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Household wastewater as a sentinel for community-level antimicrobial resistance: a cross-sectional study in Gombe, Nigeria

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Antimicrobial resistance (AMR) represents a critical global health challenge, with projections suggesting 10 million annual deaths by 2050. Environmental transmission routes, particularly through wastewater, remain understudied despite their significant role in resistance development and spread. This study investigated household wastewater as a sentinel for community-level AMR patterns in Gombe, Nigeria. A cross-sectional study was conducted between December 2024 and February 2025, collecting 320 household wastewater samples across seven districts in Gombe using multi-stage sampling techniques. Bacterial isolation followed standard conventional methods. Antibiotic susceptibility testing was performed using the disc diffusion method. Extended-spectrum beta-lactamase (ESBL) production was confirmed using double-disc synergy tests, and PCR detected key resistance genes in selected isolates. Microbiological analysis yielded 402 bacterial isolates, with 81% classified as multidrug-resistant (MDR). MDR prevalence across districts ranged from 60.3% to 95.9% ($p < 0.01$). Gram-negative bacteria predominated, with *Escherichia coli* (32.7%), *Klebsiella pneumoniae* (19.2%), and *Pseudomonas aeruginosa* (11.2%) being the most common. ESBL production was detected in 54% of tested isolates. MDR isolates demonstrated resistance to approximately 8 antibiotics (median), while non-MDR isolates showed resistance to only 1–2 antibiotics. Molecular analysis revealed a high prevalence of clinically significant resistance genes, with *blaCTX-M* detected in 100% of tested isolates. This study demonstrates household wastewater's value as a community-level antimicrobial resistance indicator. The high prevalence of MDR bacteria (81%) highlights significant environmental reservoirs that could contribute to community AMR transmission. Wastewater-based epidemiology can serve as a cost-effective complement to traditional clinical surveillance, especially in resource-limited settings.

Keywords Antimicrobial resistance, Wastewater surveillance, Environmental stewardship, Multi-drug resistance, Extended-spectrum beta-lactamase, Carbapanemase

Abbreviations

AMR	Antimicrobial Resistance
MDR	Multidrug-Resistant
ESBL	Extended-Spectrum Beta-Lactamase
PCR	Polymerase Chain Reaction
CLSI	Clinical Laboratory Standards Institute
EUCAST	European Committee on Antimicrobial Susceptibility Testing
LGA	Local Government Area
MAR	Multiple Antibiotic Resistance
LMICs	Low-and Middle-Income Countries
AST	Antimicrobial Susceptibility Testing

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DDST Double Disc Synergy Test

Antimicrobial resistance (AMR) has emerged as one of the most pressing global health challenges of the 21 st century, with conservative estimates projecting 10 million annual deaths by 2050 if current trends continue unchecked[1]. This crisis transcends traditional healthcare boundaries, threatening the foundation of modern medicine and jeopardising routine medical procedures that depend on effective antimicrobial therapy[2].

While clinical antimicrobial stewardship programs have received substantial attention and resources, environmental transmission routes of AMR, particularly through wastewater systems, remain significantly understudied despite mounting evidence of their critical role in resistance development, maintenance, and dissemination[3,4]. This knowledge gap is particularly pronounced in low- and middle-income countries (LMICs) such as Nigeria, where inadequate wastewater treatment infrastructure and poor sanitation systems create ideal conditions for the proliferation and spread of resistant organisms[5,6].

Conventional AMR surveillance strategies have traditionally focused on clinical isolates obtained from healthcare settings, creating significant blind spots in our understanding of community-level resistance dynamics and environmental reservoirs[7,8]. The One Health framework, which recognises AMR as an interconnected phenomenon spanning human, animal, and environmental domains, emphasises the critical importance of environmental surveillance[9]. However, environmental monitoring remains substantially underutilised as both a surveillance tool and a stewardship intervention[8,10].

Household wastewater represents a complex microbial ecosystem containing human excreta, pharmaceutical residues, personal care products, and organic waste materials. This mixture serves as an ideal reservoir for resistant bacteria and provides optimal conditions for horizontal gene transfer events that facilitate the spread of resistance determinants[11]. When improperly managed or inadequately treated, household wastewater introduces resistant organisms directly into environmental ecosystems, where they can interact with indigenous microbial communities and facilitate the exchange of genetic material across diverse microbiomes[12,13].

The concept of wastewater-based epidemiology has gained significant traction in recent years, particularly following its successful application in COVID-19 surveillance programs worldwide[14,15]. This approach offers several advantages over traditional clinical surveillance methods, including the ability to capture population-level trends, detect asymptomatic infections, and provide early warning signals for emerging health threats[16,17]. In the context of AMR, wastewater surveillance offers unique opportunities to monitor community-level resistance patterns, track the effectiveness of stewardship interventions, and identify environmental hotspots of resistance development.

Nigeria, Africa's most populous country with over 200 million inhabitants, faces significant challenges in antimicrobial stewardship and resistance monitoring. The country's healthcare system is characterised by limited laboratory capacity, inadequate surveillance infrastructure, and widespread availability of antimicrobials without prescription[2]. Additionally, poor sanitation infrastructure and limited wastewater treatment capacity create ideal conditions for environmental AMR amplification and dissemination.

This study addresses the critical knowledge gap regarding environmental AMR surveillance in Nigeria by investigating household wastewater as a sentinel system for community-level antimicrobial resistance patterns in Gombe. Gombe, located in northeastern Nigeria, represents a typical Nigerian state with mixed urban and rural populations, limited healthcare infrastructure, and challenges in waste management that are representative of broader Nigerian contexts.

We hypothesised that household wastewater in Gombe would harbor high levels of multidrug-resistant bacteria reflective of community-level antimicrobial resistance patterns, and that these could be effectively detected and characterised using standard microbiological and molecular methods. Specifically, we sought to answer three key research questions[1]: What is the prevalence and distribution of MDR bacteria in household wastewater across different districts[2]? What resistance mechanisms and genes are present in these environmental isolates[3]? Can household wastewater surveillance serve as a practical and cost-effective tool for monitoring community-level AMR in resource-limited settings?

To address these questions, the primary objectives of this research were threefold: first, to quantify the prevalence of multidrug-resistant bacteria in household wastewater across different districts in Gombe; second, to characterise the resistance profiles of isolated organisms through phenotypic and molecular analytical methods; and third, to evaluate the potential of wastewater surveillance as a practical tool for AMR monitoring and stewardship that could extend antimicrobial stewardship programs beyond traditional clinical settings.

Materials and methods

Study design and setting

This cross-sectional study was conducted between December 2024 and February 2025 in Gombe State, northeastern Nigeria. The study focused on households within the 11 wards of Gombe Local Government Area (LGA), which comprises a total of 46,112 households distributed across various districts[18]. Gombe LGA was selected as the study site due to its representative demographic profile, a mix of urban and rural communities, and the typical wastewater management challenges it shares with other parts of Nigeria. A multi-stage random sampling technique was employed to select specific communities and households, ensuring representation of different socio-economic and geographic characteristics. Each household served as a unit for examining wastewater samples, associated practices, and potential public health impacts. Seven districts were randomly selected from the state's administrative divisions, representing both urban and rural settings. Within each district, proportional sampling was used to ensure that each district's representation in the sample is proportional to its population size. Two wards were randomly selected from each of the seven districts by balloting and simple random sampling technique was applied to select the number of households in each ward to be included in the study. This minimises selection bias and ensures geographical representativeness.

Sample size determination

To determine the required sample size for this research, Cochran's formula was applied. This formula is widely used in prevalence studies to ensure that the sample is large enough to provide statistically valid results[19,20]. The formula is as follows:

$$n_0 = \frac{Z^2 + P + (1 - P)}{e^2}$$

Where:

- Z is the Z-score corresponding to the desired confidence level (1.96 for 95% confidence).
- P is the estimated prevalence of MDR bacteria in wastewater, which in this case is 75% (0.75) (Yusuf et al., 2023).
- 1 – P1 - P1 – P represents the proportion without the phenomenon, calculated as 0.25.
- e is the margin of error, set at 5% (0.05).

Substituting these values into the formula:

$$n_0 = \frac{(1.96)^2 \times 0.75 \times 0.25}{(0.05)^2}$$

This results in an initial sample size of 288 households.

To account for potential non-response, a 10% non-response rate was included. The adjusted sample size was calculated as follows:

$$n_{adjusted} = n_0 \div (1 - \text{non} - \text{responserate})$$

Substituting the values:

$$n_{adjusted} = 288 \div (1 - 0.10) = 288 \div 0.90 = 320$$

Ethical considerations

Ethical approval for this study was obtained from the Gombe State Environmental Protection Agency before sample collection (approval number: ES/GOSEPA/ADM/S/38/V.I). The study protocol adhered to international guidelines for environmental research and ensured that all sampling activities were conducted in compliance with local regulations and community consent procedures.

Sample collection

A total of 320 household wastewater samples were collected across seven districts over the three-month study period. Sample collection was standardised to minimise temporal variation, with collections conducted during consistent periods (early morning hours) to capture similar household activities. Approximately 20 ml of wastewater sample was collected from each household's wastewater into wide-mouthed sterile plastic containers with screw cap tops and corked tightly. The containers were labelled with date, time and sites of collection, and transported to the laboratory within 4 h of collection using appropriate cold chain procedures to maintain sample integrity.

Bacterial isolation and identification

Bacterial isolation was performed using standard conventional microbiological methods as described by Cheesbrough (2009)[21]. First, the water samples were incubated in sterile peptone water overnight and the enriched samples were then streaked on to differential selective media namely, Eosin Methylene blue agar and MacConkey agar. After 24 h of incubation, colonies were sub-cultured onto freshly prepared solidified nutrient agar and incubated for 24 h at 37 °C to get pure and distinct colonies. This was repeated several times until satisfactory pure isolates were obtained.

The bacterial isolates were tentatively identified using morphological characteristics, cellular and biochemical tests. Given that household wastewater typically contains enteric bacteria of public health significance, we anticipated isolating members of the Enterobacteriaceae family (*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Salmonella* spp., *Shigella* spp., *Serratia* spp.) and other gram-negative organisms such as *Pseudomonas aeruginosa*, which are commonly associated with fecal contamination and known for harbouring antimicrobial resistance genes.

Morphological characteristics were observed for each bacterial colony after 24 h of growth. The appearance of each colony of each isolate on the media was noted and the characteristics observed include cell shape, elevation, edge, consistency, colony surface and pigmentation. To confirm the cellular morphology and classification of the isolates, the Gram staining technique, with 100X optical microscopy visualisation, was used to determine the shape, arrangement and classification of isolates.

A standardised panel of biochemical tests was selected to differentiate between the expected gram-negative organisms. The tests included: motility (to distinguish motile organisms like *E. coli*, *Proteus* spp., and *Salmonella* spp. from non-motile ones like *Klebsiella* spp. and *Shigella* spp.); indole production (to differentiate indole-positive *E. coli* and *Proteus vulgaris* from indole-negative organisms); urease test (to identify *Proteus* spp. and some *Klebsiella* spp.); citrate utilisation (to distinguish *Klebsiella* spp., *Enterobacter* spp., and *Salmonella* spp. from *E. coli* and *Shigella* spp.); oxidase test (to differentiate oxidase-positive *Pseudomonas aeruginosa* from oxidase-negative Enterobacteriaceae); methyl red test (to distinguish *E. coli* from *Enterobacter* spp. and *Klebsiella* spp.); and lactose fermentation (to differentiate lactose fermenters like *E. coli* and *Klebsiella* spp. from non-lactose fermenters like *Pseudomonas* spp., *Proteus* spp., *Salmonella* spp., and *Shigella* spp.). The combination of these tests allowed for reliable differentiation and identification of the target organisms. The results were compared with Bergey's Manual of Determinative Bacteriology[22] to ensure accurate species identification.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed on Mueller-Hinton agar (MHA) plates using the disc diffusion method (Kirby-Bauer technique) according to guidelines established by the National Committee for Clinical Laboratory Standards Institute (CLSI) 2024[23]. A standardised panel of antimicrobial agents was selected to represent major antibiotic classes commonly used in clinical practice and available in the Nigerian healthcare system.

Bacterial suspensions equivalent to 0.5 McFarland standard were prepared and evenly distributed on MHA plates. The commercially available gram negative antibiotic discs containing the following antibiotics: cefotaxime (CTX, 25 µg), cefuroxime (CXM, 30 µg), gentamicin (GEN, 10 µg), ceftriaxone (CRO, 30 µg), imipenem (IMP, 10 µg), ampiclox (ACX, 10 µg), ofloxacin (OFX, 5 µg), amoxicillin clavulanate (AUG, 30 µg), cefepime (ZEM, 5 µg), nitrofurantoin (NF, 300 µg), nalidixic acid (NA, 30 µg) and levofloxacin (LEV, 5 µg) (www.celtechproducts.com) were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and incubated at 37 °C for 18 h. Zones of inhibition after incubation were observed and the interpretation was made using susceptibility breakpoints of CLSI (2024). The diameters of the zone of inhibition around the discs were measured to the nearest millimetre using a metre rule and the isolates were classified as sensitive, intermediate or resistant.

Multidrug resistance definition and analysis

Multidrug resistance (MDR) was defined according to international consensus criteria as resistance to at least one agent in three or more antimicrobial categories[24,25]. This definition ensures consistency with global surveillance programs and facilitates comparison with other studies.

The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate using the formula: MAR index = Number of antibiotics to which the isolate is resistant/Total number of antibiotics tested[26]. MAR index values greater than 0.2 indicate high-risk sources of contamination and significant antimicrobial pressure.

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

ESBL production was investigated using the double-disc synergy test (DDST), following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2006 guidelines[27]. This method involves placing discs containing ceftriaxone, ceftazidime, and cefotaxime at a standardised distance from an amoxicillin-clavulanate disc. The presence of ESBL is indicated by the expansion of the inhibition zone around beta-lactam antibiotics toward the amoxicillin-clavulanate disc, creating a characteristic "keyhole" appearance.

Quality control was maintained using standard reference strains: *Escherichia coli* ATCC 25,922 (ESBL-negative control) and *Klebsiella pneumoniae* ATCC 700,603 (ESBL-positive control).

Suspected carbapenemase-resistant *Pseudomonas aeruginosa* isolates were selected based on preliminary identification using standard biochemical tests and confirmed by antimicrobial susceptibility testing (AST), which revealed resistance to carbapenems such as imipenem.

Molecular analysis

Polymerase Chain Reaction (PCR) was employed to detect key antimicrobial resistance genes in selected isolates representing three species (*E. coli*, *Klebsiella pneumoniae and Pseudomonas aeruginosa*) and resistance phenotypes. DNA extraction was performed using standard protocols, followed by PCR amplification targeting clinically significant resistance genes[28].

The molecular analysis focused on detecting key antimicrobial resistance genes, including extended-spectrum β-lactamase (ESBL) genes (*blaTEM*, *blaSHV*, *blaCTX-M*) and carbapenemase genes (*blaKPC*, *blaVIM*, *blaNDM*). These genes were selected based on their clinical importance and reported prevalence in similar geographical regions.

PCR amplification was performed under optimised conditions specific to each gene target. Reference strains previously confirmed to harbor the respective genes were used as positive controls, while nuclease-free water served as the negative control. Amplification products were separated on 1.5% agarose gels stained with ethidium bromide and visualised under UV illumination. The presence of target genes was confirmed based on the expected amplicon sizes.

Statistical analysis

Statistical analysis was performed using R Statistics version 4.4.0. Descriptive statistics were calculated for all variables, including frequencies, percentages, medians, and interquartile ranges as appropriate for data distribution.

Comparative analyses between groups (MDR vs. non-MDR isolates, different districts, different species) were performed using appropriate statistical tests. Chi-square tests were used for categorical variables, while Mann-

Whitney U test (Wilcoxon rank-sum test) or the Kruskal-Wallis test were employed for continuous variables depending on data distribution and number of groups compared.

Correlation analyses were performed to examine relationships between variables such as the number of resistant antibiotics and MAR index values. Statistical significance was set at $p < 0.05$ for all analyses, and confidence intervals were calculated where appropriate.

Results

Bacterial isolation and identification

The analysis of 320 household wastewater samples collected across seven districts in Gombe State yielded a total of 402 bacterial isolates, representing an average of 1.26 isolates per sample. On MacConkey agar, pink colonies indicated lactose fermenters such as *Escherichia coli*, *Klebsiella spp.*, and *Enterobacter spp.*, while colourless colonies were characteristic of non-lactose fermenters including *Proteus spp.*, *Pseudomonas aeruginosa*, *Salmonella spp.*, and *Shigella spp.*. Similarly, metallic green sheen on Eosin Methylene Blue (EMB) agar confirmed the presence of *E. coli*.

Gram staining revealed that all isolates were Gram-negative bacilli, consistent with members of the family *Enterobacteriaceae* and other related genera. Subsequent biochemical characterisation using standard tests (motility, indole, methyl red, citrate, oxidase, urease, and lactose fermentation) enabled differentiation and tentative identification of the isolates. Based on the combination of reactions and comparison with Bergey's Manual of Determinative Bacteriology, the predominant isolates were identified.

The summarised biochemical reactions of these isolates are presented in Table 1. All isolates showed distinct patterns consistent with their known biochemical profiles, confirming the reliability of the identification procedure.

Species distribution

Among the identified isolates, *Escherichia coli* emerged as the most prevalent species, accounting for 32.7% (131/402) of all bacteria isolated. *Klebsiella pneumoniae* represented the second most common species at 19.2% (77/402), followed by *Pseudomonas aeruginosa* at 11.2% (45/402). Other significant species included *Salmonella spp.* at 11% (44/402), *Klebsiella oxytoca* at 6.5% (26/402), *Enterobacter spp.* at 5.5% (22/402), *Proteus mirabilis* at 5% (20/402), *Proteus vulgaris* at 4.2% (17/402), Shigella spp. at 4% (16/402) while *Serratia marcescens* represented less than 1% of the total isolates (3/402). The frequency and percentage distribution of all bacterial species isolated is shown in Fig. 1.

Multidrug resistance prevalence

The analysis revealed a concerning prevalence of MDR among wastewater isolates. Of the 402 bacterial isolates analysed, 326 (81.1%) were classified as MDR according to the established criteria of resistance to at least one agent in three or more antimicrobial categories.

Widespread multi-drug resistance across all bacterial species was examined, with resistance rates consistently exceeding 60% (Fig. 2). While *Serratia marcescens* showed complete resistance and *Proteus mirabilis* demonstrated the highest rates among larger sample sizes, the overall pattern indicated uniformly high MDR prevalence throughout the bacterial population. There was no significant difference in resistance patterns between species ($p = 0.13$), as illustrated in Fig. 2.

District-level variation in MDR prevalence

Significant geographical variation in MDR prevalence was observed across the study region, with a notable 24.7% point difference between the highest and lowest performing districts (Fig. 3). MDR prevalence ranged from 60.3% to 95.9% across the seven districts studied. Statistical analysis confirmed highly significant inter-district differences ($p < 0.01$), as shown in Fig. 3.

Bacterial Specie	Gram Stain Reaction	Lactose Fermentation	Citrate Utilisation	Methyl Red	Oxidase	Motility	Indole Production	Urease
E. coli	-	+	-	+	-	+	+	-
Enterobacter spp	-	+	+	-	-	+	-	-
Klebsiella oxytoca	-	+	-	+	-	+	+	W
Klebsiella pneumoniae	-	+	-	+	-	+	+	-
Proteus mirabilis	-	-	+	+	-	+	-	+
Proteus vulgaris	-	-	+	+	-	+	+	+
Pseudomonas aeruginosa	-	-	+	-	+	+	-	+
Salmonella spp	-	-	-	+	-	+	-	-
Serratia marcescens	-	+	-	-	-	+	-	+
Shigella spp	-	-	-	+	-	-	-	-

Table 1. Biochemical characterization and identification of bacterial Isolates. **Key:** + = Positive reaction; – = Negative reaction; **W** = Weak reaction.

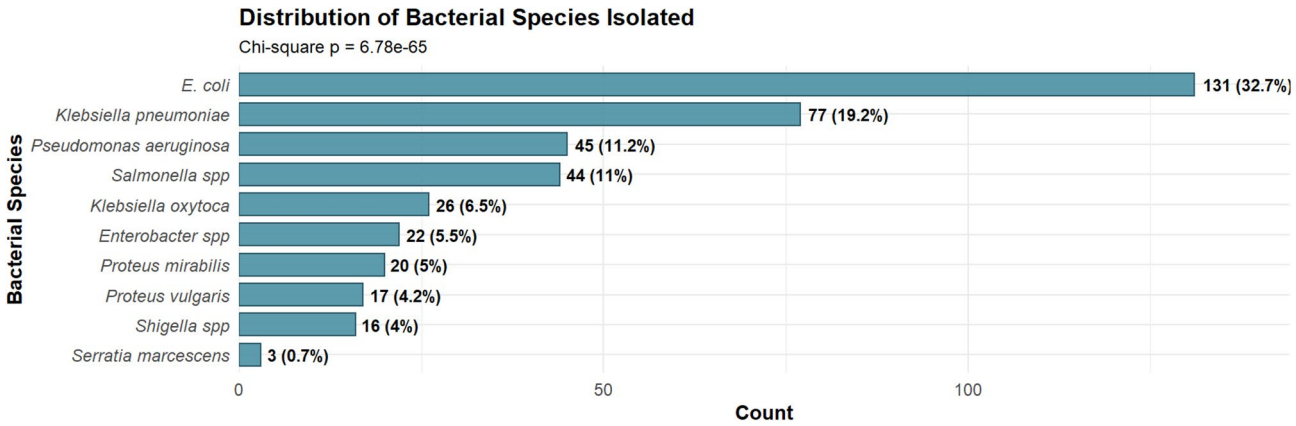

Fig. 1. The Frequency and Percentage Distribution of Bacterial Isolates from Household Wastewater Samples.

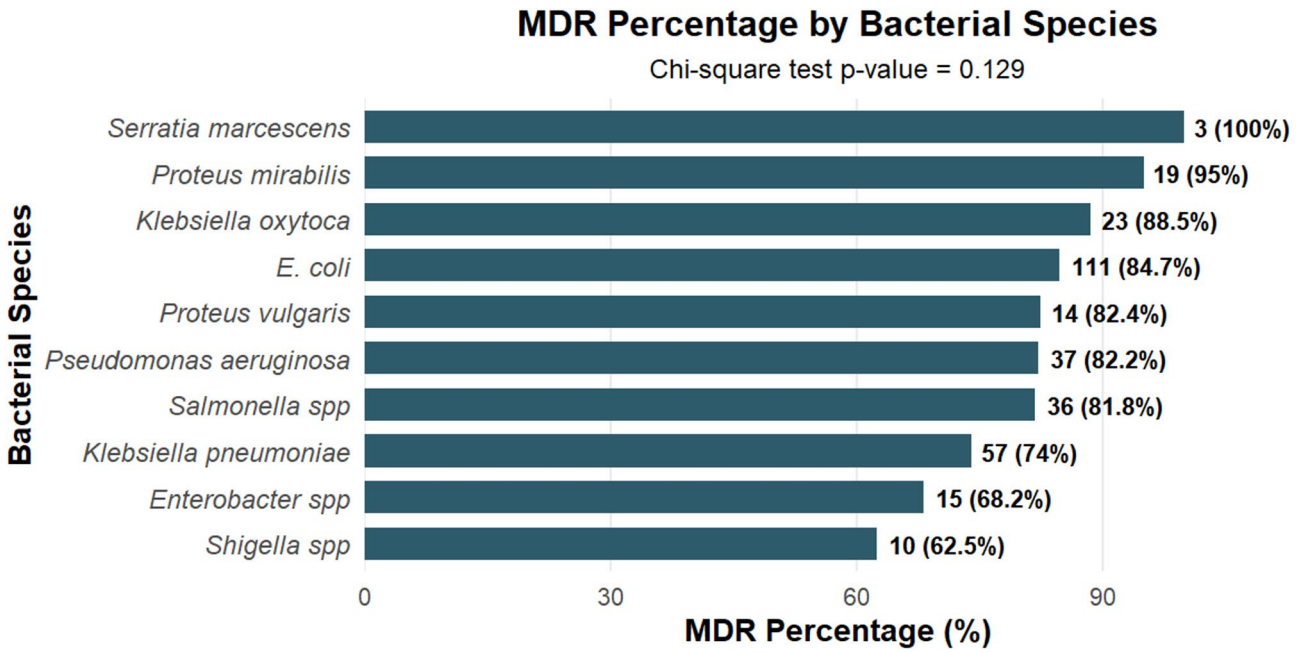

Fig. 2. Specie-specific MDR Prevalence Across Ten Bacterial Isolates.

Extended-spectrum beta-lactamase (ESBL) production

ESBL testing of 235 selected isolates from three key Enterobacteriaceae species revealed substantial enzymatic resistance capacity, with over half demonstrating this clinically significant mechanism. Among the species tested, *E. coli* showed ESBL production in 55% of isolates, *Klebsiella pneumoniae* in 52%, and *Klebsiella oxytoca* in 58%, as shown in Fig. 4. The observed inter-species variation in ESBL production rates highlights differential resistance strategies among these closely related bacterial populations, with certain species showing greater propensity for this particular resistance mechanism (Fig. 4). Representative images of ESBL detection using the double disc synergy test are shown in Fig. 5, while antimicrobial susceptibility testing demonstrating imipenem resistance in *Pseudomonas aeruginosa* is illustrated in Fig. 6.

Resistance metrics comparison

Antimicrobial susceptibility testing revealed a concerning pattern of widespread resistance across the antibiotic panel, with resistance rates exceeding 40% for most agents tested (Fig. 7). The highest resistance was observed against commonly used antibiotics, with amoxicillin-clavulanate (59%), cefotaxime (58%), and ampiclox (57%) showing the highest resistance rates. A notable gradient in resistance patterns emerged, with newer or more specialised antimicrobials such as levofloxacin (21%) and ofloxacin (26%) demonstrating substantially lower resistance rates, as shown in Fig. 7.

To complement the resistance pattern analysis, MAR indices were computed to quantify the extent of multidrug resistance among isolates. The mean MAR index values ranged from 0.29 to 0.55, indicating that most

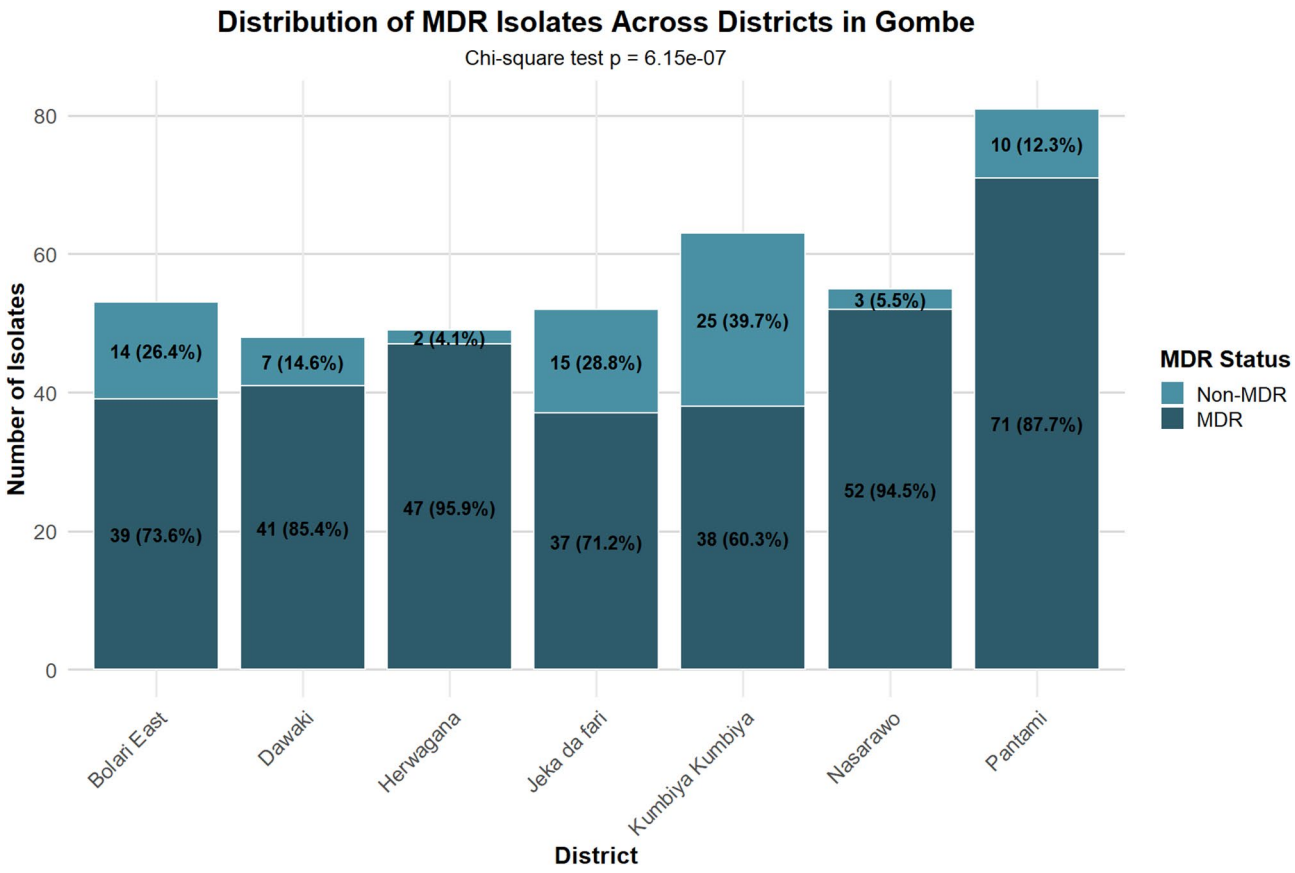

Fig. 3. Distribution of MDR Isolates Across Seven Districts in Gombe.

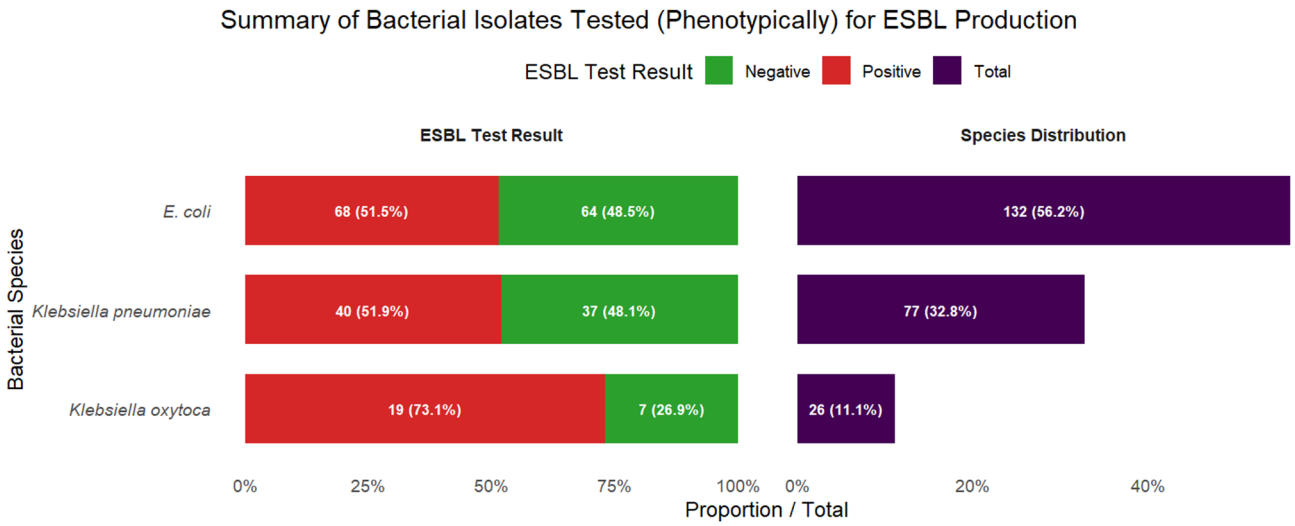

Fig. 4. Phenotypic Detection of ESBL Production in Some Isolates.

isolates originated from environments with high antibiotic exposure and selective pressure (Table 2). *Proteus mirabilis* demonstrated the highest mean MAR index (0.55), followed by *Pseudomonas aeruginosa* (0.532) and *Salmonella spp.* (0.5), while *Serratia marcescens* showed the lowest value (0.289) among the isolates analyzed (Table 2).

Statistical comparison between MDR and non-MDR isolates revealed profound differences in resistance burden, with MDR bacteria demonstrating approximately four-fold greater antibiotic resistance capacity (Fig. 8). MDR isolates showed resistance to a median of 8 antibiotics compared to only 1–2 antibiotics in non-

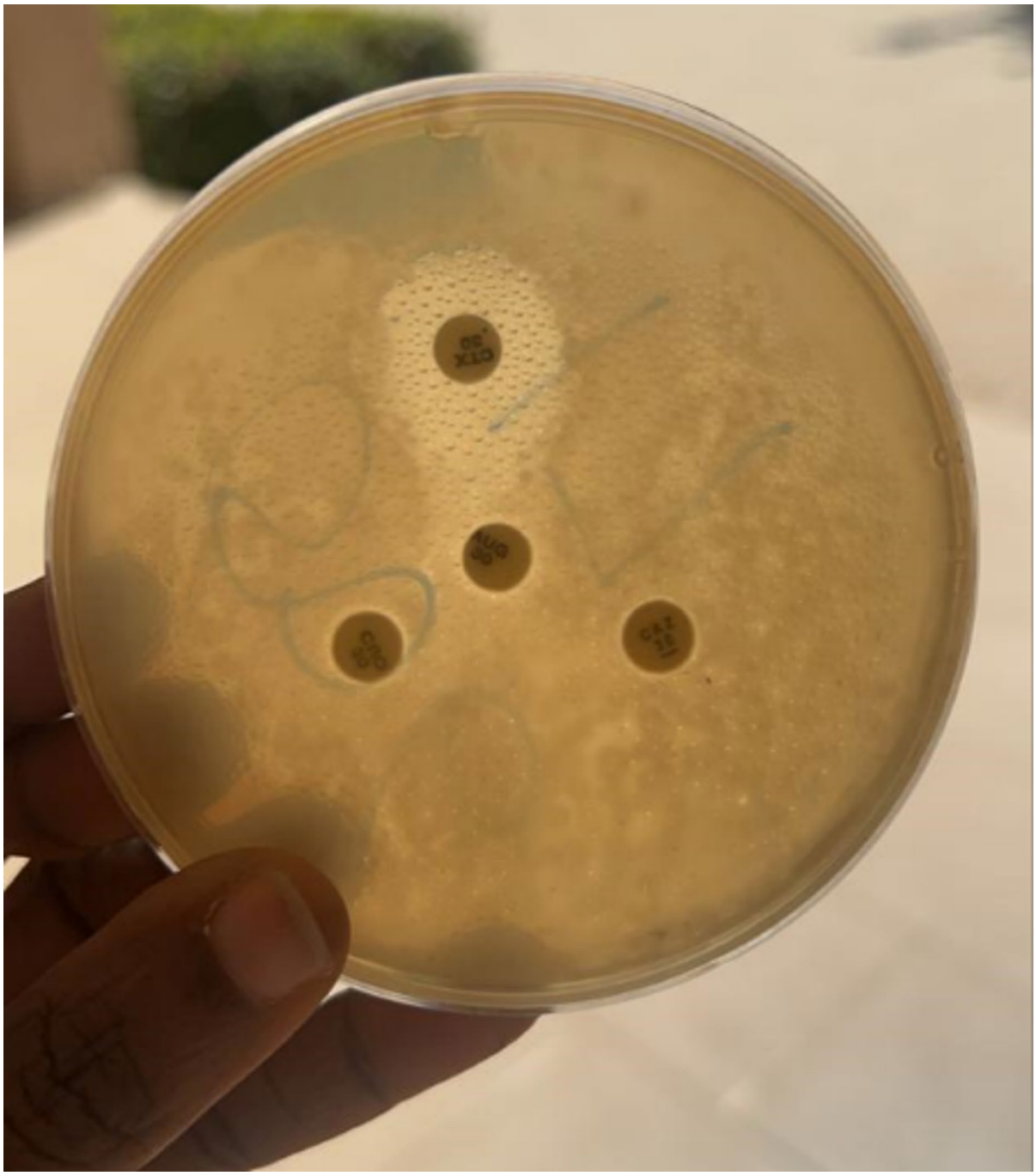

Fig. 5. Detection of ESBL Production in *E. coli* and *Klebsiella spp* Using the Double Disc Synergy Test (DDST).

MDR isolates. A strong positive correlation ($r = 0.99$) was observed between the number of resistant antibiotics and MAR index values, as illustrated in Fig. 8.

Molecular resistance gene detection

Representative PCR gel electrophoresis results for selected isolates are shown in Figs. 9 and 10. Distinct bands corresponding to the expected amplicon sizes were observed in positive isolates, while no amplification was detected in the negative control lane, confirming the absence of contamination. Although the positive control bands were not captured in the presented gel image, each PCR assay was validated using appropriate positive and negative controls during analysis.

PCR-based molecular analysis confirmed the genetic foundation of observed resistance phenotypes, revealing universal or near-universal distribution of extended-spectrum beta-lactamase genes among tested isolates (Table 3). The *blaCTX-M* gene was detected in all tested isolates (100%), while *blaTEM* and *blaSHV* were present in 90% and 60% of isolates, respectively (Fig. 9). Among carbapenemase genes, *blaVIM* was detected in

Fig. 6. AST Plate of *Pseudomonas aeruginosa* Showing Resistance to Imipenem.

60% of *Pseudomonas aeruginosa* isolates tested, while *blaKPC* was found in 20% (Fig. 10), representing a critical finding indicating potential resistance to last-resort antibiotics (Table 3).

Discussion

The bacterial diversity observed in household wastewater systems reflects the characteristic microbiological composition of domestic effluents, consistent with findings from similar environmental studies[29,30]. The predominance of enteric gram-negative bacteria, particularly *E. coli*, aligns with established understanding of wastewater microbiology, where these organisms naturally dominate due to their fecal origin and environmental persistence[31]. The predominance of gram-negative bacteria represents the higher end of documented ranges internationally, which is particularly alarming given the limited therapeutic options available for treating infections caused by these organisms.

The absence of significant differences in multi-drug resistance patterns between bacterial species suggests that antimicrobial resistance has become widespread across diverse bacterial populations, consistent with current understanding of resistance genes transfer between different bacterial species in environmental settings[32,33]. The overall MDR prevalence represents one of the highest rates reported in environmental surveillance studies from Sub-Saharan Africa[34], substantially exceeding the 70% MDR rates documented in clinical settings from some West African countries[35].

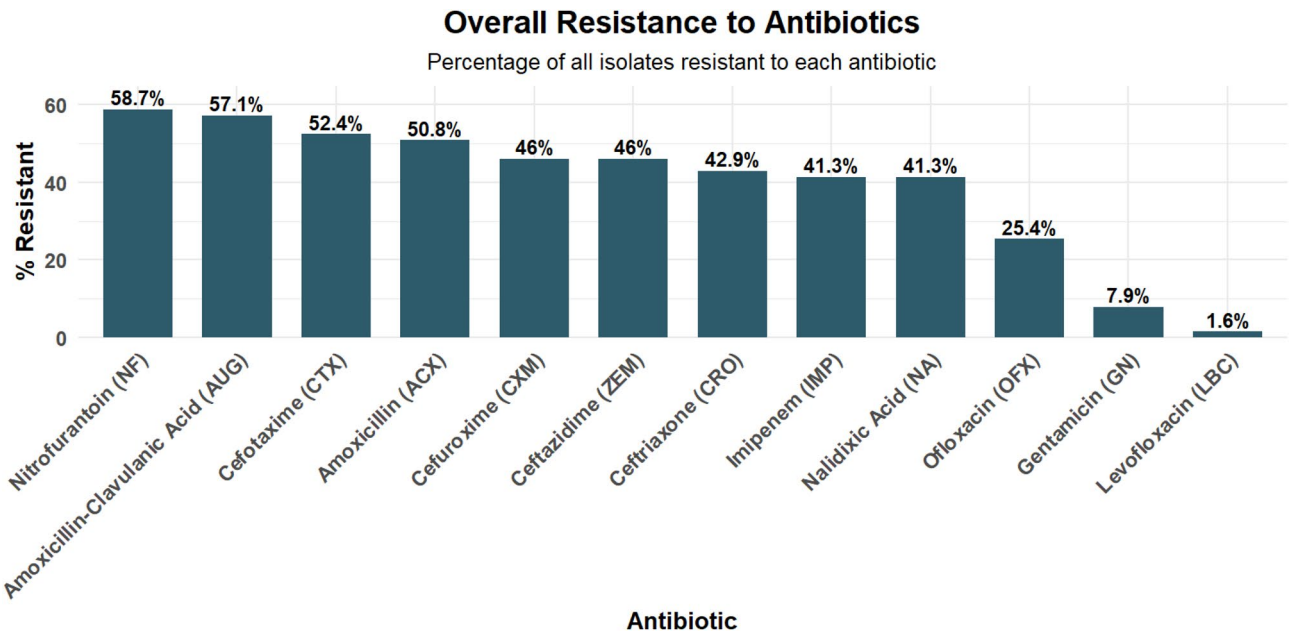

Fig. 7. Bacterial Resistance Rates to Twelve Commonly Used Antibiotics7.

Bacterial Specie	Number of Antibiotics Tested	Number of Isolates	mean MARI	sd MARI	MDR Count and (%)	Resistant Antibiotics
Proteus mirabilis	12	20	0.55	0.205	20(100)	AUG, CTX, IMP, NA, NF, CXM, CRO, ACX, ZEM
Pseudomonas aeruginosa	12	45	0.532	0.224	43(95.6)	AUG, CTX, IMP, NA, NF, CXM, CRO, ACX, ZEM
Salmonella spp	12	44	0.5	0.203	41(93.2)	AUG, CTX, IMP, NA, NF, CXM, CRO, ACX, ZEM
Proteus vulgaris	12	17	0.471	0.191	15(88.2)	AUG, CTX, NA, NF, CXM, CRO, ACX, ZEM
Klebsiella oxytoca	12	26	0.451	0.173	23(88.5)	AUG, CTX, IMP, NA, NF, CXM, CRO, ACX, ZEM
Shigella spp	12	16	0.45	0.28	14(87.5)	AUG, GN, NF, CXM, CRO, ACX, ZEM
E. coli	12	131	0.434	0.209	113(86.3)	AUG, CTX, IMP, NA, NF, CXM, CRO, ACX, ZEM
Klebsiella pneumoniae	12	77	0.385	0.24	58(75.3)	AUG, CTX, IMP, NF, CXM, ACX, ZEM
Enterobacter spp	12	22	0.345	0.246	15(68.2)	AUG, CTX, IMP, NA, NF, CXM, CRO
Serratia marcescens	12	3	0.289	0.102	3(100)	AUG, CTX, CXM, ACX

Table 2. Multiple antibiotic resistance index summary of Isolates.

When contextualized within the broader Sub-Saharan African AMR landscape, these findings take on heightened significance. The region already bears the highest global mortality burden from AMR, with 27.3 deaths per 100,000 attributed to antimicrobial resistance in 2019, and Western Sub-saharan Africa experiencing death rates exceeding 100 per 100,000 individuals[35]. The environmental burden of resistance demonstrated suggests that traditional clinical surveillance significantly underestimates the true scope of the AMR problem in Nigerian communities.

Our findings align with limited but growing evidence from similar wastewater surveillance studies in Sub-Saharan Africa. A recent study from Lagos, Nigeria documented ESBL-producing Enterobacteriaceae in 68% of wastewater canal samples, with *E. coli* and *Klebsiella* spp. predominating[36]. Similarly, wastewater surveillance in Niger identified high levels of antimicrobial resistance in community wastewater systems, though specific MDR prevalence rates were lower than observed in our study[37]. In Burkina Faso, ESBL-producing bacteria were detected in 97.6% of healthcare centre wastewater samples, with 95% confirmed as ESBL producers and all isolates exhibiting multidrug resistance[38], while South African wastewater studies documented similar gram-negative bacterial dominance, though wastewater AMR surveillance remains concentrated in few African countries[39].

However, our study's 81% MDR prevalence and 100% *blaCTX-M* detection rate exceed these regional findings, suggesting that Gombe may represent a particularly high-burden setting or that household-level sampling captures resistance patterns not fully reflected in municipal wastewater systems. The methodological similarity across these studies relying on culture-based isolation and phenotypic susceptibility testing strengthens the validity of cross-study comparisons and highlights the concerning uniformity of high resistance burdens across diverse Sub-Saharan African settings.

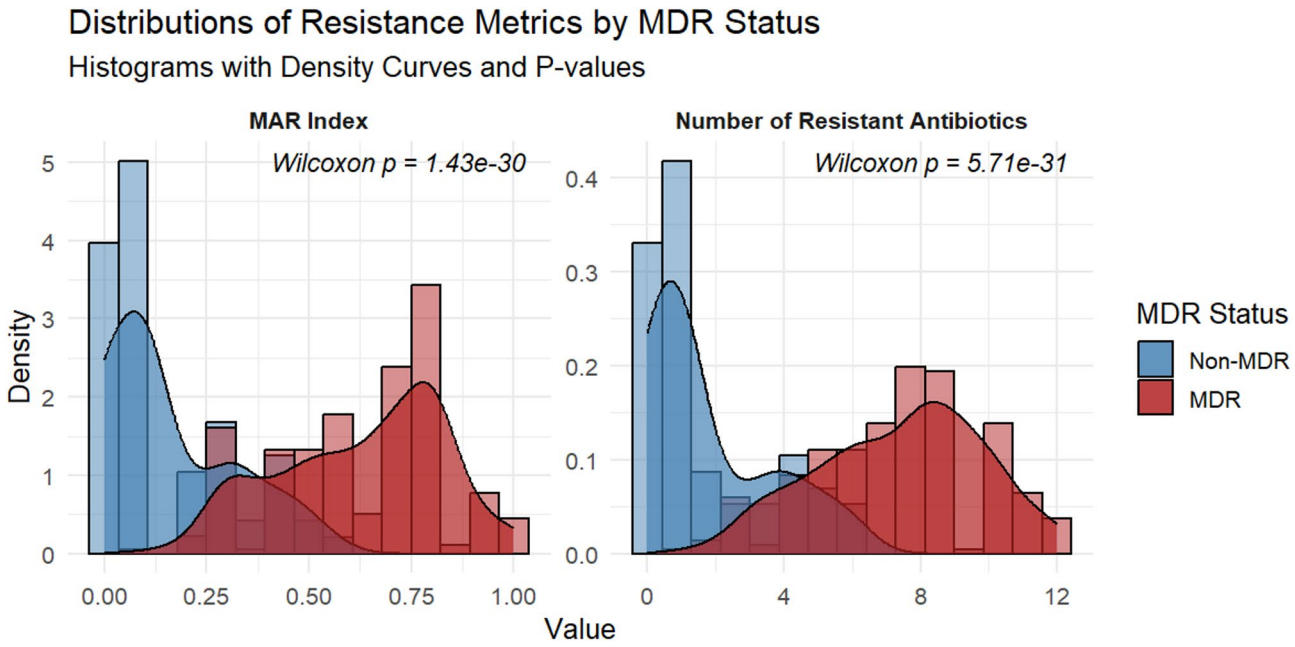

Fig. 8. Distribution of Multiple MAR Index and Resistant Antibiotic Count in MDR versus Non-MDR Bacterial Isolates.

Understanding the drivers of these high resistance rates is crucial for developing effective interventions. The high prevalence of MDR bacteria in household wastewater likely reflects multiple contributing sources. Household antibiotic use represents a primary driver, particularly given Nigeria's context of widespread over-the-counter antibiotic availability without prescription requirements[2]. When antibiotics are consumed, a significant proportion is excreted unchanged or as active metabolites in urine and faeces, introducing both residual antibiotics and resistant bacteria into household wastewater systems[3,11]. Additionally, livestock-related contamination may contribute to the resistance burden, as many households in Gombe keep small livestock (chickens, goats) in close proximity to living areas, and veterinary antibiotic use in Nigeria is largely unregulated[33].

Environmental contamination from inadequate waste disposal practices, proximity to healthcare facilities, and cross-contamination between wastewater and drinking water sources further compound the problem[5,6]. The district-level variation observed in our study supports this multifactorial etiology, as districts with different demographic profiles, healthcare access patterns, and sanitation infrastructure demonstrated significantly different MDR prevalence rates.

The detection of ESBL production in 54% of tested isolates represents a critical finding that aligns with documented rates from both clinical and environmental surveillance programs in similar settings[40–42]. When compared to regional studies from Burkina Faso healthcare centre wastewater[38] and South African wastewater treatment plants[39], the prevalence rates observed in Gombe confirm the widespread presence of ESBL-producing bacteria in environmental settings. The clinical implications are profound, as ESBL-producing bacteria are associated with increased mortality, prolonged hospitalisation, and elevated healthcare costs[43,44]. In resource-limited settings like Nigeria, where third-generation cephalosporins serve as first-line therapy for many serious infections[45], this environmental prevalence suggests widespread therapeutic challenges.

Molecular characterisation provided deeper insights into the genetic basis of the observed resistance patterns. The universal presence (100%) of *blaCTX-M* genes in our tested isolates substantially exceeds prevalence rates documented in international wastewater surveillance studies. A comprehensive wastewater genomic surveillance study from Finland reported *blaCTX-M* detection in 86.3% of ESBL-producing *E. coli* isolates from municipal wastewater treatment plants[46], while a global meta-analysis of wastewater studies found an overall pooled prevalence of *blaCTX-M* genes of 66.56% across 57 international wastewater surveillance[47]. A study from Marrakech, Morocco found *blaCTX-M* genes in 81.25% of ESBL-producing *E. coli* from municipal wastewater treatment plant influent samples, with 78.35% of isolates exhibiting multidrug resistance phenotypes[48].

Our finding of universal *blaCTX-M* prevalence in Gombe household wastewater represents one of the highest rates documented and is particularly concerning given that this gene family has become the predominant ESBL type globally and is associated with rapid horizontal transfer between bacterial species[49], suggesting intense selective pressure and widespread dissemination of these resistance determinants in the local environment. Most alarming is the detection of carbapenemase genes, particularly *blaVIM* in *P. aeruginosa* isolates. Given that carbapenems are considered last-resort antibiotics for treating multidrug-resistant infections[50,51], the environmental circulation of these resistance mechanisms suggests that carbapenem resistance may be more widespread in Nigerian communities than clinical surveillance indicates.

This study demonstrates the feasibility and exceptional value of wastewater-based epidemiology for AMR surveillance in resource-limited settings, contributing to the growing international evidence base supporting

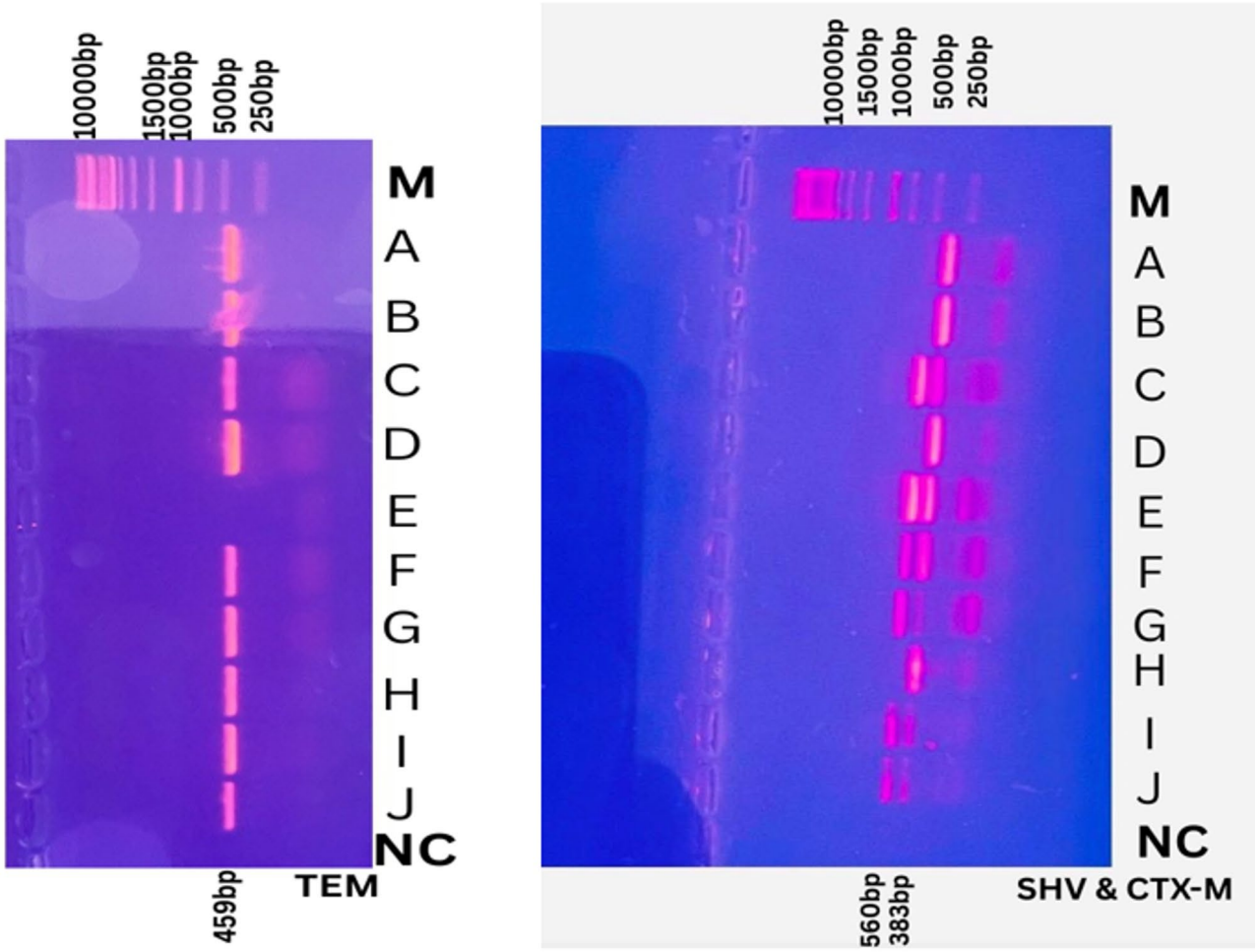

Fig. 9. PCR Detection of *blaCTX-M*, *blaTEM*, and *blaSHV* Genes in Selected *Escherichia coli* and *Klebsiella pneumoniae* isolates.

this approach[52]. The methodology aligns with successful surveillance studies in Taiwan[53], Nigeria[36], and Niger[37], while providing novel insights specific to household-level surveillance in Sub-Saharan Africa. The strong correlation between resistance gene detection and phenotypic resistance patterns validates the biological relevance of wastewater surveillance findings for monitoring clinically relevant resistance patterns[7,54,55].

These findings have critical implications for local health authorities and public health practice in Nigeria. First, wastewater surveillance can serve as a cost-effective early warning system for emerging resistance threats, allowing health authorities to detect community-level resistance patterns before they manifest as treatment failures in clinical settings. Local governments in Gombe and similar settings could implement periodic household wastewater monitoring in representative districts to track resistance trends over time and evaluate the impact of antibiotic stewardship interventions. Second, the district-level variation documented suggests that geographically targeted interventions such as community education campaigns about appropriate antibiotic use, improved sanitation infrastructure, or regulated disposal of pharmaceutical waste, could yield measurable improvements in specific high-burden areas. Third, these data should inform empirical treatment guidelines for community-acquired infections, as the high prevalence of ESBL-producing organisms and carbapenemase genes suggests that commonly prescribed beta-lactam antibiotics may have limited effectiveness.

Local health authorities could use wastewater surveillance data to update antimicrobial formularies and treatment protocols, prioritising antibiotics less affected by prevalent resistance mechanisms. Finally, this approach supports the integration of environmental health into broader One Health antimicrobial stewardship frameworks, providing actionable data that bridges clinical, veterinary, and environmental sectors[9]. Implementation would require modest investment in laboratory capacity and training but could be integrated into existing environmental health surveillance programs[10].

Study limitations include the cross-sectional design, which captures only a "snapshot" of antimicrobial resistance patterns and does not assess temporal variations. In addition, reliance on classical biochemical and culture-based identification methods may have led to misidentification of some bacterial isolates compared with molecular confirmation. The focus on culturable bacteria may also underestimate the true microbial diversity in wastewater. Furthermore, molecular analysis was limited to selected isolates and specific resistance genes. The

Fig. 10. PCR Detection of *blaNDM*, *blaVIM*, and *blaKPC* Genes in Selected Carbapenem-Resistant *Pseudomonas aeruginosa*.

Resistance Gene	Number Tested	Present (*n*, %)	Absent (*n*, %)
bla_CTX-M	10	10 (100%)	0 (0%)
bla_SHV	10	6 (60%)	4 (40%)
bla_TEM	10	9 (90%)	1 (10%)
bla_NDM	5	0 (0%)	5 (100%)
bla_KPC	5	1 (20%)	4 (80%)
bla_VIM	5	3 (60%)	2 (40%)

Table 3. Antimicrobial resistance genes detected by PCR.

study did not investigate the viability or infectivity of resistant bacteria in wastewater, which would be important for assessing direct transmission risks.

Conclusion

These findings demonstrate that household wastewater systems serve as significant reservoirs of antimicrobial resistance, with complex interactions between bacterial communities, environmental factors, and human activities driving the emergence and maintenance of resistance phenotypes. This study provides important environmental AMR surveillance data from Sub-Saharan Africa, documenting resistance rates that are among the higher levels reported internationally and highlighting the critical importance of environmental surveillance in understanding community-level AMR patterns.

The molecular characterisation provides novel insights into resistance gene circulation in African communities and establishes wastewater surveillance as a feasible and valuable tool for AMR monitoring in resource-limited settings. The district-level variation documented offers hope that targeted interventions could have measurable impacts on community resistance patterns, while these results emphasise the need for coordinated, multi-sectoral responses to address the substantial environmental AMR challenge documented in this Nigerian setting.

While this cross-sectional study has limitations in temporal scope and molecular characterisation depth, these findings contribute significantly to the global understanding of environmental AMR and provide crucial baseline data for monitoring the effectiveness of future intervention strategies in one of the world's most affected regions.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author upon reasonable request.

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Author contributions

ZM conducted the sample collection, laboratory and data analysis, and jointly conceptualised the research with MTA, LG, and TAU. MTA, LG, and TAU also contributed to the study design and methodology. MKU, SSA, IY critically reviewed and revised the manuscript. SSA and IY provided technical advice and support throughout the study. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This study was approved by the Gombe State Environmental Protection Agency (GOSEPA), Nigeria. The ethical approval reference number is **ES/GOSEPA/ADM/S/38/V.I**. All procedures involving environmental sampling were conducted in accordance with national and international guidelines for environmental health research. Although no human participants or biological tissues were directly involved in the study, verbal informed consent was obtained from the heads of households before wastewater sample collection. Participants were informed about the purpose of the study, assured of confidentiality, and their voluntary participation was respected. The need for written consent was waived by the ethics committee due to the non-invasive nature of environmental sampling and absence of identifiable human data.

Consent for publication

Not applicable. This manuscript does not contain data from any individual person, including individual details, images, or videos.

Additional information

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