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# Extended-spectrum β-lactamase Enzymes (ESBLs) and Slime Production of some Gram-negative Bacilli Isolated from Human, Animal and Environmental Sources in Port Harcourt, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors SDA, CKW and EGN designed the study and wrote the protocol, author SJH wrote the first draft of the manuscript, managed the analyses and the literature searches of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To determine extended-spectrum β-lactamase enzymes (ESBLS) and slime production of some Gram-negative Bacilli isolated from human, animal and environmental 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

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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. Isolates from the susceptibility testing with zone diameters found to be resistant to the Cephalosporins were suspected to be ESBL Positive (β-lactamase positive isolates). All isolates were also subjected to detection of slime production by Congo red agar plate method. 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:** The results showed that out of the 224 isolates, 38 (17.0%) were ESBL-producing. ESBL-producers were more frequently isolated from Hospital Wastewaters (26.3%), followed by isolates from Poultry dung samples 12(24.0%) and Abattoir Effluent waters (15.0%). No ESBL-producing bacteria was recovered from the Butchers' Hand swabs. Again, of the 38 ESBL-producing organisms, *E. coli* accounted for 24 (63.2%), followed by *Klebsiella* spp. 8 (21.1%), while *Pseudomonas* sp. recorded 6 (15.8%). Of the 224 isolates, 98 (43.8) were slime-producing, while 23 (60.5%) of the 38 ESBL-producing isolates produced slime.
**Conclusion:** ESBL and slime producing organisms (mostly *E. coli)* were more frequently isolated from Hospital Wastewaters compared to other sample areas.

*Keywords: Extended-spectrum β-lactamase enzymes (ESBLs); slime production; gram-negative bacilli, human; animal, environmental; port harcourt; Nigeria.*

## 1. INTRODUCTION

The emergence and spread of resistance to third-generation cephalosporins (3GCs), mediated mainly by extended-spectrum β-lactamases (ESBLs) [1], is an increasing health problem. An important component of this emergence is mediated by the spread of plasmid-borne ESBL-encoding genes [2]. The CTX-M family of ESBLs currently predominant have appeared in both community and nosocomial settings and has taken over from the SHV and TEM type ESBLs that were predominant in the 1990s [3]. Among these, CTXl-M-15 belonging to the CTX-M-1 group appears to be the most widespread, followed by CTX-M-14, another common variant of the CTX-M enzymes. [4][5].

A specific type of antibiotic resistance that currently represents a major public health concern is the 3$^{rd}$ generation Cephalo-sporin resistance induced by extended spectrum β-lactamase (ESBL) production (5PLOS) [6]. ESBL-producing bacteria are resistant to almost all β-lactam antibiotics, and often to other classes of antibiotics as well. This results in difficult to treat infections and additionally compels the use of so-called last-resort antibiotics; for example, Carbapenems, resulting in increased resistance to these types of antibiotics as well [6]. Carbapenems are broad-spectrum β-lactam antibiotic. They are active against many aerobic and anaerobic Gram-positive and Gram-negative organisms. Globally, the rate of carbapenem hydrolyzing β-lactamase in the midst of bacterial isolates is increasing from various clinical locations [7]. Majority of the bacteria isolate that shelter this catalyst also known as enzymes are of the group Enterobacteriaceae, which are also known to have the ability to live in the human intestinal region as commensals. Similarly, this class of bacteria can easily infect one person to another through portable hand carriage and infected food and water that are consumed by humans, thereby resulting to widespread epidemics and can likely acquire hereditary materials as a result of horizontally transferred gene [8].

In general, hospitals provide an environment conducive for multi-drug resistant bacteria and especially the ESBL-producing bacteria making the treatment options limited and expensive. [9]. The World Health Organization (WHO) published a list of the most critical antimicrobial resistant microorganisms (ARMs) against which new antibiotics need to be developed urgently [10]. Among the highest priority pathogens, extended-spectrum β-lactamases (ESBLs)-producing Enterobacteriaceae were identified as an emerging global threat due to their increasing prevalence in livestock in recent years after being mainly identified in human medicine in the past [11].

Extended-spectrum β-lactamase enzymes (ESBLs) are currently considered one of the major public health concerns throughout the world [10]. The emergence and wide dissemination of this resistance have important implications in public health due to the risk of

clinical treatment failure. The β-lactamases are enzymes expressed by some enzymes which act on, and inactivate the β-lactam antibiotics. All β-lactam antibiotics possess a Carbon 4 atom ring in their structure known as the β-lactam ring [12]. The β-lactamase enzyme breaks open (by hydrolysis). β-lactam moiety of the compound, thereby rendering the drugs ineffective against the bacteria. [13].

Several factors contribute to the spread of ESBLs within and outside of hospital; the overuse of antibiotics in humans and in food producing animals [14] water environment [15] or healthy fecal carriers [16]. All these different sources where ESBL bacteria have been isolated were defined as reservoirs that contribute to ESBL transmission.

Extended spectrum β-lactamases (ESBLs) are of different complex and plasmid-mediated class or enzymes that represent a key therapeutic difficulty in the curing of the affected patients. They consist of β-lactamase that can hydrolyze a more extensive range of β-lactamase antibiotics far more than the simple parent β-lactamases, thus the emergence of the term extended spectrum. An extensive variety of Gram-negative rods have been observed to be producers of ESBLs where most of them are part of the Enterobacteriaceae family [17]. ESBLs are more often generated by E-coli, Klebsiella species, while Klebsiella pneumonia is apparently the Chief producer of ESBL [17].

In a regular microbiological work, the necessity of identify ESBL-producing Gram-negative bacteria is on the increase. Quick identification of ESBL is vital not just for the principles and treatment but to encourage enhanced avoidance of nosocomial diseases (Shah *et al*., 2004). Inability to identify this ESBL-mediated resistance has led to therapeutic failure thereby aiding the quick dissemination of organisms that are generating ESBL [18]. For the sake of efficient medical treatment which is not financially expensive as well, there is very necessity that patients must be screened to detect ESBL production [19].

With the transmission of ESBL-producing types in various environmental sources, it is important to evaluate the predominance of ESBL positive types in Obio/Akpor, and Port-Harcourt Local Government Area in Rivers State in order to make plans for experimental treatment in units where these micro-organisms are found. Therefore, the aim of this study wasup to determine extended-spectrum β-lactamase enzymes (ESBLS) and slime production of some Gram-negative Bacilli isolated from human, animal and environmental sources in Port Harcourt, 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.

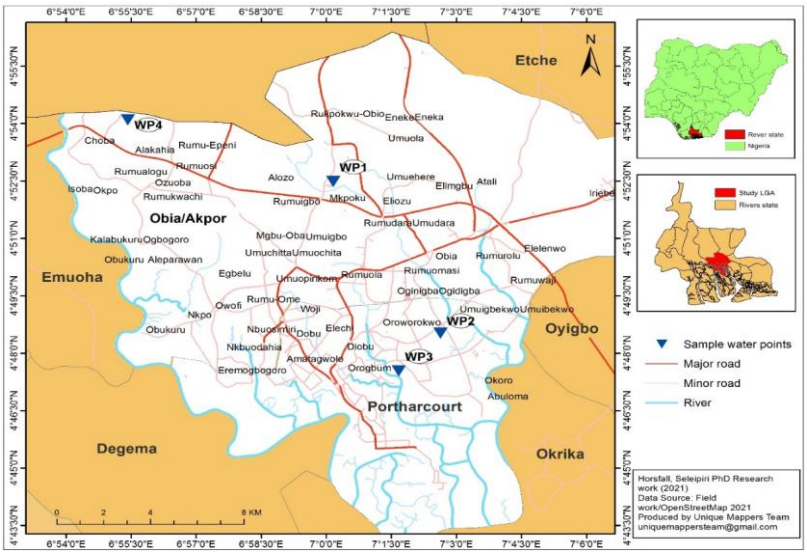


**Fig.1. Map of Port Harcourt Metropolis showing location of Sampled Points**

## 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 Samples

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 samples were 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

### 2.6.1 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.

### 2.6.2 Laboratory Analysis

*2.6.2.1 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 [20].

*2.6.2.2 Detection of ESBL Producing Stains*

Isolates from the susceptibility testing with zone diameters found to be resistant to the Cephalosporins such as Cefpodoxime (≤ 27mm), Cefotaxime, (≤ 27mm) Ceftazidime (≤ 22mm) and Aztreonam (≤ 27mm) were suspected to be ESBL Positive (β-lactamase positive isolates).

*2.6.2.3 Double Disc Synergy Test for ESBL*

Isolates found to be resistant to Ceftazidime, Cefotaxime were constrained to the Double Disc Synergy Test to test for the presence of ESBL producing enzymes. Isolates were streaked on the surface of Muller-Hinton agar plates and disc containing the standard concentration of Azteronam, Ceftazidime, Cefpodoxime, Cefotaxime were placed 15mm from Amoxicillin/Clavulanic acid combination disc. The inoculated media were incubated all night at a temperature of 37℃. An improved zone of inhibition between that of the β-lactam discs and the β-lactamase inhibitor combination disc was explained as confirmation for the existence of an ESBL.

*2.6.2.4 Detection of Slime Production*

All isolates *(E. coli, K. pneumoniae and P. aeruginosa)* were subjected to detection of slime production by Congo red agar plate method as described by Freeman et al. [21].

## 2.7 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. Prevalence of Extended Spectrum β-lactamase-Producing Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent water, Poultry dung and Butchers Hand swab from different locations in Port Harcourt**

| Sample | Number Tested | Number of Bacterial Isolates | Number of ESBL Producers (%) |
|---|---|---|---|
| Hospital Waste Water | 84 | 38 | 10(26.3) |
| Chicken Cloaca | 76 | 44 | 4(9.0) |
| Abattoir Effluent Water | 182 | 80 | 12(15.0) |
| Poultry Dung | 96 | 50 | 12(24.0) |
| Butchers Hand Swab | 44 | 12 | 0(0.00) |
| **Total** | **482** | **224** | **38(17.0)** |

**Table 2. Distribution of ESBL-Producers among Bacterial Isolates**

| Sample Type | Total Isolates Tested | *E. coli* | | *Klebsiella* spp | | *Pseudomonas* spp | |
|---|---|---|---|---|---|---|---|
| | | No. Tested | No. positive (%) | No. Tested | No. positive (%) | No. Tested | No. positive (%) |
| Hospital Waste Water | 38 | 18 | 6 (33.3) | 12 | 0 (0) | 8 | 4 (50) |
| Chicken Cloaca | 44 | 24 | 2 (8.3) | 16 | 2 (12.5) | 4 | 0 (0) |
| Abattoir Effluent Water | 80 | 48 | 8 (16.7) | 30 | 4 (13.3) | 2 | 0 (0) |
| Poultry Dung | 50 | 26 | 8 (30.8) | 18 | 2 (11.1) | 6 | 2 (33.3) |
| Butchers Hand Swab | 12 | 8 | 0 (0.0) | 4 | 0 (0.0) | 0 | 0 (0.0) |
| **TOTAL** | 224 | 124 | 24 (19.4) | 80 | 8 (10) | 20 | 6 (30) |
| **% of 38 ESBL Producers** | | | 24 (63.2) | | 8 (21.1) | | 6 (15.8) |

**Table 3. Distribution of ESBL-Producing Bacterial Isolates by One-Health Approach (Human, Animal and Environment)**

| Sample Type | Total Isolates Tested | *E. coli* | | *Klebsiella spp* | | *Pseudomonas spp* | |
|---|---|---|---|---|---|---|---|
| | | No. Tested | No. positive (%) | No. Tested | No. positive (%) | No. Tested | No. positive (%) |
| Environment | 168 | 92 | 22 (23.9) | 60 | 6 (10) | 16 | 6 (37.5) |
| Animal | 44 | 24 | 2 (8.3) | 16 | 2 (12.5) | 4 | 0 (0) |
| Human | 12 | 8 | 0 (0.0) | 4 | 0 (0.0) | 0 | 0 (0.0) |
| **TOTAL** | 224 | 124 | 24 (19.4) | 80 | 8 (10) | 20 | 6 (30) |
| **% of 38 ESBL Producers** | | | 24 (63.2) | | 8 (21.1) | | 6 (15.8) |

**Table 4. Occurrence of ESBL Producing organisms according to location in PH**

| Location | No. of Isolates | Number of ESBL Organisms | | | Total No. (%) n = 38 |
|---|---|---|---|---|---|
| | | *E. coli* | *Klebsiella sp* | *Pseudomonas spp* | |
| UPTH Waste Water | 22 | 2 | 0 | 4 | 6(15.8) |
| RSUTH Waste Water | 16 | 4 | 0 | 0 | 4(10.5) |
| Trans Amadi Abbatoir Effluent water | 34 | 4 | 2 | 0 | 6(15.8) |
| Rumuodomaya Abbatoir Effluent water | 46 | 4 | 0 | 2 | 6(15.8) |
| Trans Amadi Poultry Farm Dung | 28 | 4 | 2 | 0 | 6(15.8) |
| Rumuodomaya Poultry Farm Dung | 22 | 4 | 0 | 0 | 4(10.5) |
| Chicken Cloaca (Trans Amadi) | 20 | 2 | 2 | 0 | 4(10.5) |
| Chicken Cloaca (Rumuodomaya) | 24 | 0 | 2 | 0 | 2(5.3) |
| Hand Swab (Trans Amadi) | 8 | 0 | 0 | 0 | 0 |
| Hand Swab (Rumuodomaya) | 4 | 0 | 0 | 0 | 0 |
| **Total (%)** | **224** | **24** | **8** | **6** | **38(100.0)** |

**Table 5. Number of *E. coli* Positive for Extended Spectrum β-Lactamase Production**

| Sample | No. of Isolates | Positive Number (%) |
|---|---|---|
| Hospital Waste Water | 18 | 6(33.3) |
| Chicken Cloaca | 24 | 2(8.3) |
| Abattoir Effluent Water | 48 | 8(16.7) |
| Poultry Dung | 26 | 8(30.7) |
| Butchers' Hand Swab | 8 | 0(0.0) |
| **Total** | **124** | **24(19.3)** |

**Table 6. Number of *Klebsiella* Positive for Extended Spectrum β-Lactamase Production**

| Sample | No. of Isolates | Positive Number (%) |
|---|---|---|
| Hospital Wastewater | 12 | 0(0.0) |
| Chicken Cloaca | 16 | 2(12.5) |
| Abattoir Effluent Water | 30 | 4(13.3) |
| Poultry Dung | 18 | 2(11.1) |
| Hand Swab | 4 | 0(0.0) |
| **Total** | **80** | **8(10.0)** |

**Table 7. Number of *Pseudomonas* Positive for Extended Spectrum β-Lactamase Production**

| Sample | No. of Isolates | Positive Number (%) |
|---|---|---|
| Hospital Waste Water | 8 | 4(50.0) |
| Chicken Cloaca | 4 | 0(0.0) |
| Abattoir Effluent Water | 2 | 0(0.0) |
| Poultry Dung | 6 | 2(33.3) |
| Butchers' Hand Swab | 0 | 0(0.0) |
| **Total** | **20** | **6(30.0)** |

**Table 8. Distribution of Slime-Producing Gram-Negative Bacterial Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent water, Poultry dung and Butchers Hand swab from different locations in Port Harcourt**

| Population screened | No. Screened | No. Positive (%) Slime Producers | No. Negative (%) Non-Slime Producers | P– value | $X^2$-Value |
|---|---|---|---|---|---|
| **Total Isolates** | 224 | 98 (43.8) | 126 (56.2) | | |
| ESBL-Positive | 38 | 23 (60.5) | 15 (39.5) | 0.194 | 1.684 |
| ESBL-Negative | 186 | 75 (40.3) | 111 (59.7) | 0.008 | 6.968 |
| ***Escherichia coli*** | 124 | 52 (41.9) | 72 (58.1) | | |
| ESBL-Positive | 24 | 14 (58.3) | 10 (41.7) | 0.414 | 0.667 |
| ESBL-Negative | 100 | 38 (38.0) | 62 (62.0) | 0.016 | 5.760 |
| ***Klebsiella*** sp | 80 | 34 (42.5) | 46 (57.5) | | |
| ESBL-Positive | 8 | 5 (62.5) | 3 (37.5) | 0.480 | 0.500 |
| ESBL-Negative | 72 | 29 (40.3) | 43 (59.7) | 0.099 | 2.722 |
| ***Pseudomonas*** sp | 20 | 12 (60.0) | 8 (40.0) | | |
| ESBL-Positive | 6 | 4 (66.7) | 2 (33.3) | 0.667 | 0.414 |
| ESBL-Negative | 14 | 8 (57.1) | 6 (42.9) | 0.593 | 0.286 |
| **Summary** | | | | | |
| ***Escherichia coli*** | 124 | 52 (41.9) | 72 (58.1) | 0.072 | 3.226 |
| ***Klebsiella*** sp | 80 | 34 (42.5) | 46 (57.5) | 0.180 | 1.800 |
| ***Pseudomonas*** sp | 20 | 12 (60.0) | 8 (40.0) | 0.371 | 0.800 |

**Table 9. Antimicrobial Resistance between Slime-Producing and Non-Slime-Producing Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent water, Poultry dung and Butchers Hand swab from different locations in Port Harcourt**

| Antimicrobial Agent | Total Resistance | Resistance to Slime Producers (n=98) | | Resistance to Non-Slime Producers (n=126) | | | |
|---|---|---|---|---|---|---|---|
| | | R | R (%) | R | R (%) | p-value | $X^2$-Value |
| Cefotaxime(30µg) | 142 | 78 | 79.6 | 64 | 50.8 | 0.240 | 1.380 |
| Ceftazidime(30µg) | 131 | 69 | 70.4 | 62 | 49.2 | 0.541 | 0.374 |
| Ceftriaxone(30µg) | 132 | 68 | 69.4 | 64 | 50.8 | 0.728 | 0.121 |
| Cefpodoxime(10µg) | 140 | 77 | 78.6 | 63 | 50.0 | 0.237 | 1.400 |
| Nalidixic acid(30µg) | 206 | 90 | 91.8 | 116 | 92.1 | 0.070 | 3.282 |
| Gentamicin(10µg) | 112 | 61 | 62.2 | 51 | 40.5 | 0.345 | 0.893 |
| Ciprofloxacin(5µg) | 54 | 31 | 31.6 | 23 | 18.3 | 0.276 | 1.185 |
| Tetracycline(30µg) | 174 | 89 | 90.8 | 85 | 67.5 | 0.762 | 0.092 |
| Norfloxacin(30µg) | 64 | 36 | 36.7 | 28 | 22.2 | 0.317 | 1.000 |
| Trimethoprim-Sulfame-Thoxazole(1.25/23.73µg) | 177 | 92 | 93.9 | 85 | 67.5 | 0.599 | 0.277 |
| Imipenem | 5 | 3 | 7.9 | 2 | 1.1 | 0.655 | 0.200 |

**Table 10. Antimicrobial Resistance between ESBL-Producing and Non-ESBL-Producing Isolates from Hospital Wastewater, Chicken Cloaca, Abattoir Effluent water, Poultry Dung and Butchers Hand swab from different locations in Port Harcourt**

| Antimicrobial Agent | Total Resistant Isolate | Resistance to ESBL Producers (n=38) | | Resistance to Non-ESBL Producers (n=186) | | | |
|---|---|---|---|---|---|---|---|
| | | R | R (%) | R | R (%) | p-value | $X^2$Value |
| Cefotaxime(30µg) | 142 | 32 | 84.2 | 110 | 59.1 | < 0.001 | 42.845 |
| Ceftazidime(30µg) | 131 | 31 | 71.6 | 100 | 53.8 | < 0.001 | 36.344 |
| Ceftriaxone(30µg) | 132 | 27 | 71.1 | 105 | 56.5 | < 0.001 | 46.091 |
| Cefpodoxime(10µg) | 140 | 26 | 68.4 | 114 | 61.3 | < 0.001 | 55.314 |
| Nalidixic acid(30µg) | 206 | 31 | 81.6 | 175 | 94.1 | < 0.001 | 100.660 |
| Gentamicin(10µg) | 112 | 19 | 50.0 | 93 | 50.0 | < 0.001 | 48.893 |
| Ciprofloxacin(5µg) | 54 | 16 | 42.1 | 38 | 20.4 | < 0.001 | 8.963 |
| Tetracycline(30µg) | 174 | 29 | 76.3 | 145 | 78.0 | < 0.001 | 77.333 |
| Norfloxacin(30µg) | 64 | 14 | 36.8 | 50 | 26.9 | < 0.001 | 20.250 |
| Trimethoprim-Sulfame-Thoxazole(1.25/23.73µg) | 177 | 37 | 97.4 | 140 | 75.3 | < 0.001 | 59.938 |
| Imipenem | 5 | 3 | 7.9 | 2 | 1.1 | 0.655 | 0.200 |

The problem of antibiotic resistance, exacerbated by ESBL-producing organisms, is increasing on a global scale with major outbreaks being reported and this has led to the concept of one-health approach (human, animal and environment) in dealing wholistically with the problem.

The present study assessed ESBL-production among the target bacterial isolates. Of the 224 isolates tested, 38 (17%) were ESBL-producing organisms, with *E. coli* accounting for 24 (63.2%), followed by *Klebsiella* spp. 8 (21.1%), while *Pseudomonas* sp. recorded 6 (15.8%) (Table 2). Furthermore, the ESBL-producers were more frequently isolated from Hospital Wastewaters (26.3%), followed by isolates from Poultry dung samples 12(24.0%) and Abattoir Effluent waters (15.0%) (Table 4). In a study by Zakir et al*.* [22] to investigate the bacterial diversity, antimicrobial resistance patterns and types of β-lactamase genes in Gram-negative bacteria isolated from a hospital sewage treatment plant, of the 221 isolates identified, 40% were characterized as extended-spectrum β-lactamase (ESBL) producers, with the most common being *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli*. Nagano et al., [23] recovered a total of five (15.15%) ESBL producers in the order- 3 *Escherichia coli*, 2 *Pseudomonas aeruginosa* and 1 *Klebsiella pneumoniae.*

In general, an isolate is suspected to be an ESBL producer when it shows *in vitro* susceptibility to the second-generation cephalosporins (cefoxitin, cefotetan) but resistance to the third-generation cephalosporins and to aztreonam. Moreover, one should suspect these strains when treatment with these agents for Gram-negative infections fails despite reported *in vitro* susceptibility. Once an ESBL-producing strain is detected, the laboratory should report it as "resistant" to all penicillins, cephalosporins, and aztreonam, even if it is tested (in vitro) as susceptible. Associated resistance to aminoglycosides and trimethoprim - sulfamethoxazole, as well as high frequency of co-existence of fluoroquinolone resistance, creates problems. β-lactamase inhibitors such as clavulanate, sulbactam, and tazobactam *in vitro* inhibit most ESBLs, but the clinical effectiveness of β-lactam/ β-lactamase inhibitor combinations cannot be relied on consistently for therapy. *In vivo* studies have yielded mixed results against ESBL-producing *K. pneumoniae*. (Cefepime, a fourth-generation cephalosporin, has demonstrated *in vitro* stability in the presence of many ESBL/AmpC strains.) Currently, carbapenems are, in general, regarded as the preferred agent for treatment of infections due to ESBL-producing organisms. Carbapenems are resistant to ESBL-mediated hydrolysis and exhibit excellent *in vitro* activity against strains of Enterobacteriaceae expressing ESBLs, and this accounts for the higher level of susceptibility recorded against the imipenem (Table 10).

Ability of the organisms to produce slime was also evaluated using colonial morphology of isolates on Congo red agar. Formation of black colonies of dry consistency is characteristic for slime-producing strains. A biofilm is defined as a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each

other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. Bacteria in the biofilm exhibit increased resistance to components of the host's immune system and antimicrobial agents, as compared to their planktonic counterparts. Biofilm-forming ability has been increasingly recognized as an important virulence factor in many microorganisms.

Formation of a biofilm begins with the attachment of bacteria to the host cells. This specific adhesion pattern is mediated by bacterial cell wall structures containing adhesins, which is a genetically determined feature of bacterial species. Adherence of *Staphylococcus* species to the host cells is mediated by specific cell-surface proteins such as fibronectin, fibrinogen and collagen. The slime is viscous extra-capsular layer, weakly immunogenic and of labile structure which is lost or partially lost on *in vitro* subcultures [24]. Slime production is a virulence marker for clinically significant isolates Ishak et al. [25]. Slime-producing strains are considered to have increased ability of colonizing host tissue and better protection from opsonization and phagocytosis [25]. The importance of the role played by slime is further increased by its frequent association to reduced antibiotic susceptibility. The difficulty in eradicating a chronic infection associated with slime formation has been reported, and slime-producing bacteria have been shown to resist higher antibiotic concentrations than non-slime-producing bacteria Gristina et al. [26]. Antibiotics are effective in inhibiting planktonic bacterial population, whereas bacteria in biofilm survive the treatment and provide material for further growth. The mechanisms by which the biofilm provides bacteria with higher antibiotic resistance have yet to be completely elucidated.

In the present study, slime production was detected by the Congo Red Agar test. 98 (43.8%) of the study isolates tested positive (Table 8). Turkyilmaz and Eskiizmirliler, [27], using a similar method had reported a 61.1% rate of production of slime factor in all the organisms investigated. Furthermore, nature of infection or sample collection site may also have influenced the rate observed. Davenport et al. [28] had established a link between the production of slime and the resistance to antibiotics. Diaz- Mitoma et al. [29] also found an association between antibiotic failure and slime production. Turkyilmaz and Eskiizmirliler, [27] reported a higher resistance of slime-producing strains of microorganisms to antimicrobial agents tested in comparison to non-slime-producing strains. In the present study, the comparison of slime-producing strains with non-slime-producing strains revealed significant difference ($p<.05$) in resistance to antimicrobial agents investigated (Table 9). Similarly, there was significant association ($p<.05$) between ESBL production and slime production.

## 4. CONCLUSION

Based on the findings, we conclude that ESBL and slime producing organisms (mostly *E. coli)* were more frequently isolated from Hospital Wastewaters compared to other sample areas.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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