Original Research Article
Antimicrobial Resistance of some Gram-negative Bacilli Isolated from various sources in Port Harcourt, Nigeria

ABSTRACT

Aim: To isolate and detect the antimicrobial resistance of some Gram-negative Bacilli Isolated from various sources in Port Harcourt, Nigeria

Study design: Cross-sectional study.

Place and Duration of Study: University of Port Harcourt Teaching Hospital, Trans-Amadi and Rumuodomaya Slaughters and Bob-D Ventures Poultry Farms, all in Port Harcourt, Nigeria, between July and December, 2019.

Methodology: This study was conducted at Port Harcourt and Obio/Akpor local government areas of Rivers State, Nigeria from 2019-2020. Hospital wastewater was taken from different sections at the two University Teaching Hospitals- University of Port Harcourt and Rivers State University Teaching Hospitals. Abattoir effluent water samples were taken at different sites from Trans-Amadi and Rumuodumaya Abattoirs. Chicken cloaca samples as well as Hand swab samples of Butchers were collected at the two Abattoirs. All samples were processed following standard procedures and identified organisms were assessed for susceptibilities to different antibiotics following Kirby-Bauer disk diffusion and Microbroth dilution methods. Data were analyzed using SPSS version 22.0. Percentages and *Chi square* were used to summarize the data and p values less than 0.05 were considered significant

Results: Out of a total of 482 samples consisting of Hospital wastewater (84), Chicken cloaca swab (76), Abattoir Effluent water (182), Poultry Dung (96) and Butchers' Hand Swab (44) investigated for possible recovery of some Gram-Negative organisms, 224 target bacterial isolates were identified. *Escherichia coli* showed highest percentage occurrence 124 (25.7%), while *Klebsiella pneumoniae* had 80 (16.5%) and the least rate of occurrence was *Pseudomonas aeruginosa,* 20 (4.1%). A total of 38 bacteria were isolated from the wastewater samples of the two hospitals; 44 were isolated from the Chicken cloaca samples; 80 from Abattoir Effluent Water samples; 50 from Poultry dung and 12 from Butchers' hand swabs. Organisms were most susceptible to Imipenem (97%) and most resistance to Nalidixic acid (92%).

Conclusion: Multiple drug resistance to the commonly used antibiotics is high in the study area. The contamination of wastewater by antibiotics or other pollutants lead to the rise of resistance due to selection pressure. The presence of antibiotic resistance organisms in these wastewaters should not be overlooked. Proper wastewater treatment and improved sanitary measures are advocated.

Keywords: Antimicrobial Resistance, Gram-negative Bacilli, Port Harcourt, Nigeria

1. INTRODUCTION

The discovery of antibiotics marked a milestone in infectious diseases therapy saving millions of lives. However, antibiotics misuse among humans and animals has led to the emergence of antimicrobial resistance. The use of antibiotics in human and animal health care has resulted in the widespread prevalence of antibiotic resistant (ABR) bacteria not only in humans and animals but also in the environment, Example in surface water and soil [1].

As a consequence, the probability of getting exposed to ABR bacteria outside a health care setting has increased.

Antibiotics are used extensively to prevent or to treat microbial infections in humans and animal healthcare. They are also used to promote more rapid growth of livestock. Most of the compounds used in medicine are only partially metabolized by patients and are then discharged into the hospital sewage system or directly into municipal waste water if used at home. The dynamic rise and fast spreading of antimicrobial resistance has been one of the greatest difficulties confronting public health internationally. The accelerated bacterial resistance to antimicrobials has emerged as perhaps the greatest threat to the favorable outcome of infections both in the hospital settings and in the communities.

Antimicrobial resistance (AMR) is a global problem that threatens progress in health and the achievement of sustainable development. Although resistance to antimicrobial is an ancient phenomenon which evolved without human influence in the past, the current increased global presence of AMR is driven by anthropogenic activities [2]. The introduction of antibiotic resistant bacteria and genes (ARB and ARG) into the environment via waste streams from anthropogenic sources is being increasingly recognized as important contributing factor for its prevalence in the human population. There are indications that in low and lower- middle income countries (LIC and LMIC) insufficient sanitation infrastructure and the attendant release of untreated or poorly treated waste water into the environment contributes more to the prevalence of AMR than antibiotic consumption itself. Consequently, polluted environments have been recognized as important reservoirs of AMR in recent times and deserves further attention [3].

Antimicrobial substances used for livestock enter the environment when manure is applied to the fields. These antibiotics may either end up in soil, sediment or in ground water. Antimicrobial agents are also used to treat infections in intensive poultry farming where they are added directly to the water, resulting in high local concentration in the water compartment and adjoining sediments. Hospital wastewater is a key source of environmental pollution especially in the developing countries of the world, where wastewater treatment plants are either not available or ill equipped to handle effective treatment. Wastewater are generated from all the activities in the hospital including medical and non-medical activities from the operating, diagnosis, emergency, first aid, laboratory, radiology, kitchen etc. Based on these activities, wastewater containing high content of enteric pathogens such as bacteria, viruses and helminthes which can be transmitted to humans through water is of great public health concern. The wastewaters that are generated in the wards during patient's treatment are contaminated with enteric pathogens and could be particularly problematic during outbreaks of diseases such as diarrhea.

Uncontrolled and excessive use of antibiotics in human and animal's therapy has been one of the reasons for the increase in antibiotics resistance and the spread of resistance genes in environmental samples such as hospital waste water [4]. Hence, effluents from hospital could augument the number and prevalence of antibiotics resistant bacteria in the environment where the effluents are discharged; this can be by both the means of introduction and selection for resistant bacteria [5].
The use of antimicrobials in veterinary practices and animal husbandry as growth promoters and for therapeutic purposes has led to a sharp rise in antibiotic resistance, as antibiotics fed to animals are not fully metabolized and residual concentrations are passed into the environment via faeces, which could trigger the development of resistance to antibiotics in the normal flora of the intestine and receiving environmental bacteria. The aim of this study was to determine the occurrence of antibiotic resistance among some Gram-Negative Bacilli from various sources in Port Harcourt, Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The Study sites were two Local Government Areas of Rivers State (Obio-Akpor and Port Harcourt City) located in Port-Harcourt, the capital of Rivers State, Niger Delta region of Nigeria.

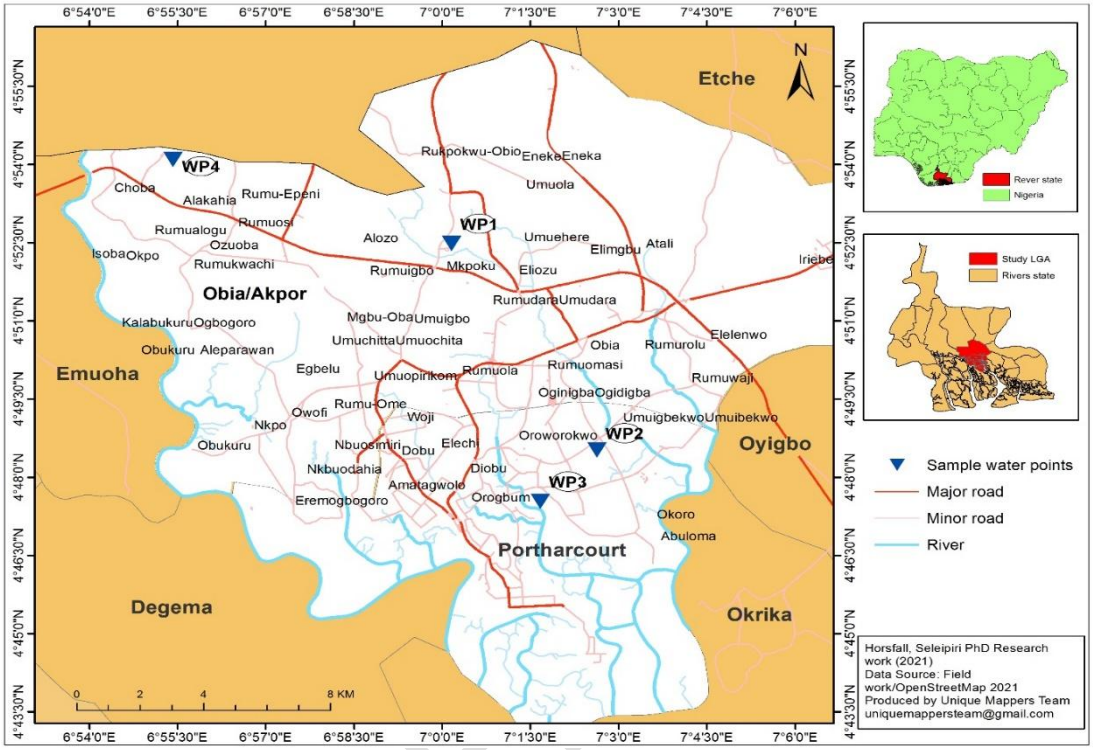

Fig.1: Map of Port Harcourt Metropolis showing location of Sampled Points

2.2 Description of Study Area

This study was undertaken in two different abattoirs located in the metropolitan city of Port Harcourt at Trans-Amadi and Rumuodomaya, in Rivers State, Nigeria (Fig. 1). Trans-Amadi abattoir is the larger and is dominated by manufacturing industries with beehive of activities. It is located at longitude 04 48.442 N and latitude 007 2.303E. Rumuodomaya abattoir is located close to the council headquarters of Obio-Akpor Local Government Area and is located at longitude 04 '52' 48.0 N and latitude 7'58'20.0 E. The two abattoirs are located within market centers. The temperature and humidity of the area is usually high all year round and experiences an annual rainfall of about 70% within April and August and 22% within September and November. Dry and wet seasons occur distinctly in the area.

2.3 Study Population

The study consisted of Hospital wastewater (84 samples), Chicken cloaca swab (76 samples), Abattoir effluent water (182 samples), Poultry dung (96 samples) and Butchers' hand swabs (44 samples). Total population was 482.

2.4 Sample Size

A total of 482 samples consisting of Hospital wastewater (84), Chicken cloaca swab (76), Abattoir effluent water (182), Poultry dung (96) and Butchers' hand swab (44) were investigated for possible recovery of some Gram-Negative organisms (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa)*. Samples were collected based on sample size calculation for Qualitative variables with the formula,

Sample size = $N = \frac{Z^2pq}{d^2}$

Where: p = 0.42, q = 0.58, z = 1.96, d = 0.05, N = 374

2.5 Materials

2.5.1 Nutrient media used for sample processing

Different types of media were: Eosin Methylene Blue Agar (EMB Oxoid, UK) for isolation and purification of *Escherichia coli,* Cysteine Lactose Electrolyte-Deficient Agar (CLED) (Lab M, UK) for the isolation of *Klebsiella* and *E. coli* species, Mac Conkey Agar (Biomark, India) for the lactose terminating organisms, Cetrimide Agar (LAB M, UK) for isolation and purification of *Pseudomonas* sp, Mueller-Jinton Agar (LAB M, UK) for antibiotic sensitivity tests, Nutrient Agar (Fluka, Spain) for the preparation of slants, Selenite-F broth and Chromogenic Agar.

2.6 Sample Collection and Analysis

2.6.2 Sample collection

All samples were collected aseptically with sterile containers/material. Each specimen was clearly labeled. All samples were collected weekly for a period of six (6) months (July–December, 2019). Seventy-Six (76) Cloaca samples were collected with sterile cotton swab from chicken. Samples from apparently healthy chicken species were collected from the cloaca immediately after slaughtering and leaning of the animal at the abattoirs aseptically and were transported to the laboratory immediately. Hospital Wastewater samples were collected in sterile containers, preserved in ice pack and was transported to the laboratory for immediate analysis. Abattoir effluent water samples were collected in sterile containers, preserved in ice pack and was transported to the laboratory immediately. Poultry dung samples from the animal house were also collected with sterile containers and was taken to the laboratory for immediate analysis. Hand Swab samples of chicken processors (Butchers) from the abattoir were also collected with sterile cotton swab and were taken to the laboratory immediately. All samples collected were taken immediately to the laboratory for processing and analysis.

2.6.3 Laboratory Analysis

2.6.3.1 Isolation and identification

Samples were cultured on EMB (OXOID, UK), Mac Conkey (BW Marik, India), SSA (OXOID, UK) and Cetrimide (LAB M, UK) Agar medium for 24hrs at 37℃ to isolate *E. coli, Klebsiella sp,* and *Pseudomonas* spp. The organisms were identified using colonial characteristics, Gram staining and standard biochemical tests such as fermentation of lactose, sucrose, glucose, mannitol, ability to produce indole, nitrated and urease utilization, motility of organisms along with oxidase, methyl red and Voges Proskauer according to Cheesbrough [6].

2.6.3.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility assay was carried out with the use of Kirby-Bauer (1966) disc diffusion technique to assess how sensitive the test organisms was to series of antibiotics such as ciprofloxacin (5µg), cefotaxime (30µg), gentamicin (10µg), aztreonam (30µg), cefpodoxime (10µg), ceftazidime (30µg), tetracycline (10µg), ertapenem (10µg) imipenem (10µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg) (Oxoid, UK).

2.6.3.3 Multiple Antibiotic Resistance (MAR)

Multiple antibiotic resistance (MAR) Index of the selected isolates was analyzed by employing a procedure described by Krumperman et al. [7], which the index is usually estimated thus: dividing the number of antibiotics to which the isolates were resistant to by the total number of antibiotics to which the isolate was exposed to for antibiotic resistance testing.

3.7 Statistical Analysis

Data were analyzed using SPSS version 22.0. Percentages and *Chi square* were used to summarize the data and p values less than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Table 1: Occurrence of Bacteria Isolated from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent water, Poultry Dung and Butchers Hand Swab

No. of Samples	No. Tested	*E. coli* Number (% Isolate)	*Klebsiella* sp Number (% Isolate)	*Pseudomonas sp* Number (% Isolate)
Hospital Waste Water	84	18(21.4)	12(14.2)	8(9.5)
Chicken Cloaca	76	24(31.5)	16(21.0)	4(5.2)
Abattoir Effluent Water	182	48(26.4)	30(16.5)	2(1.1)
Poultry Dung	96	26(27.0)	18(18.7)	6(6.2)
Butchers Hand Swab	44	8(18.1)	4(9.0)	0(0.0)
Total	482	124(25.7)	80(16.5)	20(4.1)

Table 2: Occurrence of Bacteria Isolated from the Hospital Waste Water Samples n (%)

Organism	Sampling Sites UPTH (n=22)	RSUTH (n=16)	χ^2	*p*-value	Total (n=38)
Escherichia coli	12(54.5)	6(37.5)	4.000	0.0455	18(47.3)
***Klebsiella* sp**	6(27.2)	6(37.5)	0.000	>0.9999	12(31.5)
***Pseudomonas* sp**	4(18.1)	4(25.0)	0.000	>0.9999	8(21.0)

χ^2 is the Chi-square value of the comparison between the both locations. P-value is considered significant at 95% confidence interval.

Table 3: Occurrence of Bacteria Isolated from Cloaca of Chicken Samples

Organism	Sampling Site Trans Amadi Abattoir n=20 (%)	Rumuodomaya Abattoir n=24 (%)	χ^2	*p*-value	Total n=44 (%)
Escherichia coli	14(70.0)	10(41.6)	1.333	0.2482	24(54.5)
***Klebsiella* sp**	4(20.0)	12(50.0)	8.000	0.0047	16(36.3)
***Pseudomonas* sp**	2(10.0)	2(8.3)	0.000	>0.9999	4(9.0)

χ^2 is the Chi-square value of the comparison between the both locations. P-value is considered significant at 95% confidence interval.

Table 4: Occurrence of Bacteria Isolated from Abattoir Effluent Water

Organism	Sampling Site Trans Amadi Abbatoir n=34 (%)	Rumuodomaya Abbatoir n=46 (%)	χ^2	*p*-value	Total n=80 (%)
Escherichia coli	20(58.8)	28(60.9)	2.667	0.1025	48(60.0)
***Klebsiella* sp**	14(41.2)	16(34.8)	0.2667	0.6056	30(37.5)
***Pseudomonas* sp**	0(0.0)	2(4.3)	4.000	0.0455	2(2.5)

χ^2 is the Chi-square value of the comparison between the both locations. P-value is considered significant at 95% confidence interval.

Table 5: Occurrence of Bacteria Isolated from Poultry Dung Samples

Organism	Sampling Site: Trans-Amadi Abattoir n=28 (%)	Rumuodomaya Abattoir n=22 (%)	χ^2	*p*-value	Total n=50 (%)
Escherichia coli	16(57.1)	12(54.5)	1.143	0.2850	28(56.0)
***Klebsiella* spp**	8(28.5)	8(36.3)	0.000	>0.9999	16(32.0)
***Pseudomonas* spp**	4(14.2)	2(9.0)	1.333	0.2482	6(12.0)

χ^2 is the Chi-square value of the comparison between the both locations. P-value is considered significant at 95% confidence interval.

Table 6: Occurrence of Bacteria Isolated from Butchers Hand Swab Samples

Organism	Sampling Site: Trans-Amadi Abattoir n=8 (%)	Rumuodomaya Abattoir n=4 (%)	χ^2	*p*-value	Total n=12 (%)
Escherichia coli	6(75.0)	2(50.0)	4.000	0.0455	8(66.6)
***Klebsiella* spp**	2(25.0)	2(50.0)	0.000	>0.9999	4(33.3)
***Pseudomonas* spp**	0(0.0)	0(0.0)	-	-	0(0.0)

χ^2 is the Chi-square value of the comparison between the both locations. P-value is considered significant at 95% confidence interval.

Table 7: Antimicrobial Resistance of 124 *E. coli* Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent Water, Poultry Dung and Butchers' Hand Swab.

Antibiotics	Resistance (%)	Susceptibility (%)
Cefotaxime(30µg)	70(56.4)	54(43.6)
Ceftazidime(30µg)	76(61.2)	48(38.7)
Ceftriaxone(30µg)	68(54.8)	56(45.2)
Cefpodoxime(10µg)	82(66.1)	42(33.9)
Nalidixic acid(30µg)	106(85.5)	18(14.5)
Gentamicin(10µg)	40(32.3)	84(67.7)
Ciprofloxacin(5µg)	26(21.0)	98(79.0)
Tetracycline(30µg)	74(59.7)	50(40.3)
Norfloxacin(30µg)	30(24.2)	94(75.8)
Trimethoprim-Sulfame-Thoxazole(1.25/23.73µg)	92(74.2)	32(25.8)
Imipenem (10µg)	0(0.0)	124(100)

Table 8: Antimicrobial Resistance of 80 *Klebsiella* spp Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent Water, Poultry Dung and Butchers' Hand Swab.

Antibiotics	Resistance (%)	Susceptibility (%)
Cefotaxime(30µg)	56(70.0)	24(30.0)
Ceftazidime(30µg)	42(52.5)	38(47.5)
Ceftriaxone(30µg)	58(72.5)	22(27.5)
Cefpodoxime(10µg)	44(55.0)	36(45.0)
Nalidixic acid(30µg)	80(100)	0(0.0)
Gentamicin(10µg)	54(67.5)	26(32.5)

Ciprofloxacin(5µg)	11(13.7)	69(86.3)
Tetracycline(30µg)	80(100)	0(0.0)
Norfloxacin(30µg)	18(22.5)	62(77.5)
Trimethoprim-Sulfame-Thoxazole(1.25/23.73µg)	68(85.0)	12(15.0)
Imipenem (10µg)	2(2.5)	78(97.5)

Table 9: Antimicrobial Resistance of 20 *Pseudomonas* Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent Water, Poultry Dung and Butchers' Hand Swab.

Antibiotics	Resistance (%)	Susceptibility (%)
Cefotaxime(30µg)	16(80.0)	4(20.0)
Ceftazidime(30µg)	13(65.0)	7(35.0)
Ceftriaxone(30µg)	6(30.0)	14(70.0)
Cefpodoxime(10µg)	14(70.0)	6(30.0)
Nalidixic acid(30µg)	20(100)	0(0.0)
Gentamycin(10µg)	18(90)	2(10)
Ciprofloxacin(5µg)	17(85.0)	3(15.0)
Tetracycline(30µg)	20(100)	0.00
Norfloxacin(30µg)	16(80.0)	4(20.0)
Trimethoprim-Sulfame-Thoxazole(1.25/23.73µg)	17(85.0)	3(15.0)
Imipenem (10µg)	3(15.0)	17(85.0)

Table 10: Antimicrobial Resistance of 224 Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent Water, Poultry Dung and Butchers' Hand Swab.

	***Pseudomonas* sp**		***Klebsiella* sp**		***Escherichia coli***		**All Organisms**	
Antimicrobial Agent	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Cefotaxime(30µg)	16(80.0)	4(20.0)	56(70.0)	24(30.0)	70(56.4)	54(43.6)	142 (63.4)	82 (36.6)
Ceftazidime(30µg)	13(65.0)	7(35.0)	42(52.5)	38(47.5)	76(61.2)	48(38.7)	131 (58.5)	93 (41.5)
Ceftriaxone(30µg)	6(30.0)	14(70.0)	58(72.5)	22(27.5)	68(54.8)	56(45.2)	132 (58.9)	92 (41.1)
Cefpodoxime(10µg)	14(70.0)	6(30.0)	44(55.0)	36(45.0)	82(66.1)	42(33.9)	140 (62.5)	84 (37.5)
Nalidixic acid(30µg)	20(100)	0(0.0)	80(100)	0(0.0)	106(85.5	18(14.5)	206 (92.0)	18 (8.0)
Gentamycin(10µg)	18(90)	2(10)	54(67.5)	26(32.5)	40(32.3)	84(67.7)	112 (50.0)	112 (50.0)
Ciprofloxacin(5µg)	17(85.0)	3(15.0)	11(13.7)	69(86.3)	26(21.0)	98(79.0)	54 (24.1)	170 (75.9)
Tetracycline(30µg)	20(100)	0.00	80(100)	0(0.0)	74(59.7)	50(40.3)	174 (77.7)	50 (22.3)
Norfloxacin(30µg)	16(80.0)	4(20.0)	18(22.5)	62(77.5)	30(24.2)	94(75.8)	64 (28.6)	160 (71.4)
TrimethoprimSulfameThoxazole(1.25/23.73µg)	17(85.0)	3(15.0)	68(85.0)	12(15.0)	92(74.2)	32(25.8)	177 (79.0)	47 (21.0)
Imipenem (10µg)	3(15.0)	17(85.0)	2(2.5)	78(97.5)	0(0.0)	124(100)	5 (2.2)	219 (97.8)

Legend: R= Resistance, S= Susceptible

The use of antimicrobials in veterinary practices and animal husbandry as growth promoters and for therapeutic purposes has led to a sharp rise in antibiotic resistance, as antibiotics fed to animals are not fully metabolized and residual concentrations are passed into the environment, especially, via faeces, which could trigger the development of resistance to antibiotics in the normal flora of the intestine and receiving environmental bacteria. This is in addition to inappropriate use of antibiotics in human medicine.

Out of a total of 482 samples consisting of Hospital wastewater (84), Chicken cloaca swab (76), Abattoir Effluent water (182), Poultry Dung (96) and Butchers' Hand Swab (44), investigated for possible recovery of some Gram-Negative organisms (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa)* (Table 1), total of 224 bacterial isolates were identified. Among the isolates obtained, *Escherichia coli* showed highest percentage occurrence 124 (25.7%), while *Klebsiella pneumoniae* had 80 (16.5%) and the least rate of occurrence was *Pseudomonas aeruginosa,* 20 (4.1%). Several studies have

revealed an array and diversity of bacterial organisms from different environments. In a study on Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia, 113 bacterial isolates were recovered and of these 65 (57.5%) were from hospital environment and 48 (42.5%) were from non-hospital environment [8]. Furthermore, Moges et al. [8] also reported that among the Gram-Negative bacilli, *Klebsiella* spp. 30 (26.6%), was the most frequently identified bacterium, followed by *Pseudomonas* spp. 19(16.8%), *E. coli* (11.5%) and *Citrobacter* spp (11.5%), while the overall prevalence of multiple drug resistance (MDR) in their study was 79/113 (69.9%). In a related study to characterize medically important bacterial diversity, isolated from staff hands, hospital surfaces and wastes in healthcare settings in Kenya, Maina et al. [9] highlighted the presence of *Escherichia coli* (13%), *Pseudomonas aeruginosa* (9.3%) and *Klebsiella pneumonia* (6.36%), amongst others. Similarly, in another study conducted to determine the resistant profile of isolates from hospital waste water of two hospitals in Delta State, Nigeria, *Escherichia coli* was the most prevalent in the two locations, out of 123 Gram-negative isolates. In a study on multiple antibiotic resistance, antibiogram and phenotypic detection of metallo-beta-lactamase (MBL) from *E. coli* of Poultry origin by Ejikeugwu et al. [10], a total of 29 (72.5%) *E. coli* isolates was recovered from 40 cloacal swab samples.

Although the present study did not dwell on diversity of microorganisms in the study population, however reports confirm that diversity varies from one environment to another and one geographical location to another, depending on what substrates are introduced into the environment through anthropogenic and other activities. Antimicrobial resistance has been recognized as a global challenge both in developed and developing countries. Most of the isolates in this study were resistant to one or more antibiotic used, an indication that the organisms have been well exposed to these antibiotics and have developed resistance to them. Organisms in the present study were most susceptible to Imipenem (97%) and most resistance to Nalidixic acid (92%). Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, although carbapenem-resistant isolates have recently been reported.

From the report of Ejikeugwu et al. [10], 29 (72.5%) *E. coli* isolates recovered from 40 poultry cloacal swab samples, showed varying levels of resistance to the carbapenems- imipenem (31%), meropenem (58.6%), ertapenem (75.9%) the quinolone- ciprofloxacin (89.7%) and the cephalosporins – cefoxitin (93.1%), ceftazidime (69.0%) and cefotaxime (55.2%) (Table 7). Multiple antibiotic resistance was detected in 3 (10.3%) of the 29 *E. coli* isolates. Of the 124 *E. coli* isolates from the present work, 24(31.5%) were recovered from chicken cloaca, with high resistances to the cephalosporins - Cefpodoxime (66.1%), Ceftazidime (61.2%), Cefotaxime (56.4%) and Ceftriaxone (54.8%) (Table 7). The *β*-lactam antibiotics are one of the treatment choices for bacterial infections. However, more than half of the organisms were resistant to the common *β*-lactam antibiotics (Table 10). Resistance to these antibiotics have been previously reported. This is a pointer to inappropriate use of antibiotics, especially as growth promoters in poultry farms. One of the most effective mechanisms of resistance to *β*-lactams is the production of *β*-lactamase enzymes such as ESBLs. ESBLs are derived as a result of mutation in as few as one amino acid of their progenitors, resulting in a profound change in enzyme activities of the ESBLs. The ESBLs are able to hydrolyze extended-spectrum cephalosporins (third generation antibiotics) with an oxyimino side chain – aztreonam, hence the extension of spectrum of activity when compared to the parent enzymes. Genes for ESBL enzymes are often carried on plasmids facilitating their rapid dissemination. These genes also carry resistance to other antimicrobial agents. There has been a rapid increase in the number of studies describing the prevalence of ESBL-producing Enterobacteriaceae, worldwide.

4. CONCLUSION

Multiple drug resistance to the commonly used antibiotics is high in the study area. The contamination of wastewater by antibiotics or other pollutants lead to the rise of resistance

due to selection pressure. The presence of antibiotic resistance organisms in these wastewaters should not be overlooked. Proper wastewater treatment and improved sanitary measures are advocated.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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