Sahel Journal of Life Sciences FUDMA 3(4): 450-456, 2025

Sahel Journal of Life Sciences FUDMA (SAJOLS)
December 2025 Vol. 3(4): 450-456
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2025-0304-52*

Research Article

Prevalence and Antibiotic Susceptibility Profiles of *Klebsiella* spp. in African Catfish (*Clarias gariepinus*) in Maiduguri, Borno State, Northeastern Nigeria

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ABSTRACT
The emergence and spread of antibiotic-resistant bacteria in aquatic systems presents a growing threat to public health, food safety, and sustainable fish farming worldwide. This study investigated the prevalence and antibiotic susceptibility profiles of *Klebsiella* species isolated from African catfish (*Clarias gariepinus*) in Maiduguri, Borno State, Northeastern Nigeria. A total of 300 catfish samples were randomly collected from three major markets, and both skin and intestinal specimens were analysed using standard microbiological and biochemical methods. *Klebsiella* spp. were detected in 50% of all samples, with a significantly higher prevalence in intestinal samples (72%) compared to skin samples (28%) ($p < 0.001$), while no significant differences were observed across market locations. Antibiotic susceptibility testing using the Kirby–Bauer disc diffusion method revealed high sensitivity to ofloxacin (99.3%), streptomycin (96.0%), and chloramphenicol (95.3%), but moderate susceptibility to ampicillin, ciprofloxacin, and nalidixic acid. Alarmingly, all the isolates were resistant to amoxicillin–clavulanic acid, and over 90% were resistant to pefloxacin, indicating that the pathogen has developed substantial resistance to these agents. Multi-drug resistance (MDR) was present in 20% of isolates, with some exhibiting resistance to up to seven antibiotics. These results highlight the widespread presence of Klebsiella and multi-drug resistance in catfish, reinforcing public health concerns and the need for improved antibiotic management and surveillance in aquatic environments.

Keywords: Antibiotic; Biochemical tests; *Clarias gariepinus; Klebsiella;* Prevalence

Citation: Mohammed, A., Nur, Z.S., Ngoshe, I.Y., Saidu, A.S., Mohammed, S., Idris, A.A., Jidda, D., Bukar, A.T., Shettima, S., Mustapha, F.A. & Goniri, B. (2025). Prevalence and Antibiotic Susceptibility Profiles of *Klebsiella* spp. in African Catfish (*Clarias gariepinus*) in Maiduguri, Borno State, Northeastern Nigeria. *Sahel Journal of Life Sciences FUDMA*, 3(4): 450-456. DOI: *https://doi.org/10.33003/sajols-2025-0304-52*

INTRODUCTION
Aquatic environments play a critical role in the maintenance and dissemination of various bacterial pathogens, including those of public health significance. Among these, members of the genus *Klebsiella* have emerged as important opportunistic

pathogens, not only in clinical settings but also in environmental reservoirs such as freshwater fish ((Cho *et al.,* 2023; Araújo *et al.,* 2025). *Klebsiella* species, particularly *Klebsiella pneumoniae*, are increasingly recognized as significant pathogens in both human and aquatic animal health, with notable implications for food safety and public health. In fish, *Klebsiella* spp. are part of the normal microbiota but can act as opportunistic pathogens, causing disease outbreaks in aquaculture and contributing to spoilage and reduced shelf life of fish products (Cortés-Sánchez *et al*., 2025; Oliviera *et al.*, 2017). *Klebsiella* can be transmitted from fish to humans through direct contact, handling, or consumption of contaminated products. The zoonotic potential is heightened by the bacterium's ability to acquire and disseminate resistance genes, making infections harder to treat and control. Environmental factors, such as water quality and farm management practices, play a crucial role in the spread of *Klebsiella* and other Enterobacteriaceae (Dong *et al*., 2022). *Klebsiella* spp. are Gram-negative, non-motile, rod-shaped bacteria commonly found in water, soil, plants, and the mucosal surfaces of animals and humans. They can cause a range of infections, including pneumonia, septicaemia, urinary tract infections, and wound infections (Gupta *et al*., 2011; Canada Public Health Agency, 2025).

The rise in antibiotic resistance among aquatic bacterial isolates is a growing concern, especially in developing countries where regulatory oversight of antibiotic usage in aquaculture and agriculture is often limited (Akinbowale *et al*., 2006; Cabello *et al*., 2013). Aquatic animals, such as wild catfish (*Clarias gariepinus*), can act as reservoirs and vectors for resistant bacteria, which may be transmitted to humans through direct contact, consumption of raw or undercooked fish, or environmental pathways (Akinbowale *et al*., 2006; Miranda *et al*., 2013). In Nigeria, catfish is a major dietary protein source, and its wild populations, particularly in regions like Maiduguri, Borno State, are subject to environmental pressures that may facilitate the proliferation and spread of pathogenic and resistant bacteria (Adedeji *et al*., 2011; Grema *et al*., 2015).

The emergence of multidrug-resistant (MDR) *Klebsiella* strains in aquaculture environments is a growing concern, as these bacteria can be transmitted to humans through the food chain, posing risks of hard-to-treat infections (Dong *et al*., 2022). Antimicrobial resistance in *Klebsiella* spp. is particularly alarming due to the organism's ability to acquire and disseminate resistance genes, especially those conferring resistance to beta-lactam antibiotics, quinolones, and aminoglycosides (Prestinaci *et al*., 2015; Pitout *et al*., 2015). The detection of multidrug-resistant strains in aquatic systems suggests a potential risk for horizontal gene transfer to other pathogenic bacteria, thereby complicating treatment outcomes in both veterinary and human medicine (Miranda *et al*., 2013).

Despite these concerns, there is a paucity of data regarding the occurrence and antibacterial susceptibility profiles of *Klebsiella* spp. in wild catfish within Maiduguri, Borno State. Most available studies have focused on clinical or farmed fish isolates, leaving a significant knowledge gap concerning wild fish populations in this region (Adedeji *et al*., 2011; Akinbowale *et al*., 2006). Understanding the prevalence of *Klebsiella* spp. and their resistance patterns in wild catfish is essential for assessing potential public health risks and guiding appropriate interventions.

Therefore, this study aims to investigate the occurrence and antibacterial susceptibility profiles of *Klebsiella* spp. isolated from wild catfish in Maiduguri, Borno State. The findings are expected to provide valuable baseline data for public health authorities, policymakers, and stakeholders involved in fisheries and food safety.

MATERIALS AND METHODS

Study Area

This research was carried out in notable fish markets in Maiduguri, the capital of Borno State in Nigeria's Northeast area. Maiduguri is situated at 11°50' N latitude and 13°09' E longitude, with a population of over 1.9 million, rendering it the largest city in Borno State. The state encompasses 69,435 square kilometres, or approximately 7.69% of Nigeria's total land area. It shares international boundaries with Niger, Chad, and Cameroon, as well as internal boundaries with Adamawa, Yobe, and Gombe states. Essential water bodies such as Lake Alau, Lake Chad, and the Ngadda River sustain the region's fisheries and fish commerce, rendering these marketplaces exemplary for analysis (NaijaPedia, 2024; Britannica Editors, 2025).

Study Design and Sampling Techniques

A cross-sectional study design was implemented. Three principal markets (Tashan Bama Market, Monday Market, and Custom Market) in the study area were purposively chosen based on the presence of fish selling establishments. A total of 300 catfish samples were randomly acquired in batches of 10 each buy over a thirty-day period from June to October 2025.

Isolation and Identification of the Isolates

Freshwater catfish skin surface and intestinal contents were aseptically swabbed with sterile swab sticks moistened with sterile normal saline and properly labelled. The specimens were immediately placed in ice-packed boxes and transported to the Zoonoses and Food Safety Laboratory, Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, and processed within four hours of collection,

In the laboratory, the specimens were inoculated into nutrient broth for enrichment. The enriched broth cultures were streaked onto nutrient agar plates and incubated aerobically at 37 °C for 24 hours for the development of bacterial colonies. Distinct greyish-white, moist colonies suggestive of *Klebsiella* species were further sub-cultured using the streak plate technique on MacConkey agar and Eosin Methylene Blue (EMB) agar plates and incubated at 37 °C for 24 hours. Sub-culturing was repeated until pure cultures were obtained

Presumptive *Klebsiella* isolates were identified based on colonial morphology, Gram staining reaction, and a series of standard biochemical tests. Biochemical characterization included catalase, indole, citrate utilization, urease, triple sugar iron (TSI), methyl red (MR), and Voges–Proskauer (VP) tests. The biochemical reactions were interpreted according to standard microbiological procedures as described by Cheesbrough (2017).

Antibiotic Susceptibility Testing

Pure cultures of confirmed isolates were emulsified in sterile physiological saline. Turbidity was adjusted to the 0.5 McFarland standard corresponding to approximately 1.5×10^8 CFU/mL. The Kirby–Bauer disc diffusion method was used on Mǔller–Hinton agar. The agar surface was swabbed in three directions to ensure uniform lawn growth. Commercial antibiotic discs were applied using sterile forceps. Antibiotic discs (ciprofloxacin, ofloxacin, pefloxacin, nalidixic acid, streptomycin, tetracycline, ampicillin, augmentin, chloramphenicol, and cefepime) were applied to the agar surface with sterile forceps. The plates were subsequently incubated at 35–37°C for 18–24 hours, after which the zones of inhibition were quantified in millimetres using a calliper. The results were classified as susceptible, intermediate, or resistant according to CLSI (2025) breakpoints, except for pefloxacin, which referenced the EUCAST (2025) breakpoints. (Table 1).

Data Analysis

The data acquired from this investigation were analysed utilizing SPSS version 20 (IBM, Amonk, NY: IBM Corp.). Positive results were computed in proportion, and variables were evaluated for associative evidence using Chi-square, yielding Odds ratios and their corresponding 95% confidence intervals. Any probability less than or equal to alpha (0.05) was deemed statistically significant.

Table 1. Antibiotic susceptibility breakpoints for Enterobacterales

Antibiotics	Susceptibility Breakpoints			Protocol
	Sensitive	Intermediate	Resistant	
Ofloxacin	≥ 16	13-15	≤ 12	CLSI (2025)
Pefloxacin	≥ 24	NA	< 24	EUCAST (2025)
Ciprofloxacin	≥ 26	22-25	≤ 21	CLSI (2025)
Augmentin	≥ 18	14-17	≤ 13	CLSI (2025)
Cefepime	≥ 25	NA	≤ 18	CLSI (2025)
Streptomycin	≥ 15	12-14	≤ 11	CLSI (2025)
Ampicillin	≥ 17	14-16	≤ 13	CLSI (2025)
Tetracycline	≥ 15	12-14	≤ 11	CLSI (2025)
Chloramphenicol	≥ 18	13-17	≤ 12	CLSI (2025)
Nalidixic acid	≥ 19	14-18	≤ 13	CLSI (2025)

EUCAST, European Committee on Antimicrobial Susceptibility Testing

RESULTS

Morphological and biochemical characteristics of isolates

The isolates were Gram-negative, non-motile rods producing large, mucoid, lactose-fermenting pink colonies on MacConkey agar and mucoid colonies without metallic sheen on EMB agar. Other characteristics are presented in Table 2.

Prevalence of *Klebsiella* in catfish

Out of the 300 fish samples, 150 were positive for *Klebsiella,* giving an overall prevalence of 50.0%.

In addition (Table 3), Tashan Bama Market, Monday Market and Custom Market had 47.0%, 51.0% and 52.0% prevalence rates, respectively. The prevalence of *Klebsiella* was significantly higher in intestinal samples (72%) compared with skin samples (28%), indicating a strong association between sample type and bacterial detection (χ^2 = 56.33, p < 0.001). The odds of isolating *Klebsiella* from the intestine were approximately 6.6 times higher than from the skin. In contrast, the prevalence across the three sampled locations (47–52%) did not differ significantly (χ^2 = 0.56, p = 0.756), and the odds ratios were approximately equal to 1.

Table 2: Morphological and biochemical characteristics of bacterial Isolates

Test Category	Characteristics/Test	Reaction
Morphological characteristics	Appearance on MacConkey agar	Pink, large, mucoid lactose-fermenting colonies
	Appearance on EMB agar	Mucoid colonies without metallic sheen
	Gram reaction	Gram-negative rods
Biochemical characteristics	Indole	−
	Methyl Red	−
	Voges–Proskauer	+
	Citrate	+
	Catalase	+
	Urease	+
	TSI reaction	A/A + Gas, No H_2S

Table 3. Distribution of *Klebsiella* in catfish samples

Variable	Total No. Tested	No. Positive (%)	OR (95% CI)	Chi-square	*P* – value
Tashan Bama Market	100	47 (47.0)	Ref.	-	-
Monday Market	100	51 (51.0)	0.85 (0.49-1.48)	0.18	0.671
Custom Market	100	52 (52.0)	0.82(0.47-1.43)	0.32-	0.574
Skin	150	42 (28.0)	Ref.	56.33	0.001
Intestine	150	108 (72.0)	6.61 (3.99-10.95)	-	-
Overall	300	150 (50%)	-	-	-

No., number; %, percent; OR, odds ratio; CI, confidence interval; Ref, reference category

Antibiotic susceptibility profiles exhibited by *Klebsiella* isolates from catfish in Maiduguri

The antibiotic susceptibility patterns of the 150 *Klebsiella* isolates are summarized in Table 4. Overall, marked variability in susceptibility was observed across the tested antibiotics. Ofloxacin (OFX) demonstrated the highest activity, with 149 isolates (99.3%) classified as sensitive and only one isolate (0.7%) showing intermediate susceptibility; no resistance to OFX was detected. Similarly, streptomycin (STEP) and clindamycin (CLM) showed high effectiveness, with sensitivities of 96.0% and 95.3%, respectively, and resistance rates below 5%.

Moderate susceptibility was observed for nalidixic acid (NAL), ampicillin (AMP), and ciprofloxacin (CIP). For NAL, 99 isolates (66.0%) were sensitive, 46 (30.7%) intermediate, and 5 (3.3%) resistant. Ampicillin showed a comparable pattern, with 66.0% sensitivity, 28.0% intermediate susceptibility, and 6.0% resistance. Ciprofloxacin demonstrated 64.0% sensitivity, a relatively high proportion of intermediate isolates (34.0%), and a low resistance rate of 2.0%.

In contrast, high levels of resistance were recorded for pefloxacin (PEF) and amoxicillin–clavulanic acid (AUG). Resistance to PEF was observed in 137 isolates (91.3%), with only 13 isolates (8.7%) remaining sensitive. All isolates (100.0%) were resistant to AUG, indicating complete loss of efficacy of this antibiotic against the *Klebsiella* isolates studied. Tetracycline (TET) showed mixed activity, with 118 isolates (78.7%) sensitive and a notable resistance rate of 20.7%.

Multi-drug resistance pattern of *Klebsiella* isolates from catfish in Maiduguri

The resistance burden per isolate, defined as the number of antibiotics to which each isolate was resistant, is presented in Table 5. Resistance to two antibiotics was the most frequent pattern, observed in 111 isolates (74.0%). Resistance to three antibiotics was detected in 24 isolates (16.0%), while resistance to a single antibiotic occurred in 8 isolates (5.3%).

A smaller proportion of isolates exhibited resistance to four or more antibiotics. Specifically, one isolate (0.7%) was resistant to four antibiotics, one isolate (0.7%) to five antibiotics, four isolates (2.7%) to six antibiotics, and one isolate (0.7%) to seven antibiotics. No isolate showed resistance to eight or nine antibiotics.

Overall, 30 isolates (20.0%) were resistant to three or more antibiotics, indicating the presence of multidrug resistance within the study population, although high-level resistance involving a large number of antibiotics was relatively uncommon.

Table 4. Antibiotic susceptibility pattern of *Klebsiella* isolated from catfish in Maiduguri

Antibiotics	Number (%)		
	Sensitive	Intermediate	Resistant
Ampicillin	99 (66.0)	42 (28.0)	9 (6.0)
Tetracycline	118 (78.7)	1 (0.7)	31 (20.7)
Streptomycin	144 (96.0)	0 (0.0)	6 (4.0)
Ciprofloxacin	96 (54.0)	51 (34.0)	3 (2.0)
Pefloxacin	13 (8.7)	NA	137 (91.3)
Ofloxacin	149 (99.3)	1 (0.7)	0 (0.0)
Nalidixic acid	99 (66.0)	46 (30.7)	5 (3.3)
Chloramphenicol	143 (95.3)	6 (4.0)	1 (0.7)
Augmentin	0 (0.0)	0 (0.0)	150 (100.0)

Table 5. Multi-drug resistance pattern of *Klebsiella* isolated from catfish in Maiduguri

No. Antibiotic	Resistance to Number Antibiotics	
	Number of Isolate	Proportion (%)
1	8	5.3
2	111	74.0
3	24	16
4	1	0.7
5	1	0.7
6	4	2.7
7	1	0.7
8	0	0
9	0	0
Overall	150	100

DISCUSSION

This study demonstrates a high prevalence (50%) of *Klebsiella* spp. in African catfish (*Clarias gariepinus*) marketed in Maiduguri, Borno State, with a significantly greater frequency from intestinal samples (72%) than from skin (28%). This intestinal predominance is consistent with the ecology of *Klebsiella* as an enteric bacterium and reinforces prior reports that the gut is a primary reservoir for this genus in aquatic animals (Adedeji *et al*., 2011). The lack of significant differences in prevalence among the three major markets suggests that *Klebsiella* colonization in catfish is widespread within the region, likely reflecting shared environmental exposures and management practices across market sources.

The observed prevalence agrees with previous Nigerian studies reporting frequent detection of *Klebsiella* in both wild and cultured catfish populations. For example, Adedeji *et al*. (2011) found *Klebsiella* spp. in over 40% of catfish sampled in Ekiti State, while Usman *et al*. (2021) reported similar rates in Oyo States. However, Grema *et al*. (2015) reported a much lower prevalence of 4.5% in Maiduguri, Borno State. These data collectively indicate that *Klebsiella* is a consistent and ubiquitous component of the microbial flora in Nigerian catfish, both in wild and cultured settings.

The antimicrobial susceptibility profiles observed in this study further corroborate the growing concern over microbial resistance in aquatic environments. The high susceptibility of isolates to ofloxacin (99.3%), streptomycin (96%), and chloramphenicol (95.3%) is encouraging, suggesting these agents may remain effective therapeutic options locally. However, the moderate susceptibility to ampicillin, ciprofloxacin, and nalidixic acid agrees with findings from Adeshina *et al*. (2016), who also reported partial resistance to these agents among fish-associated *Klebsiella* isolates in southwestern Nigeria.

Alarmingly, all isolates were resistant to amoxicillin–clavulanic acid, and over 90% were resistant to pefloxacin. This mirrors the results of Usman *et al*. (2021), who observed widespread β-lactam resistance among Enterobacteriaceae (including *Klebsiella*) from catfish, and supports the hypothesis that intensive and indiscriminate use of β-lactams and certain fluoroquinolones in Nigerian aquaculture and human medicine has driven the selection of resistant strains (Adeshina *et al*., 2016; Usman *et al*., 2021). The universal resistance to amoxicillin–clavulanic acid underscores a worrisome trend of declining efficacy for this crucial drug combination.

The multidrug resistance (MDR) pattern detected, 20% of isolates resistant to three or more antibiotics, echoes broader studies in Nigeria. Usman *et al*. (2021) found that all *Klebsiella* isolates in their survey of catfish farms were MDR, while Okon (2022) highlighted MDR Enterobacteriaceae as a major problem in Nigerian aquaculture and food animals. The presence of isolates resistant to up to six or seven antibiotics in the present study, though infrequent, signals the potential for escalation and horizontal gene transfer events that could further complicate treatment and containment.

Some inter-study variability was noted in susceptibility profiles, particularly for tetracycline and selected fluoroquinolones. For instance, Adeshina *et al*. (2016) reported a slightly higher proportion of tetracycline-sensitive *Klebsiella* isolates than observed here, likely reflecting differences in local antibiotic usage patterns, sampling periods, or the specific susceptibility testing methodologies employed. These discrepancies highlight the complex interplay between local prescribing practices, antibiotic availability, and resistance evolution.

Overall, the findings from Maiduguri fit within the continuum of antimicrobial resistance (AMR) trends previously described in Nigerian fish and food-animal studies. *Klebsiella* spp. in catfish show high resistance to multiple antibiotic classes, particularly β-lactams and some fluoroquinolones, and bear a significant MDR burden. This underscores the urgent need for coordinated antimicrobial stewardship, routine surveillance, and public education to mitigate the further spread of resistant pathogens through the food chain and aquatic environments.

CONCLUSION

In summary, this study highlights a substantial prevalence of *Klebsiella s*pp. in African catfish from Maiduguri, with a marked predominance in intestinal samples. The findings reveal a concerning pattern of multidrug resistance, particularly against commonly used antibiotics such as amoxicillin–clavulanic acid and pefloxacin and confirm that resistance profiles observed in this region are in line with previous reports from other parts of Nigeria. The persistence of multidrug-resistant *Klebsiella* in catfish not only poses a potential risk to public health through the food chain, but also underscores the urgent need for improved antimicrobial stewardship and regulatory oversight in aquaculture. Continued surveillance and targeted interventions are essential to curb the spread of resistant pathogens and safeguard both animal and human health in Nigeria.

A key limitation of this research is the reliance on phenotypic identification and susceptibility testing, without molecular characterization of isolates. The absence of genotypic analysis restricts the ability to determine specific resistance genes, clonal relationships, or the potential for horizontal gene transfer. Future studies incorporating molecular methods will be essential for a more comprehensive understanding of the epidemiology and mechanisms of resistance among *Klebsiella* spp. in aquatic systems.

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