Original Research Article

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

.

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 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 (Beta 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 3rd generation Cephalo-sporin resistance induced by extended spectrum beta-lactamase (ESBL) production (5PLOS) [6]. ESBL-producing bacteria are resistant to almost all beta-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; Example, Carbapenems, resulting in increased resistance to these types of antibiotics as well [6]. Carbapenems are broad-spectrum beta-lactam antibiotic. They are active against many aerobic and anaerobic Gram-positive and Gram-negative organisms. Globally, the rate of carbapenem hydrolyzing beta-lactamase in the midst of bacterial isolates is increasing from various clinical locations [7]. Majority of the bacteria isolate that shelter this catalyst alsoknown 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 Beta-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 Beta-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 beta-lactamases that can hydrolyze a more extensive range of beta-lactam antibiotics far more than the simple parent beta-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.

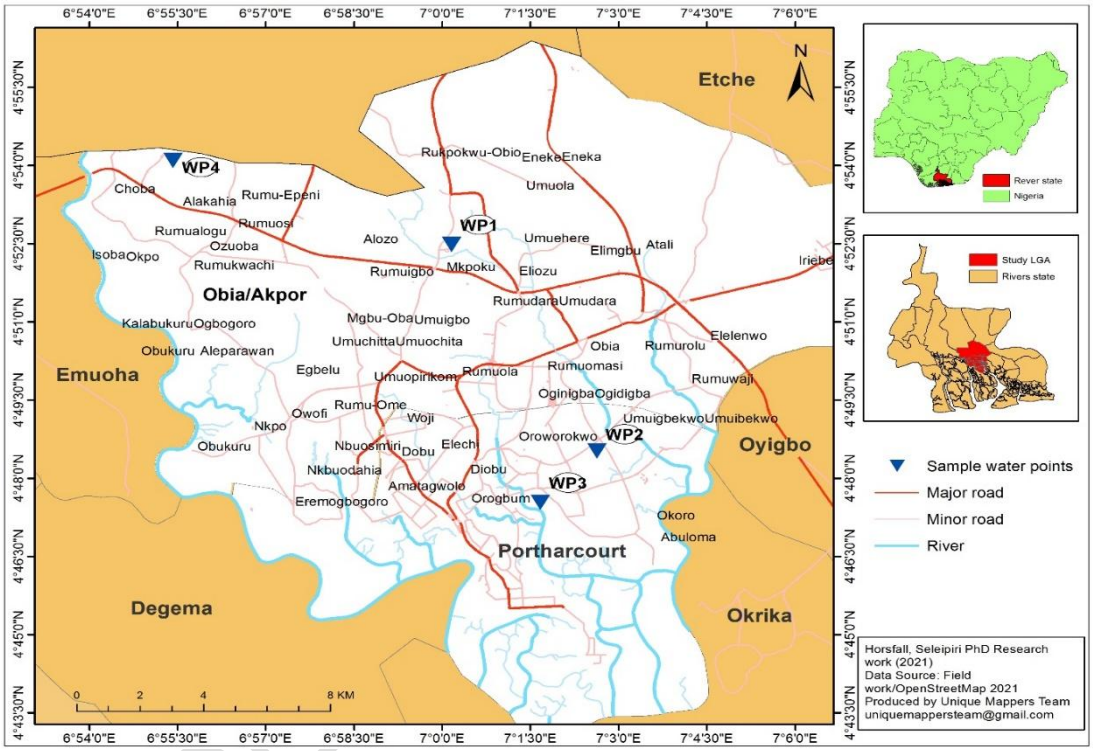

Fig.1: Map of Port Harcourt Metropolis showing location of Sampled Points

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 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 [20].

2.6.3.2 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 (Beta lactamase positive isolates).

2.6.3.3 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 beta-lactamase inhibitor combination disc was explained as confirmation for the existence of an ESBL.

2.6.3.4 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].

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: Prevalence of Extended Spectrum Beta-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.	No.	No.	No.	No.	No.

		Tested	positive (%)	Tested	positive (%)	Tested	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 Beta-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 Beta-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 Beta-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^2Value
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 beta-lactamase genes in Gram-negative bacteria isolated from a hospital sewage treatment plant, of the 221 isolates identified, 40% were characterized as extended-spectrum beta-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. Beta-lactamase inhibitors such as clavulanate, sulbactam, and tazobactam *in vitro* inhibit most ESBLs, but the clinical effectiveness of beta-lactam/beta-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.

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.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. 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.

REFERENCES

1. Coque, T. M., Novais, Â. Carattoli, A., Poirel, L., Pitout, J., Peixe, L., … Nordmann, P. Dissemination of clonally RelatedEscherichia coliStrains expressing extended-spectrum β-lactamase CTX-M-15. *Emerging Infectious Diseases*, 2008; 14(2): 195-200.
2. Carattoli, A. Plasmids and the spread of resistance. *International Journal of Medical Microbiology*, 2013; 303(6-7): 298-304.
3. Cantón, R., & Coque, T. M. The CTX-M β-lactamase pandemic. *Current Opinion in Microbiology*, 2006; *9*(5): 466-75.
4. Pitout, J. D., & Laupland, K. B. Extended-spectrum β-lactamase-producing Enterobacteriaceae: An emerging public-health concern. *The Lancet Infectious Diseases*, 2008; 8(3): 159-66.
5. Woerther, P., Burdet, C., Chachaty, E., & Andremont, A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: Toward the globalization of CTX-M. *Clinical Microbiology Reviews*, 2013; *26*(4): 744-58.
6. Cantón, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F., & Coque, T. Prevalence and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae in Europe. *Clinical Microbiology and Infection*, 2008; 14: 144-53.
7. Nordmann, P., Naas, T., & Poirel, L. Global spread of carbapenemase-producingenterobacteriaceae. *Emerging Infectious Diseases*, 2011; 17(10): 1791-8.
8. Akinniyi, A., Afolabi, O., Iwalokun, B., Oluwaseun, E., & Onagbesan, K. Clonal dissemination of blaTEM S-lactamase strains among enteric isolates in Abeokuta, Nigeria. *Research Journal of Microbiology*, 2011; *6*(12): 919-25.
9. Magiorakos, A., Srinivasan, A., Carey, R., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S., Hindler, J., Kahlmeter, G., … Monnet, D. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, 2012; 18(3): 268-81.
10. World Health Organization. World Health Organization publishes list of bacteria for which new antibiotics are urgently needed. News release, Geneva. 2017.
11. Enoch, D., Brown, F., Sismey, A., Mlangeni, D., Curran, M., Karas, J., Cone, D., Aliyu, S., Dhanji, H., Doumith, M., Maharjan, S., Meunier, D., & Woodford, N. Epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae

in a UK district hospital; an observational study. *Journal of Hospital Infection*, 2012; 81(4): 270-7.
12. Holten K. B. and Onusko E. M. Appropriate prescribing of oral beta-lactam antibiotics. American Family Physician, 2000; 62(3): 611-20
13. Rubtsova M. Y., Ulvashora M. M., Bachmann T. T., Schid R. D. and Egorov A. M. Multiparametric determination of genes and their point mutations for identification of beta-lactamases. Biochemistry, 2010; 75(13): 1628-49.
14. Angulo, F. J., Nargund, V. N., & Chiller, T. C. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. *Journal of Veterinary Medicine Series B*, 2004; 51(8-9): 374-9.
15. Korzeniewska, E., & Harnisz, M. Extended-spectrum beta-lactamase (ESBL)-positive Enterobacteriaceae in municipal sewage and their emission to the environment. *Journal of Environmental Management*, 2013; 128: 904-11.
16. Livermore, D. M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G. M., Arlet, G., Ayala, J., Coque, T. M., Kern-Zdanowicz, I., Luzzaro, F., Poirel, L., & Woodford, N. CTX-M: Changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy*, 2006; 59(2): 165-74.
17. Bradford, P. A. Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*, 2001; 14(4): 933-51.
18. Paterson, D. L., & Bonomo, R. A. Extended-spectrum β-lactamases: A clinical update. *Clinical Microbiology Reviews*, 2005; 18(4): 657-86.
19. Siegel, J. D., Rhinehart, E., Jackson, M., & Chiarello, L. Management of multidrug-resistant organisms in health care settings, 2006. *American Journal of Infection Control*, 2007; 35(10): 165-93.
20. Monica, C. District Laboratory Practice in Tropical countries, part 2 Cambridge University Press, Great Britain. 132-143. 2006.
21. Freeman, D.J., Falkiner, F.R., Keane and C.T. New method for detecting slime production by coagulase negative staphylococci, J Clin Pathol, 1989; 42: 872-4.
22. Zakir, A., Dadi, B.R., Aklilu, A. and Oumer, Y. Investigation of Extended-Spectrum β-Lactamase and Carbapenemase Producing Gram-Negative Bacilli in Rectal Swabs Collected from Neonates and Their Associated Factors in Neonatal Intensive Care Units of Southern Ethiopia. Infect Drug Resist, 2021; 23 (14): 3907-17.
23. Nagano, N., Suzuki M., Wachino J.I., Kimura K. and Arakawa Y. ESBL-producing Escherichia coli and Its Rapid Rise among Healthy People. Food Safety, 2017; 5(4):122-50
24. Martínez-Aguilar, G., Alpuche-Aranda, C. M., Anaya, C., Alcantar-Curiel, D., Gayosso, C., Daza, C., Mijares, C., Tinoco, J. C., & Santos, J. I. Outbreak of nosocomial sepsis and pneumonia in a newborn intensive care unit by Multiresistant extended-spectrum β-lactamase-Producing klebsiella pneumoniae high impact on mortality. *Infection Control & Hospital Epidemiology*, 2001; *22*(11): 725-8.
25. Ishak, M.A., Groschel, D.H., Mandell, G.L. and Wenzel, R.P. Association of slime with pathogenicity of coagulase-negative staphylococci causing nosocomial septicemia, J Clin Microbiol, 1985; 22: 1025- 9.
26. Gristina AG, Hobgood CD, Webb LX, Myrvik QN. Adhesive colonization of biomaterials and antibiotic resistance. Biomaterials, 1987; 8: 423-6.
27. Turkyilmaz, S. and Eskiizmirliler, S. Detection of slime factor production and antibiotic resistance in Staphylococcus strains isolated from various animal clinical samples. Turkish Journal of Veterinary and Animal Sciences, 2006; 30(2): 201-6
28. Davenport, A.J., Cross, R.S., Watson, K.A., Liao, Y., Shi, W., Prince, H.M., Beavis, P.A., Trapani, J.A., Kershaw, M.H., Ritchie, D.S., Darcy, P.K., Neeson, P.J. and Jenkins, M.R. Chimeric antigen receptor T cells form non-classical and potent immune synapses driving rapid cytotoxicity. Proc Natl Acad Sci U S A, 2018; 27:115-9.

29. Diaz-Mitoma, F., Harding, G.K.M., Hoban, D.J., Roberts, R.S., and Low, D.E. Clinical significance of a test for slime production in ventriculoperitoneal shunt infections caused by coagulase-negative staphylococci. J Infect Dis, 1987; 156: 555–9.