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DETECTION AND MOLECULAR IDENTIFICATION OF MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA FROM HOSPITALS' SEWAGE IN SOKOTO METROPOLIS

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Article Info

Abstract

Resistance to antibiotics is a global threat., increasingly linked to environmental dissemination through hospital sewage. This study assessed the prevalence and multidrug resistance patterns of Gram-negative bacteria in hospital sewage in Sokoto Metropolis, Northern Nigeria. From 100 samples collected across 10 hospitals, 216 Gram-negative isolates were identified, including Escherichia coli (28.24%), Klebsiella spp. (18.98%), Salmonella spp (15.74%), Pseudomonas spp (14.35%), Shigella spp (13.42%), and Proteus spp (9.26%). All isolates exhibited multidrug resistance, with Proteus spp. showing the highest resistance (75%) and a Multiple Antibiotic Resistance (MAR) index ranging from 0.3 to 0.75. Ceftazidime and cefuroxime had the highest resistance rates (100%), while ofloxacin showed 100% sensitivity across all isolated species. The consistent resistance patterns suggest common resistance genes, indicating the role of hospital sewage in spreading antibiotic resistance.

Keywords:

Sokoto, Hospital, Molecular, Gram-negative, Bacteria

1.0 INTRODUCTION

The existence and propagating of antibiotic particles,bacterial species resistant to antibiotics (ARB), and antimicrobial resistance genes (ARG) in aquatic environments have become critical concerns in public health and environmental science (Voigt et al., 2019). Hospital wastewater is a significant contributor to this issue, as it often contains high concentrations of antibiotics excreted by patients, This would cause multidrug-resistant (MDR) species to proliferate (Parkes and Hota, 2018). These resistant bacteria can spread through various routes, including contaminated water sources,seriously endangering public health. (Giani et al., 2015; Weterings et al., 2015).

Hospital sewage systems are particularly concerning as they continuously discharge sub-therapeutic doses of antibiotics, fostering the exchange of resistance genes among bacterial populations (Aali et al., 2014). This has led to the increasing isolation of MDR Gram-negative bacteria, such as Escherichia coli and Klebsiella pneumoniae, from hospital sewage in various studies (Brennan and Everman, 2012; Jeukens et al., 2019). These bacteria, which often show resistance to multiple clinically important antibiotics, represent a significant challenge in treating infections and contribute to rising Healthcare expenses and extended hospital stays (Sib et al., 2019).

Despite the recognized threat of MDR bacteria, there is limited understanding of the role hospital sewage plays in their persistence and spread. This research aims to address this gap by isolating, characterizing, and detecting MDR Gram-negative bacteria (MDR-GNB) from Sokoto Metropolitan Hospital sewage. However, the study's objectives are to: (i) isolate and identify Gram-negative bacteria from hospital sewage, (ii) determine the frequency of these bacteria, and (iii) assess their antimicrobial susceptibility profiles.

2.0 MATERIALS AND METHODS

2.1 Study Area

This work was undertaken in Sokoto Metropolitan area of, Sokoto State, situated in the northwestern region of Nigeria, is known for its semi-arid climate and historical significance as the seat of the Sokoto Caliphate. The state spans longitudes 3°E to 7°E and latitudes 10°N to 14°N of the Equator, featuring a landscape dominated by savannah vegetation and seasonal rivers. This research encompasses ten healthcare facilities: Maryam Abacha Women and Children Hospital Sokoto, Specialist Hospital Sokoto, Usmanu Danfodiyo University Teaching Hospital (UDUTH), PHC Kasarawa, PHC Durbawa, Kofar Kade clinic, Kofar rini clinic, Women and Children welfare clinic (WCWC), Gagi Clinics, and Army Barracks Hospital, scattered throughout the metropolis.

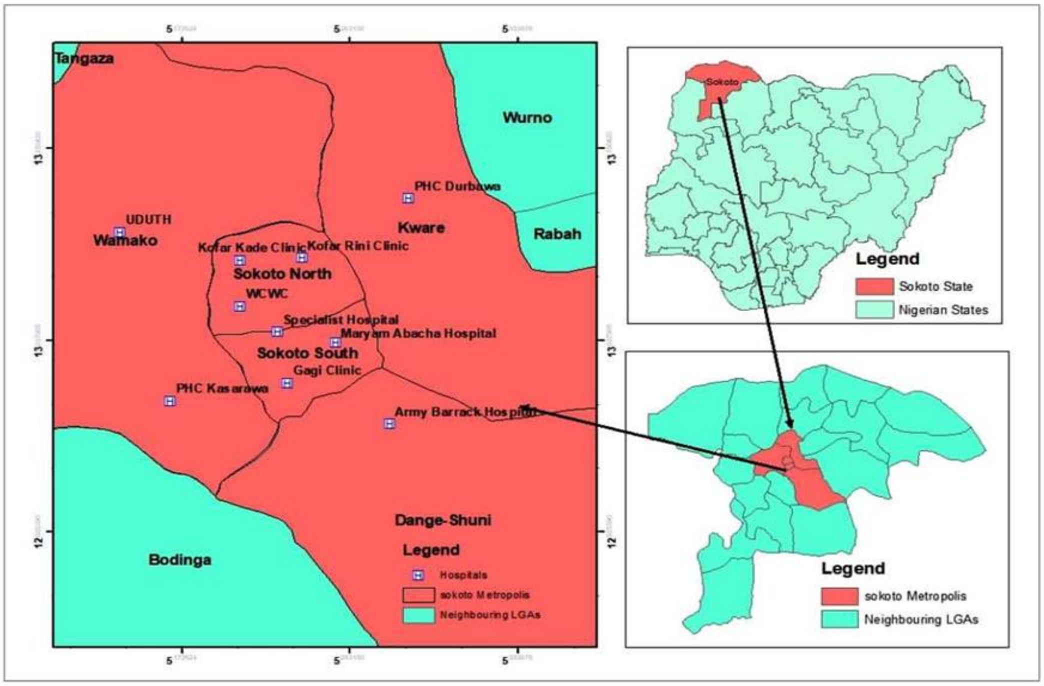

Figure 1: Map highlighting Sokoto Metropolis within Sokoto State, Nigeria, derived from the Sokoto State Government's Strategic Health Development Plan (2019).

2.2 Research Design

This cross-sectional study, conducted between June and August 2022 in Sokoto Metropolis, involved collecting 100 sewage samples from 10 hospitals using random sampling and aseptic techniques. Gram-negative bacteria were isolated, identified via biochemical tests, and their antimicrobial resistance was assessed. Conventional PCR further characterized selected isolates. Statistical analysis such as; Chi-square tests was used to assess the prevalence and patterns of multidrug resistance.

2.3 Sample Collection

A total of 100 sewage samples were systematically collected from 10 hospitals in Sokoto Metropolis over 12 weeks (June to August 2022). Using sterile bottles, 100 ml grab samples were taken twice daily (morning and evening) on Mondays and Thursdays, ensuring consistent spacing. Samples were collected at ambient temperatures (24°C to 31°C) between 7:00-8:00 a.m. and 5:00-6:00 p.m., then promptly transported on ice to the Public Health Laboratory at Usmanu Danfodio University for microbiological analysis within 2 hours.

2.4 Isolation and Characterization of Gram-Negative Bacteria from Hospitals' Sewage

Representative composites of the sewage samples were created through homogenization. Using the pour plate method, dilutions were plated on nutrient agar (Sigma-Aldrich) and incubated at 37°C for 24 hours. After colony counting, the isolated colonies were sub-cultured onto MacConkey agar (Sigma-Aldrich) and incubated again at 37°C for 24 hours. The bacterial isolates were then identified based on their morphology and biochemical reactions in accordance with established protocols (Whitman, 2015).

2.5 Biochemical Characteristics

The acquired isolates underwent several biochemical analyses in order to pinpoint their physiological and metabolic traits. These comprised the Triple Sugar Iron (TSI) Test, Urease Test, Gram staining, Indole Production, Methyl Red (MR) Test, Voges-Proskauer (VP) Test, Under oil immersion, Gram staining was carried out as detailed by Riddell and Sanyal (2019), applying crystal violet, iodine, ethanol for decolorization, and counterstaining with safranin to separate Gram-positive and Gram-negative bacteria. Using urea agar slants, urease activity was measured; a pink tint indicated a positive result and further incubation confirmed negative outcomes.

Inoculating peptone water and adding Kovac's reagent following incubation assessed indole synthesis; the absence of a red/pink layer indicates a negative outcome. After incubation, methyl red added to glucose phosphate peptone water validated acid generation for the MR test by turning the water a brilliant red color. The VP test consisted in adding potassium hydroxide and α-naphthol to VP broth so that a red color indicated a positive outcome. Using TSI agar slants, the TSI test finally evaluated carbohydrate fermentation, gas generation, and hydrogen sulfide generation with characteristic color changes and black precipitates suggesting particular metabolic activity (Riddell and Sanyal, 2019).

2.6 Antimicrobial Susceptibility Test

Following CLSI (2015) recommendations, the antimicrobial sensitivity test was carried out using the Kirby-Bauer disk diffusion technique.. Bacterial isolates were prepared to 0.5 McFarland standards and uniformly spread over Mueller–Hinton agar plates. Antibiotic disks includingamoxicillin/clavulanate (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nitrofurantoin (300 µg), cefuroxime (30 µg), ceftazidime (30 µg), and ampicillin (10 µg) were placed on the plates, which were then incubated at 37°C for 18-24 hours. Zones of inhibition were measured and categorized as sensitive, intermediate, or resistant. Multidrug-resistant (MDR) isolates were those that were resistant to at least two antibiotics from distinct classes.

2.6.1 Multiple Antibiotic Resistance Index (MARI) calculation

The formula MARI = (a/b), was used to compute the Multiple Antibiotic Resistance Index (MARI), where 'a' represents the number of antibiotics to which the bacterial isolate exhibits resistance, and 'b' refers to the total number of antibiotics tested. An isolate with a MARI value greater than 0.2 suggests high levels of antibiotic resistance and indicates the presence of highly resistant bacteria (Mustapha and Imir, 2019).

2.7 Molecular Identification of Gram-Negative Bacteria from Hospital Sewage

2.7.1 DNA Extraction

As directed by the manufacturer, DNA was extracted using the Qiagen DNA extraction kit. Bacteria suspension (200 µl) was mixed with 400 µl Buffer AVL, vortexed, and incubated at room temperature for

10 minutes. After adding 300 µl ethanol, the mixture was incubated at -20°C for 1 hour. The solution was then transferred to a QIAamp Mini column, centrifuged at 8,000 rpm for 1 minute, and washed sequentially with 500 µl Buffer AW1 and Buffer AW2, followed by centrifugation at 13,000 rpm. Finally, 50 µl Buffer AE was added to elute the DNA, which was stored at -20°C (Iliyasu et al., 2019).

2.7.2 Molecular Identification via 16S rDNA Gene Amplification

PCR was performed to amplify the 16S rDNA gene using a 25 µL mix containing 12.5 µL Master Mix (Qiagen), 2.5 µL coral load, 1 µL each of 27-F and 1942-R primers, 3 µL nuclease-free water, and 5 µL DNA template. 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 2 minutes, extension at 72°C for 1 minute 30 seconds, and final extension at 72°C for 10 minutes comprised the PCR conditions. The initial denaturation lasted 5 minutes at 94°C. According to Iliyasu et al. (2019), PCR products were kept at 4°C.

Primers Used:

- **27-Forward**: AGAGTTTGATCMTGGCTCAG
- **1492-Reverse**: TACGGYTACCTTGTTACGACTT

2.7.3 DNA Visualization Using Gel Electrophoresis

A 1.5% agarose gel was prepared by dissolving 0.75 gm agarose in 50 ml 1X Tris-Acetate-EDTA (TAE) buffer, heating until clear, and cooling to 50°C before adding 10 µL ethidium bromide (0.5 µg/ml). The gel was cast, allowed to solidify for 30 minutes, and then placed in an electrophoresis tank with 1X TAE buffer. Amplicons (8 µL) mixed with 2 µL 6X gel loading dye were loaded into the wells, alongside a 100-bp DNA ladder. Electrophoresis was performed at 70 V for 1 hour, and the gel was visualized under UV light using a Bio-Rad gel documentation system.

2.8 Data Analysis

Data was analyzed using Microsoft Excel 2019 and Statistical Products and Services Solutions (SPSS) version 25. The species of microorganisms, their distribution, and patterns of antibiotic resistance were compiled using descriptive statistics. Chi-square tests assessed significant associations between the distribution of Gram-negative bacteria and different hospital sewage samples, aiming to identify distinct bacterial profiles or prevalence rates across hospitals, with a significance level set at $\alpha = 0.05$.

3.0 RESULTS

3.1 Distribution of Gram Negative Bacteria in Hospital Sewages

Out of a total of 100 samples collected and analyzed, 86 Sewage sample were positive for Gram-negative bacteria. Hence, a total of 216 gram negative bacteria (GNB) isolated from the from hospital sewages. Gagi Clinic had the highest 28 GNB while Women and Children welfare Clinic (WCWC) had the least of 15 GNB (Table 1).

Table 1: The overall distribution of GNB in Hospital Sewages

Sample	H_A	H_B	H_C	H_D	H_E	H_F	H_G	H_H	H_I	H_J	Total
1	3	2	0	3	3	1	2	2	2	2	20
2	2	3	0	3	4	2	3	2	2	2	23
3	2	3	0	3	4	2	3	2	2	2	23
4	4	4	2	2	2	0	2	3	3	3	25
5	2	3	0	0	2	2	4	2	3	3	21
6	0	2	3	3	3	2	3	2	2	4	24
7	0	3	4	4	2	2	2	2	3	2	24
8	2	2	2	0	2	0	2	0	2	4	16
9	2	0	3	2	2	2	3	0	4	2	20
10	1	0	2	2	3	2	2	3	4	1	20
Total	**18**	**21**	**20**	**24**	**23**	**15**	**25**	**18**	**28**	**24**	**216**

Keywords: H_A= PHC Kasarawa, H_B= Uduth, H_C= PHC Durbawa, H_D= Kofar Kade Clinic, H_E= Kofar Rini Clinic, H_F= WCWC, H_G= Specialist Hospital Sokoto, H_H= Maryam Abacha Hospital, H_I= Gagi Clinic, H_J= Army Barrack Hospital.

3.2 Morphological and Metabolic Features of the Bacterial Isolates in the hospital Sewages

The findings of the morphological and biochemical traits of the bacterial isolates found in hospital sewages are shown in Table 2.. The two hundred and sixteen (216) isolates were found to be Gram-negative rod-shaped bacteria. Hence, the biochemical tests carried revealed these isolates to be Eschericia coli, Shigella spp, Klebsiella spp, Pseudomonas spp, Salmonella spp, and Proteus spp.

Table 2: Morphological and Metabolic Features of the Bacterial Isolates in the hospital Sewages

G/R	Shape	Ind	Suc	Cit	Mot	Mr	Vp	H_2S	Gas	Lac	Glu	Bacteria
-ve	Rod	-	+	-	+	-	+	-	+	+	-	Escherichia coli
-ve	Rod	-	-	-	-	+	-	-	+	+	+	Shigella spp
-ve	Rod	+	+	-	+	+	-	+	+	-	+	Proteus spp
-ve	Rod	-	+	-	+	+	-	-	+	-	-	Salmonella spp
-ve	Rod	-	+	+	-	-	+	-	+	+	+	Klebsiella spp
-ve	Rod	-	-	+	+	-	-	-	+	-	-	Pseudomonas spp

Key: IND: Indole Test, SUC: Sucrose Test, CIT: Citrate Test, MOT: Motility Test, Mr: Methyl red Test, Vp: Voges-proskauer Test, H_2S: Hidrogen Sulfide production Test, Lac: Lactose Test, Glu: Glucose Utilization Test,

3.3 Frequency of Occurrence of the Isolated GNB Species from the Hospital Sewages

Figure 2 shows the distribution of the GNB species isolated from the hospital sewages. The GNB identified in the samples belonged to six different genera. The most prevalent species were Escherichia coli, which accounted for 28.24% of the isolates, followed by Klebsiella spp. at 18.98% and Salmonella spp. at 15.74%. Escherichia coli had the highest prevalence among the identified microbes.

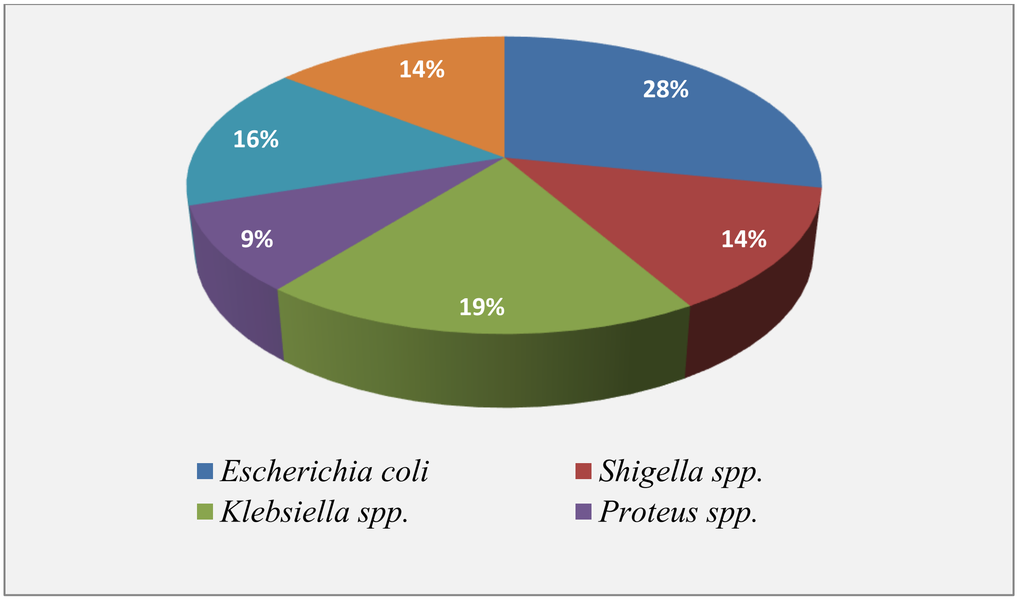

Figure 2: The frequency of occurrence of the isolated GNB from Hospital Sewages

3.4 Molecular Identification of Gram-Negative Bacteria from Hospital's Sewage

Figure 3 presents agarose gel electrophoresis analysis of 16s rDNA genes from six bacterial isolates, PCR amplified products were run on 1% agarose gel. Lane M indicates the DNA ladder (Biolabs UK, DNA ladder mix100-3000bp). Markers were properly indicated based on their sizes as described by the manufacturer. Lane 1-6 indicates the PCR amplified 16s rDNA genes of the respective bacterial isolates (Eschericia coli, Shigella spp, Klebsiella spp, Proteus spp, Salmonella spp, Pseudomonas spp respectively). Lane NC indicates negative control.

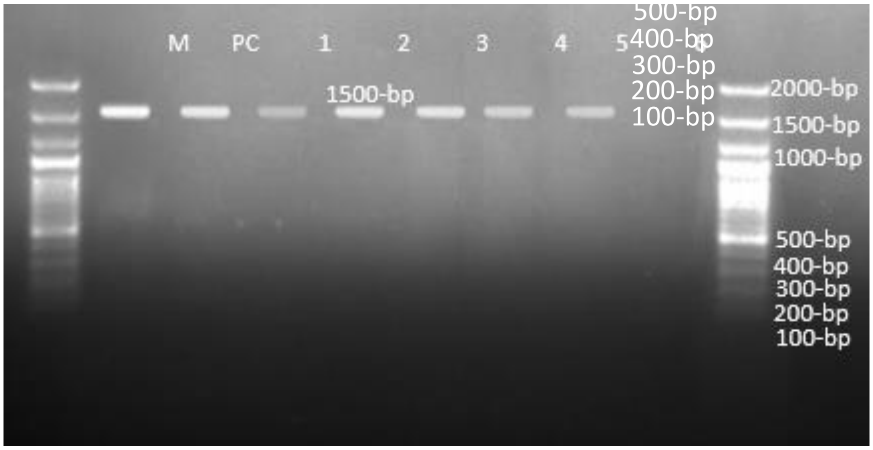

Figure 3: One percent (1%) Agarose gel Electrophoresis Image of 16S amplicons of the six bacterial species isolated

Key: PC=Positive control, NC= Negative control, Sample wells 1 to 6 = GNB isolates as Eschericia coli, Shigella spp, Klebsiella spp, Proteus spp, Salmonella spp, Pseudomonas spp respectively.

3.5 Antibiotic Resistance Pattern of Gram-negative from Hospital Sewage in Sokoto Metropolis

Table 3 summarizes the antibiotic resistance patterns of Gram-negative bacteria isolated from hospital sewage in Sokoto Metropolis. Escherichia coli isolates exhibited high resistance to ceftazidime (81.96%), cefuroxime (93.4%), and amoxicillin/clavulanate (98.4%), with no resistance to ciprofloxacin, ofloxacin, nitrofurantoin, or ampicillin. Shigella spp showed resistance ranging from 34.8% to 86.2% against various antibiotics, while Klebsiella spp demonstrated resistance between 46.34% and 78.09%. Proteus spp were resistant to multiple antibiotics except for ciprofloxacin, ofloxacin, and nitrofurantoin. Salmonella spp were sensitive to gentamicin, ofloxacin, and ampicillin, whereas Pseudomonas spp showed significant resistance to ceftazidime (83.9%), cefuroxime (96.8%), and amoxicillin/clavulanate (83.9%).

Table 3: Antibiotic Resistance Pattern of Gram-negative from Hospital Sewage in Sokoto Metropolis

Bacteria	**Number Tested**	**Antibiotics (%)**							
		CAZ	**CRX**	**GEN**	**CPR**	**OFL**	**AUG**	**NIT**	**AMP**
Escherichia coli	61	81.96	93.4	65.6	0.00	0.00	98.4	0.00	0.00
Shigella spp	29	86.20	68.96	0.00	51.72	0.00	44.82	34.8	0.00
Klebsiella spp	41	78.9	95.12	0.00	0.00	0.00	0.00	46.34	0.00
Proteus spp	20	90.00	100.0	80.00	60.00	0.00	70.00	0.00	20.00
Salmonella spp	34	85.30	82.35	0.00	41.18	0.00	94.11	61.80	0.00
Pseudomonas spp	31	83.90	96.80	0.00	0.00	0.00	83.90	29.03	0.00

Keywords: Nitrofurantoin (NIT), Amoxicillin/Clavulanate (AUG), Ofloxacin (OFL), Ciprofloxacin (CPR), Ampicillin (AMP), Cefuroxime (CRX), Gentamicin (GEN), and Ceftazidime (CAZ)

3.6 MDR – GNB from Hospital Sewage in Sokoto Metropolis

Table 4 highlights the Multi-Drug Resistant (MDR) Gram-negative bacteria (GNB) from hospital sewage in Sokoto Metropolis. All GNB isolates were resistant to both ceftazidime and cefuroxime, with E. coli (65.6%) and Proteus spp (80%) also showing resistance to gentamicin. Proteus spp (20%) alone exhibited resistance to ampicillin, while all isolates were susceptible to ofloxacin. The Multiple Antibiotic Resistance Index (MARI) of the isolates ranged from 0.375 to 0.75, with most showing resistance to three or more antibiotics.

Table 4: MDR – GNB from Hospital Sewage in Sokoto Metropolis

MDR – GNB	MARI	List OF Antibiotics Resisted
E. coli	0.50	CAZ,CRX,GEN,AUG
Shigella spp	0.625	CAZ,CRX,CPR,AUG,NIT
Klebsiella spp	0.375	CAZ,CRX,NIT
Proteus spp	0.75	CAZ,CRX,GEN,CPR,AUG,AMP
Salmonella spp	0.625	CAZ,CRX,CPR,AUG,NIT
Pseudomonas spp	0.50	CAZ,CRX,AUG,NIT

KEY: Nitrofurantoin (NIT), Amoxicillin/Clavulanate (AUG), Ofloxacin (OFL), Ciprofloxacin (CPR), Ampicillin (AMP), Cefuroxime (CRX), Gentamicin (GEN), and Ceftazidime (CAZ). > 0.2 = MDR, MARI = Multiple Antibiotic Resistance Index, MDR-GNB = Multi-Drug Resistance Gram-Negative Bacteria.

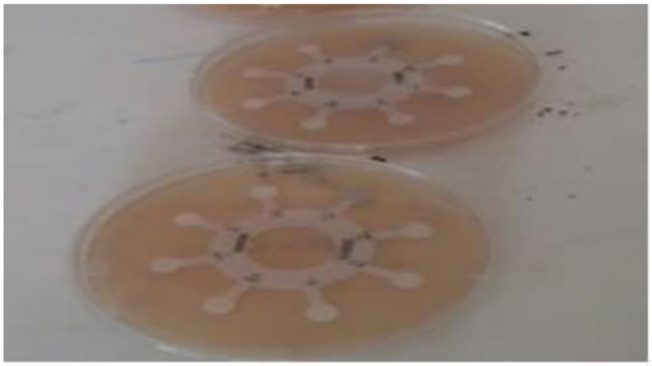

Figure 4: Sensitivity test plates showing zone of inhibitions

3.0 DISCUSSION

This study focused on isolating and identifying Gram-negative bacteria (GNB) from hospital sewage within Sokoto metropolis. By examining the hospital wastewater, the study aimed to understand the prevalence of different bacterial species and their antimicrobial susceptibility profiles. The study founds high bacterial count in hospital sewage within the Sokoto metropolis, which aligns with findings by Lien et al. (2017) in Vietnam, where high bacterial counts were also observed in hospital sewage and effluents. This concentration of bacteria in sludge creates favorable conditions for their growth, and discharging untreated sewage into larger water bodies could further spread bacterial resistance. Similar observations of high bacterial counts in hospital wastewater have been reported by Sib et al. (2019) and Mustapha and Imir (2019).

The study identified Escherichia spp as the most predominant species, constituting 28.24% of the isolates, which aligns with global concerns about its persistence in healthcare wastewater systems, highlighting the need for continuous vigilance in infection control practices. However, this study contrasts with some earlier research (Karungamye et al., 2023; Davidova-Gerzova et al., 2023), which found different species to be more dominant in various geographic areas. This discrepancy emphasizes the variability in MDR-GNB prevalence across regions, likely influenced by local factors such as patient demographics, antibiotic usage, and healthcare practices.

Similarly, the detection Klebsiella spp (18.98%) and Salmonella spp (15.74%) are in significant numbers. These findings are consistent with studies from South Africa and Ghana (Okafor and Nwodo, 2023; Addae-Nuku et al., 2022) and India (Kumawat et al., 2023), emphasizing the global presence of these antibiotic-resistant pathogens in hospital sewage systems. This implies that different MDR-GNB as more prevalent, emphasizing the influence of local epidemiological factors on MDR-GNB composition in healthcare environments.

The study also detected Pseudomonas spp (14.35%), Shigella spp (13.42%), and Proteus spp (9.26%). These results are align with patterns observed by Fiaz et al. (2021) and Aleem et al. (2021), suggesting that these bacteria persist in healthcare wastewater systems worldwide, which emphasized the need for targeted antimicrobial awarenes programs to mitigate their spread.

The study's results correspond to the growing concern over antibiotic resistance as a public health crisis. This study identified a high prevalence of multidrug resistance among Gram-negative bacteria (GNB) isolated from hospital sewage, with varying degrees of resistance observed across different bacterial species to Ceftazidime and Cefuroxime, suggesting the presence of common resistance genes across the species.

Proteus spp exhibited the highest resistance level (75%), which can be attributed to its inherent resistance mechanisms and environmental factors (Stock, 2003; Kanamori et al., 2016). This result disagrees with the findings of the researches conducted by Mustapha and Imir (2019) in which the most resistant organism is the E. coli which showed resistance to all the antibiotics tested (100%).

Klebsiella spp showed the least resistance (37.9%) and remained susceptible to several antibiotics, including gentamicin and ciprofloxacin from the aminoglycoside and quinolone classes, respectively. Additionally, these isolates demonstrated susceptibility to ofloxacin and ampicillin. The final antibiotic to which the Klebsiella spp isolates were susceptible was amoxicillin/clavulanate, with clavulanic acid acting as a β-lactamase inhibitor. This susceptibility profile suggests that these antibiotics remain effective treatment options against Klebsiella spp in this context.

Other species that exhibited multidrug resistance (MDR) included Shigella spp and Salmonella spp, each resisting more than half of the antibiotics tested (62.5%). E. coli and Pseudomonas spp also demonstrated significant MDR, with both resisting 50% of the antibiotics tested. The resistance patterns of Salmonella spp (62.5%) and Pseudomonas spp (50%) align with findings from Ayodele et al. (2023) but contrast with other studies, such as Mustapha and Imir (2019) and Dassy et al. (2022), which reported higher resistance in Klebsiella spp. The results indicate that most of the isolated species are of public health importance, displaying high multiple antibiotic resistance indices (MARI), which suggests extensive antibiotic use in these environments. This finding is consistent with previous studies (Mustapha and Imir, 2019; Daisy et al., 2022; Kanamori et al., 2016). Notably, three of the six species isolated—Pseudomonas spp, Salmonella spp, and Shigella spp—are listed as global priority pathogens by the World Health Organization (WHO, 2017), indicating the significance of these findings in the context of hospital infections.

In this study, all bacterial species isolated from hospital sewage exhibited 100% resistance to both Ceftazidime and Cefuroxime, suggesting the presence of common resistance genes across these species. This aligns with findings from North-Eastern Nigeria, where similar resistance patterns and high MARI values were reported (Mustapha and Imir, 2019). While ciprofloxacin resistance was observed in 50% of the species, other studies have similarly noted increased resistance to amoxicillin/clavulanate due to the continuous release of antibiotics into wastewater, which promotes the selection of resistant bacteria. Although this study could not directly link the sewage isolates to human pathogens treated in the hospitals, the potential for these multidrug-resistant organisms to spread into the environment remains a significant concern. Statistical analysis indicated significant differences between the six bacterial genera, suggesting a non-random distribution and highlighting the predominance of certain genera in the sewage samples.

4.0 CONCLUSION

This research aimed to detect multidrug-resistant Gram-negative bacteria (MDR-GNB) in hospital sewage within Sokoto metropolis. A total of 216 bacterial isolates from six genera—E. coli, Shigella spp, Salmonella spp, Klebsiella spp, Pseudomonas spp, and Proteus spp—were identified. E. coli was the most prevalent, with 61 isolates. All bacteria exhibited resistance to two or more antibiotics, notably showing complete resistance to ceftazidime and cefuroxime. Only Proteus spp and E. coli demonstrated resistance to gentamicin, while all isolates were susceptible to ofloxacin. The number of isolates varied among hospitals, with PHC Durbawa having the highest (29) and PHC Kasarawa the lowest (18). Gagi Clinic, Army Barracks Hospital, and Kofar Kade Clinic also had significant numbers of isolates (28, 24, and 24, respectively). This study underscores the presence of MDR-GNB in hospital sewage, highlighting the urgent need for effective surveillance and control measures in healthcare settings.

5.0 Recommendations

i. Conduct in-depth studies on the genetic mechanisms of antibiotic resistance in prevalent bacteria such as Escherichia coli, Shigella spp, Salmonella spp, Klebsiella spp, Pseudomonas spp, and Proteus spp, with an emphasis on monitoring potential horizontal gene transfer.
ii. Implement rigorous infection control measures in Sokoto hospitals to curb the intra-facility transmission of multidrug-resistant Gram-negative bacteria.
iii. Advocate for an integrated sewage and rainwater management strategy in Sokoto Metropolis to mitigate the spread of multidrug-resistant Gram-negative bacteria.
iv. Develop and enforce tailored antibiotic guidelines for Sokoto hospitals, grounded in local antimicrobial susceptibility profiles, to promote effective and judicious antibiotic use.

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