaceae infections have been reported to be hospital-acquired, linked to healthcare institutions [7], and increasingly isolated from individuals with community-onset disorders and extended-care facilities [8].

Given the increasing antimicrobial resistance in Enterobacteriaceae, we hypothesize that *Citrobacter freundii* isolates from clinical and animal sources in Lagos, Nigeria, exhibit a significant level of ESBL production, contributing to treatment challenges. This study aims to screen *Citrobacter freundii* isolates from clinical and animal samples for ESBL production and evaluate their antimicrobial resistance patterns. The findings will provide insights into the prevalence of ESBL-producing *Citrobacter freundii* and support the development of improved antimicrobial agents in Nigeria.

Material and Methods

Study area

Samples were collected from various locations across Lagos State, Nigeria, including both clinical and animal sources. Clinical samples were obtained from Randle General Hospital (Latitude 6.50832°N, Longitude 3.35745°E) and Lagos State University Teaching Hospital (LASUTH) (N 6° 27' 11.0016", E 3° 23' 44.9988"). Animal samples were collected from poultry farms at Odo-Eran, Agege Abattoir (Latitude 6°35′N, Longitude 3°45′E), Ojo Barracks area poultry, and Sagamu poultry (Latitude 6° 27' 59.99" N, Longitude 3° 10' 60.00" E). Ethical clearance for this study was obtained from the LASUTH Ethical Review Committee (LASUTH/EC/2019/045).

Sample collection

A total of ninety-five (95) samples were collected over period of two (2) months from both hospitals and animal farm. Thirty (30) blood samples were collected from patients using sterile syringes by venopuncture and transferred into a vacuum ethylene diamine tetra acetic acid (EDTA) bottles while 20 urine samples were collected into sterile universal bottles and all samples were labeled accordingly. Animal droppings from cattle, chickens such as broilers, layers, cockerel and turkey were collected. 20 cattle feaces and 25 poultry feaces were collected using sterile universal bottles and labeled accordingly. The samples were transported to the laboratory in cold condition by placing them in a cold box; the collected samples were refrigerated and stored at 4°C until analysis was carried out.

Isolation and identification of bacterial pathogens

Selective media and initial culturing

Each sample was cultured on selective and differential media to facilitate the isolation of Gram-negative and Gram-positive pathogens. Blood and urine samples were directly inoculated onto MacConkey Agar and Eosin Methylene Blue (EMB) Agar, which support the selective growth of Gram-negative bacteria [9]. For fecal samples, a 1:10 dilution in sterile normal saline was prepared before inoculation on the selective media to reduce background flora and enhance isolation of the target pathogens. MacConkey Agar was employed to distinguish lactose fermenters, such as *Escherichia coli* and Klebsiella spp., from non-fermenters. EMB Agar was further used to confirm lactose fermenters by the presence of metallic sheen, indicative of *E. coli* colonies [10]. Mannitol Salt Agar (MSA) was used specifically for Staphylococcus spp., which tolerate high salt concentrations [11]. Pseudomonas Agar was employed to enhance isolation of *Pseudomonas aeruginosa*.

Enrichment and colony selection

Each blood samples were inoculated into Brain heart infusion broth which was prepared in McCartney bottles in ratio 1:9 which were prepared so to enrich the growth of the organisms present in the blood samples and then incubated at 37℃ for 18-24 hours after incubation sub-culture was made on a solid media. After overnight incubation, positive plates were observed for colonial morphological characteristics (sizes, colour, texture, etc.). The district colonies were inoculated on Nutrient agar slant and incubated at 37℃ for 18-24 hours for further identification and preservation.

Species differentiation and biochemical testing

To confirm the identity of the bacterial isolates, biochemical tests were performed as follows:

- *Citrobacter freundii* and *Citrobacter koseri*: Both species were initially screened on MacConkey Agar for lactose fermentation. Further differentiation was done using Triple Sugar Iron (TSI) agar, Citrate utilization test, and Urease test (Christensen's urea agar) [12].
- *Escherichia coli* and *E. coli* O157: Positive lactose-fermenting colonies on EMB were subjected to the Indole test, with additional testing for sorbitol fermentation on Sorbitol MacConkey Agar to identify *E. coli* O157 specifically [13].
- *Klebsiella oxytoca*: Identified by its mucoid colonies on MacConkey Agar, positive citrate utilization, and lack of motility [11].
- Staphylococcus spp.: Colonies that grew on MSA were Gram-stained, and catalase tests were performed to confirm genus identity. Coagulase tests were subsequently conducted to identify pathogenic strains such as *Staphylococcus aureus* [10]. *Pseudomonas aeruginosa*: Identified on Pseudomonas Agar based on colony pigmentation and verified by positive oxidase and catalase tests [11].

Antibiotic susceptibility testing

A standardized Kirby-Bauer disk diffusion method utilizing the Mueller Hinton Agar (MHA) (Oxoid, Basingstoke, Hampshire, England) plate technique was used to to assess the antibiotic susceptibility profiles of each bacterial isolate according to the recommendations of the CLSI guidelines [14]. The following antibiotics were incorporated including Ceftazidime CAZ (30µg), Cefuroxime CRX (30µg), Gentamicin GN (10µg), Cefixime CXM (5µg), Ofloxacin OFX (5µg), Augmentin AU (30µg), Nitrofurantoin N (300µg) and Ciprofloxacin CPX (5µg). Zones of inhibition were measured after 18-24 hours of incubation at 37°C, and results were interpreted following CLSI [14].

Phenotypic detection of extended-spectrum beta-lactamase (ESBL) production

The double-disk synergy test, as established by [15], was used to phenotypically corroborate the discovery of extended-spectrum beta-lactamases (ESBL). On a Mueller Hinton Agar plate swabbed with test isolate, a 30µg disc of amoxicillin-clavulanate and a 30µg disc of each third-generation cephalosporin test antibiotic (Ceftazidime and Cefotaxime) were positioned 20 mm from the centre to detect synergy. It was determined that ESBL production was positive when the edge of the cephalosporin inhibition zone clearly extended towards the amoxicillin-clavulanate disc [16] [17].

Results

It was observed that poultry dropping had the highest number of isolated pathogens with 16(33.33%) followed by cow dung 11(22.92), blood 11(22.92) and urine 10(20.83%) as shown in **Figure 1**.

Figure 2 shows the morphological characteristics of *C. freundii* on Xylose Lysine Deoxycholate agar (A), its resistance and susceptibility to different antibiotics (B and C), and ESBL double-disk synergy test results (D and E). While *C. freundii* isolates exhibited a negative ESBL reaction (D), the control strain showed a positive ESBL reaction (E).

Antimicrobial susceptibility test was done for all the 4(4.21%) *C. freundii* isolates which revealed that

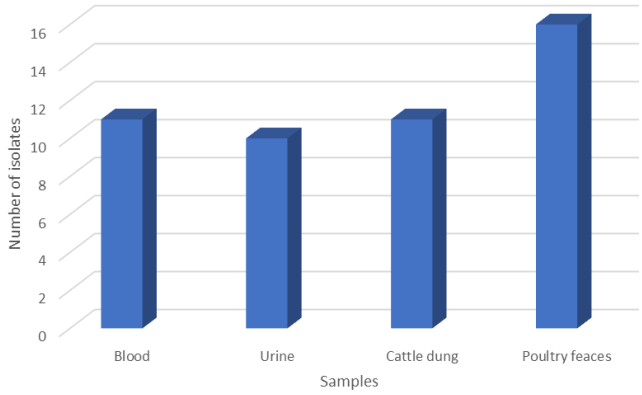

Figure 1. Bar chart showing the number of isolates in each sample

(100%), cefixime (75%) and ofloxacin (75%) as shown in **Figure 3**. Two resistance patterns were exhibited by *C. freundii* with CAZ, CRX, and AU, followed by CAZ, CRX, CXM, OFX, AU, CPR and CAZ, CRX, AU, CPR, in *C. freundii* strains as shown in **Figure 3**. In this study, all strains of *Citrobacter freundii* obtained from both clinical and animal isolates were all ESBL negative as shown in **Table 1**.

Table 1 presents the distribution of *Citrobacter freundii* isolates in different sample types based on ESBL production. Among the samples tested, only poultry droppings showed positive isolates for ESBL-producing *C. freundii*, with a total of three isolates. A total of 4(8.33%) of the 48 positive

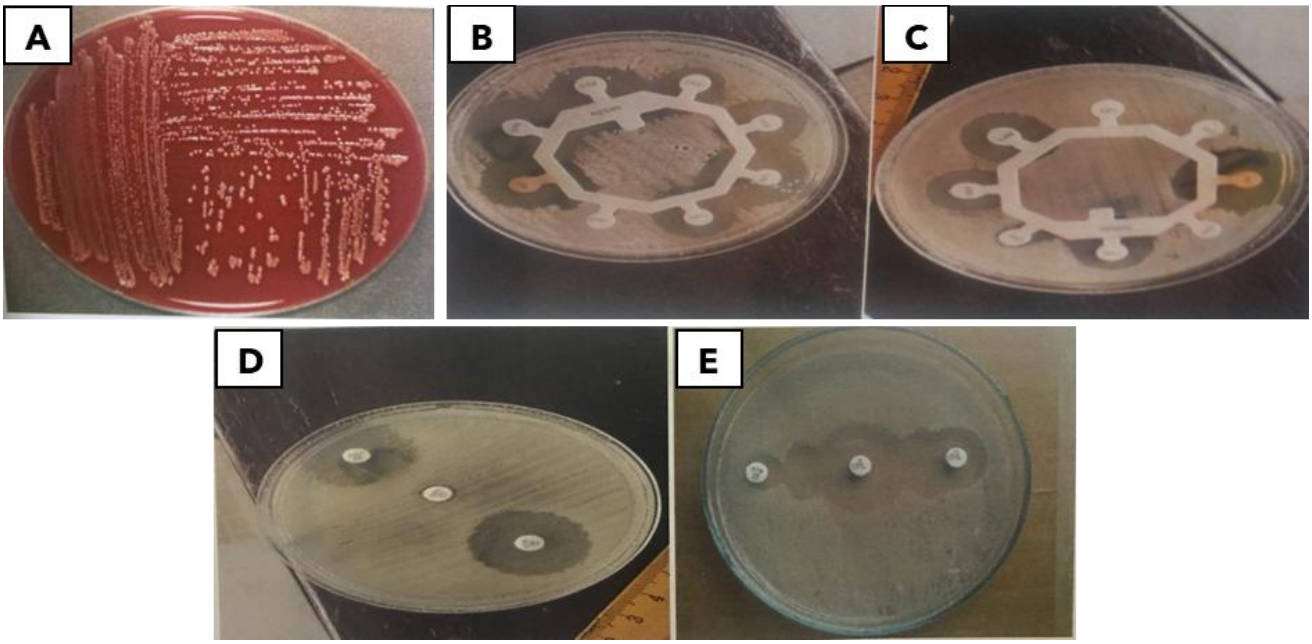

Figure 2. Morphological Characteristics, Antibiotic Susceptibility, and ESBL Screening of *Citrobacter freundii*. A: *Citrobacter freundii* on Xylose Lysine Deoxycholate agar. B and C: *Citrobacter freundii* resistant and susceptible to different antibiotics. D: ESBL antibiotics double disk synergy negative for *Citrobacter freundii*. E: ESBL antibiotics double disk synergy positive for control strain.

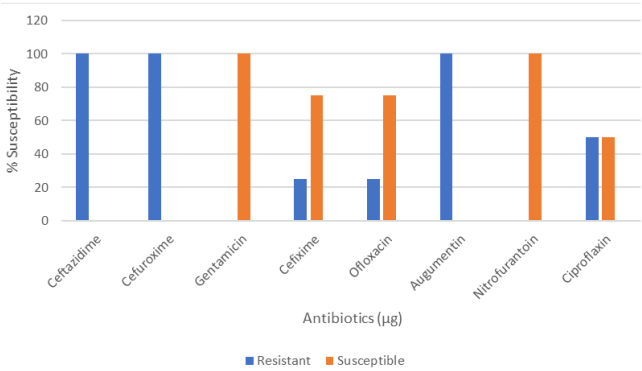

Figure 3. Bar chart showing the percentage of resistance and susceptibility of antibiotics to *Citrobacter freundii*.

high antibiotic resistance was observed in ceftazidime (100%), cefuroxime (100%), augmentin (100%) and ciprofloxacin (50%). Susceptibility was observed in gentamicin (100%), nitrofurantoin

samples were found to be *Citrobacter freundii* positive isolates, and all other isolates were identified by various biochemical tests as shown in **Table 4**.

Table 1. Distribution of *Citrobacter freundii* in Samples According to ESBL.

Type of Sample	Number of Positive Isolate	ESBL negative
Blood	1	0
Stool	0	0
Cattle dung	0	0
Poultry dropping	3	0
Total	4	0

Table 2 provides a comprehensive overview of bacterial pathogens which include *Citrobacter freundii* 4(8.33%), *Citrobacter koseri* 7(14.58%), E.

Table 2. Distribution of Bacterial Pathogens Isolated from Samples.

Type of Sample	Blood	Urine	Cattle dung	Poultry dropping	Total	% Distribution
Number of Sample	30	20	20	25	95	100
Number of Positive Sample	11	10	11	16	48	50.53
Citrobacter freundii	1	0	0	3	4	8.33
Citrobacter koseri	3	0	2	2	7	14.58
E. coli	3	6	0	7	16	33.33
E. coli 0157:H7	1	4	0	0	5	10.42
Klebsiella oxytoca	2	2	2	4	10	20.83
Staphylococcus sp.	0	0	6	0	6	12.5
Pseudomonas aeruginosa	1	0	0	0	1	2.08

Table 3. Multi-drug Resistance Pattern Exhibited by *Citrobacter freundii*.

Antibiotics	**P08**	**P10**	**P11**	**B39**
Ceftazidime	I	I	I	R
Cefuroxime	I	R	R	I
Gentamicin	S	S	S	S
Cefixime	S	S	I	S
Ofloxacin	S	S	R	S
Augmentin	R	R	R	R
Nitrofurantoin	S	S	S	S
Ciprofloxacin	S	S	R	I
Total number of resistances	1	2	4	2

Strains: P08, P10, P11, B39; S = Susceptible; P = Poultry; I = Intermediate; B = Blood; R = Resistant.

Table 4. Summary of result on biochemical characterization of the bacterial isolates showing gram test and morphology.

S/N	Gram	Morph	Slant	Butt	H_2S	Gas	Lactose	Manitol	Sorbitol	Urease	Indole	Motility	Catalase Ctest	COAG	Citrate	Oxidase	Isolates
1	-	Y	K	A	+++	+	+	+	+	V	-	+	+	-	+	-	*Citrobacter freundii*
2	-	Y	K	A	++	+	+	+	+	V	+	+	+	+	+	-	*Citrobacter koseri*
3	-	P	A	A	-	+	+	+	-	-	+	+	+	+	-	-	*E. coli*
4	-	P	A	A	-	+	+	+	+	-	+	+	+	+	-	-	*E. coli* 0157: H7
5	-	P	A	A	-	+	+	+	+	+	-	-	+	-	+	-	*Klebsiella* sp.
6	+	G	K	A	-	-	+	+	+	+	-	-	+	+	+	-	*Staphylococcus* sp.
7	-	C	K	K	-	+	-	+	-	+	-	V	+	-	+	-	*Pseudomonas aeruginosa*

+ (positive); - (negative); Y = Yellow; P = Pink; G = Golden yellow; C = Colourless; V = Varies; K = Alkaline; A = Acidic; COAG = Coagulase; Morph = Morphology.

coli 16(33.33%), *E. coli* 0157:H7 5(10.42%) and *Klebsiella oxytoca* 10(20.83%), Staphylococcus sp. 6(12.5%) and *Pseudomonas aeruginosa* 1(2.08%) isolated from the various sample types. It includes the total number of samples tested and the number of positive samples for each type of pathogen. Among the 30 blood samples tested, 11 were positive for bacterial pathogens, with *Citrobacter freundii* being detected in one sample.

Table 3 illustrates the multi-drug resistance pattern exhibited by *Citrobacter freundii* isolates against

different antibiotics. The strains are labeled as P08, P10, P11, and B39, with their corresponding susceptibility (S), intermediate (I), or resistance (R) status indicated for each antibiotic tested.

Table 4 summarizes the biochemical characterization of bacterial isolates, including their Gram stain, morphology, and results for various biochemical tests. *Citrobacter freundii* shows a negative result for urease production and positive results for motility and catalase activity.

Discussion

A number of illnesses, including UTIs, wound infections, gastrointestinal infections, septicaemia, and meningitis, can be caused by Citrobacter sp., particularly *C. freundii*, which is acknowledged as an emerging opportunistic pathogen, particularly in hospital settings and immunocompromised patients [18]. The discovery that *C. freundii* frequently exhibits resistance to several classes of antibiotics, such as third-generation cephalosporins and extended-spectrum beta-lactams, has coincided with this emergence, indicating that both clinical and animal strains may serve as a reservoir of antimicrobial resistance determinants [19]. A startlingly high percentage of *C. freundii* isolates from UTIs were found to be highly multi-drug resistance in a recent survey of outpatients in Bo, Sierra Leone [18].

In this study, Citrobacter sp. were surveyed from different patients with different health conditions and also different genders of cattle and chickens to provide better understanding of antimicrobial resistance especially extended spectrum cephalosporins to *Citrobacter freundii*. The distribution of various bacterial pathogens across different sample types, including blood, urine, cattle dung, and poultry droppings where highlighted. The detection of Citrobacter spp., *Escherichia coli*, and Klebsiella spp. in multiple sample types corroborates findings from similar studies demonstrating the wide distribution of these pathogens in both clinical and environmental settings [20]. Additionally, the presence of multidrug-resistant strains, such as *E. coli* 0157:H7, underscores the urgent need for antimicrobial stewardship efforts to combat the rising threat of antibiotic resistance [21].

According to reports, 0.5–36% of Citrobacter sp. worldwide have ESBLs [22]. Eighty percent of hospitalised patients' Citrobacter isolates in India produced ESBLs [23]. *Citrobacter freundii* was screened for antimicrobial resistance to different drugs. Remarkable high rates of resistance of the *Citrobacter freundii* to antibiotics ceftazidime (100%) which are third generations cephalosporins and this implies that the strains of *Citrobacter freundii* isolated from sample such as blood (1%) and poultry droppings (3.2%) in Nigeria are strains of Citrobacter that are resistant to third generation cephalosporins which conforms to the study by [24] in which it was reported that beta-lactams antibiotics such as ceftazidime, cefuroxime were ineffective against the studied bacteria. One cephalosporin-resistant strain of *Citrobacter freundii* LMO7/10, which is not an ESBL producer, was isolated from a urine sample in a June 2010 case presentation involving a 47-year-old woman who had been receiving private consultation assistance due to a history of grade II cystocele and recurrent UTIs since December 2009. Conventional biochemical testing was used to identify the strain, and the API 20E system was used to confirm it [25].

This study also observed high susceptibility to antibiotics; gentamicin (100%) and nitrofurantoin (100%) which conforms to the study done by [26]. in Tanzania. This shows that there is generally low resistance of Citrobacter strains to gentamicin which are strong aminoglycoside that are used to treat several types of bacterial infections which include UTIs sepsis, meningitis which are common infections caused by *Citrobacter freundii* and also nitrofurantoin. Multiple antibiotic resistance suggested that the isolate must have originated from environment where antibiotics are misused or often used as therapeutic measures in humans [27].

The phenotypic confirmation of ESBL producing *Citrobacter freundii* was detected negative. Extended spectrum β-Lactamases producing Enterobacteriaceae (ESBL-E) are becoming more common in hospitals and the general public around the world [28]. Their prevalence differs by nation, area, or even medical facility [29]. The Incidence of ESBL producing *Citrobacter freundii* in Nigeria has been less studied in contrary to Enterobacteriaceae such as *E. coli*, Salmonella sp., Klebsiella sp. Many developed countries have reported high case of ESBL producers in contrast to developing countries which can happen with the constant misuse of drugs in these countries. Although this rate of ESBL isolates is greater than the rate seen in recent European studies, which ranged from 2.5% to 5.1% in France [31] and 2.9% in Sweden [32], it is comparable to that reported in the Kingdom of Saudi Arabia (8.9%) [30]. An estimated 25% of the Enteriobacteriaceae family produces ESBLs, according to studies done in Ethiopia [33]. These prevalence rates are high as opposed to our study which may be related to variation in drug management policies or follow other control programs. Furthermore, ESBL prevalences across Latin America, the Middle East, Europe, and the South Pacific ranged from roughly 10 to 35% [34]

[35]. In 2011, however, almost 40% of clinical isolates from Asia produced ESBL [36]. Therefore, strains of extended spectrum beta-lactamases are less detected in *Citrobacter freundii* in Nigeria.

The strength of this study is that no strains of ESBL producing *Citrobacter freundii* can be found in Nigeria. The emergence of multidrug-resistant strains, particularly those producing ESBLs, is a global concern due to its implications for patient management and public health [36]. The findings are consistent with previous reports documenting the spread of ESBL-producing Citrobacter species in clinical and environmental settings, emphasizing the importance of effective infection control measures and antimicrobial surveillance programs [17].

Conclusion

This study revealed the presence of *Citrobacter freundii* in poultry sample as well as *C. freundii*-associated bacteraemia in human. None of the strains of *Citrobacter freundii* was ESBL producers. Gentamicin and Nitrofurantoin were found to be effective drugs for *Citrobacter freundii*-associated infections in this study. Further comprehensive study is essential to substantiate these findings. Constant surveillance is essential to determine the true prevalence of this pathogen in our environment. A need for prudent use of antibiotics to prevent emerging ESBL producing strain *C. freundii* and good environmental hygiene should be practiced to prevent the emergence of this pathogen.

Contribution of authors

Agubata Z.C: Conceptualization, experimental design, laboratory work, data analysis, manuscript writing and editing, Sodunke D.E.: sample collection, field work and report, Moro D.D.: Supervision, manuscript revision, and validation. Olusola A.O., Ukhureigbe O.M., Nzomiwu C.T., Okoh F.N.: Data interpretation, methodology validation, critical review, and proofreading. Ebi E.S.: Data interpretation. All authors have read and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Data availability

The original data presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

References

1. Kalsi, J.M.; Arya, P.; Wilson, P.; Mundy, A. Hospital-acquired urinary tract infection. Int. J. Clin. Pract. 2003, 57, 388–391.
2. Rawat, D.; Nair, D. Extended-spectrum β-lactamases in Gram Negative Bacteria. J. Glob. Infect. Dis. 2010, 2(3), 263–274.
3. Pachori, P.; Gothalwal, R.; Gandhi, P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes Dis. 2019, 6(2), 109–119.
4. Knothe, H.; Shah, P.; Krcmery, V.; Antal, M.; Mitsuhashi, S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection 1983, 11(6), 315–317.
5. Philippon, A.; Labia, R.; Jacoby, G. Extended-spectrum beta-lactamases. Antimicrob. Agents Chemother. 1989, 33, 1131–1136.
6. Pitout, J.D.D.; Gregson, D.B.; Church, D.L.; Elsayed, S.; Laupland, K.V. Community-wide outbreaks of clonally related CTX-M-14 β-lactamase-producing *Escherichia coli* strains in the Calgary health region. J. Clin. Microbiol. 2005, 43(6), 2844–2849.
7. Falagas, M.E.; Karageogopoulos, D.E. Extended-spectrum beta lactamases-producing organisms. J. Hosp. Infect. 2009, 73(4), 345–354.
8. Rodríguez-Baño, J.; Pascual, A. Clinical significance of extended-spectrum beta-lactamases. Expert Rev. Anti-Infect. Ther. 2008, 6(5), 671–683.
9. Baron, E.J.; Peterson, L.R.; Finegold, S.M. Bailey and Scott's Diagnostic Microbiology. Mosby: St. Louis, MO, 1994.
10. Winn, W.; Allen, S.; Janda, W.; Koneman, E.; Procop, G.; Schreckenberger, P. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Lippincott Williams & Wilkins: Philadelphia, PA, 2006.
11. Forbes, B.A.; Sahm, D.F.; Weissfeld, A.S.

Bailey and Scott's Diagnostic Microbiology, 12th ed. Mosby Elsevier: St. Louis, MO, 2007.
12. Greenwood, D.; Slack, R.C.B.; Barer, M.R.; Irving, W.L. Medical Microbiology, 18th ed. Elsevier Health Sciences, 2012.
13. Farmer, J.J. Enterobacteriaceae: Introduction and Identification. In Manual of Clinical Microbiology, 8th ed.; Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L., Pfaller, M.A., Eds.; ASM Press: Washington, DC, 2003; pp. 649–669.
14. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 26th ed. CLSI Document M100-S26. CLSI: Wayne, PA, 2016.
15. Jarlier, V.; Nicolas, M.H.; Fournier, G.; Philippon, A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. 1988, 10(4), 867–878.
16. Paterson, D.L.; Bonomo, R.A. Extended-Spectrum β-Lactamases: a Clinical Update. Clin. Microbiol. Rev. 2005, 18(4), 657–686.
17. Nandagopal, B.; et al. Frequency of extended spectrum β-lactamase producing urinary isolates of Gram-negative bacilli among patients seen in a multispecialty hospital in Vellore district, India. Indian J. Med. Microbiol. 2015, 33(2), 282–285.
18. Leski, T.A.; et al. High prevalence of multidrug resistant Enterobacteriaceae isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone. BMC Infect. Dis. 2016, 16, 167.
19. Stoesser, N.; et al. Genome sequencing of an extended series of NDM-producing Klebsiella pneumoniae isolates from neonatal infections in a Nepali hospital characterizes the extent of community- versus hospital-associated transmission in an endemic setting. Antimicrob. Agents Chemother. 2014, 58(12), 7347–7357.
20. Cantón, R. Antibiotic resistance genes from the environment: a perspective through newly identified antibiotic resistance mechanisms in the clinical setting. Clin. Microbiol. Infect. 2009, 15(Suppl. 1), 20–25.
21. Cattoir, V.; Leclercq, R. Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce? J. Antimicrob. Chemother. 2013, 68(4), 731–742.
22. Ranjan, K.P.; Ranjan, N. Citrobacter: An emerging health care associated urinary pathogen. Urol. Ann. 2013, *5*(4), 313–314.
23. Majewski, P.; et al. Emergence of a multidrug-resistant Citrobacter freundii ST8 harboring an unusual VIM-4 gene cassette in Poland. Int. J. Infect. Dis. 2017, 61, 70–73.
24. Paterson, D.L.; Bonomo, R.A. Extended-Spectrum β-Lactamases: a Clinical Update. Clin. Microbiol. Rev. 2005, *18*(4), 657–686.
25. Metri, B.C.; Jyothi, P.; Peerapur, B.V. Antibiotic resistance in Citrobacter spp. isolated from urinary tract infection. Urol. Ann. 2013, 5(4), 312–313.
26. Ahmed, T.; et al. Phenotypic and Genotypic Characteristics of Antimicrobial Resistance in Citrobacter freundii Isolated from Domestic Ducks (Anas platyrhynchos domesticus) in Bangladesh. Antibiotics 2023, 12(4), 769.
27. Arens, S.; Verbist, L. Differentiation and susceptibility of Citrobacter isolates from patients in a university hospital. Clin. Microbiol. Infect. 1997, 3(1), 53–57.
28. Enoch, D.A.; et al. Epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae in a UK district hospital; an observational study. J. Hosp. Infect. 2012, 81(4), 270–277.
29. Al-Zarouni, M.; et al. Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the United Arab Emirates. Med. Princ. Pract. 2008, 17(1), 32–36.
30. Sangare, S.A.; et al. Prevalence of ESBL-producing Enterobacteriaceae isolated from blood cultures in Mali. J. Infect. Dev. Ctries. 2016, 10(10), 1059–1064.
31. Søraas, A.; et al. Risk factors for community-acquired urinary tract infections caused by ESBL-producing Enterobacteriaceae: a case-control study in a low prevalence country. PLoS One 2013, 8(7), e69581.
32. Amsalu, A.; et al. Antimicrobial resistance pattern of bacterial isolates from different clinical specimens in Southern Ethiopia: A three year retrospective study. J. Bacteriol. Res. 2017, 9(1), 1–8.
33. Prestinaci, F.; Pezzotti, P.; Pantosti, A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog. Glob. Health 2015, 109(7), 309–318.
34. Schwaber, M.J.; et al. High levels of antimicrobial coresistance among extended-spectrum-beta-lactamase-producing Enterobacteriaceae. Antimicrob. Agents Chemother. 2005, 49(5), 2137–2139.
35. Lukac, P.J.; Bonomo, R.A.; Logan, L.K. Extended-spectrum β-lactamase-producing Enterobacteriaceae in children: old foe, emerging threat. Clin. Infect. Dis. 2015, 60(9),

1389–1397.
36. Weiner, L.M.; et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect. Control Hosp. Epidemiol. 2016, 37(11), 1288–1301.