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# ORIGINAL RESEARCH

# Extended Spectrum Beta-Lactamases (ESBLs)-producing Escherichia coli and Klebsiella pneumoniae among asymptomatic Out-patients in a University Health Centre Deji-Agboola AM\*1, Olaosebikan OR1, Adenipekun E2, Osinupebi OA1, Olajubu FA3

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### Abstract

**Background:** Asymptomatic carriage and spread of Extended-Spectrum Beta-Lactamase (ESBLs)-producing Enterobacteriaceae in the community are potential risk factors for transmission of infection.

**Objective:** To determine the prevalence of ESBL resistant genes in *Escherichia coli* and *Klebsiella pneumoniae* isolated from asymptomatic out-patients.

**Methods:** Using a questionnaire, demographic information, medical history, previous hospitalization and antibiotics used were obtained. Stool and urine samples were collected from 350 participants, cultured, and the susceptibility of the isolates to antibiotics and ESBL production were determined using the disk diffusion method. ESBL genes such as  $bla_{TEM}$ ,  $bla_{CTX}$ , and  $bla_{SHV}$  were identified using the Polymerase Chain Reaction.

**Results:** *Escherichia coli* and *Klebsiella pnuemoniae* were identified from the stool samples (256; 69.9% and 89; 24.4% respectively) and urine samples (15; 4.1% and 6; 1.6% respectively). The isolates were susceptible to imipenem (330; 90.6%) and nitrofurantoin (307; 80.4%), most of the isolates were resistant to fluoroquinolones, cephalosporins, and aminoglycosides while all the isolates were resistant to ampicillin. The prevalence of ESBL was 29 (8.3%) and was observed in *Escherichia coli* (19; 7.0%) and *Klebsiella pneumoniae* (11; 12.0%), including a dual carriage. The ESBL carriers were resistant to the cephalosporins, fluoroquinolones and aminoglycosides. CTX-M (20; 66.7%), TEM (14; 46.7%), CTX-M and TEM genes co-existed in 9 (30.0%) while no SHV gene was detected in the isolates. Age, sex, prior hospitalization and antibiotics use did not predispose to ESBL carriage.

**Conclusion:** Asymptomatic carriage of ESBL producing enterobacteria in the participants indicates that they can serve as a reservoir of the gene encoding for antibiotic resistance.

Keywords: Asymptomatic carriers, Expanded Spectrum Beta-Lactamase, Enterobacteriaceae, Resistant genes.

### Introduction

The emergence of Extended Spectrum Beta-Lactamases (ESBLs)-producing Enterobacteriaceae infections has become alarming because it has compromised the efficacy of major classes of antimicrobials. [1] The ESBL enzymes are driven by mobile genetic elements leading to the dissemination

of clones in community and hospital-acquired infections. Resistance to Beta-Lactam antimicrobial drugs among pathogenic Gramnegative bacteria is usually mediated by several genetic enzymatic factors of the pathogens, including the production of Extended-Spectrum Beta-Lactamases (ESBLs). [2]

ESBLs constitute a major group of enzymes that mediate resistance of Enterobacteriaceae to Beta-Lactam antimicrobial drugs, such as the third-generation cephalosporins and have been reported in Escherichia coli and Klebsiella pneumonia. [2] The enzymes are carried by mobile ESBLs genes which confer resistance to oxy-imino-cephalosporin and other Beta-Lactam antibiotics; they are also found on encoding plasmid resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole. [3] Most of the ESBLs enzymes are encoded blatem, blashv, blaCTX-M and blaOXAgene families. The CTX-M type of enzymes has shown rapid and alarming dissemination and is recognised as the most prevalent causative agent of hospital and community-acquired infections. [1] The switch from Klebsiella spp to Escherichia coli as the major ESBL-producing species is linked to the dissemination of blactx-m genes. [4]

ESBL genes have been reported in clinical isolates, normal bacteria flora from humans and animals, food and sewage, [5] indicating the widespread distribution and presence of environmental reservoirs of the genes. [6] The carriers of ESBL-producing Enterobacteriaceae serve as a reservoir for antibiotics resistance in the community, becoming a risk to other individuals through human-to-human transmission. They also enrich the environment with the resistance gene pool which may facilitate the acquisition of resistance mechanisms by susceptible bacteria. [7] The carriage of this gene is related to hospital care and antibiotic consumption. [8] Faecal carriage of these organisms during nosocomial outbreaks has been reported. [9] Report of ESBL-producing Enterobacteriaceae carriage rate is on the increase. [10] The prevalence among countries and risk factors analyses have shown conflicting and varying results as it has been reported in subjects without prior healthcare contact and in areas with lower use of antibiotics. [11] Some reported rates of community carriage of ESBLs in Africa were from Senegal (10.0%), Niger (30.9%) [9] and Nigeria (17.02%). [12]

In Nigeria, there is a paucity of information on surveillance, carriage rate, and spread of ESBLs in community settings but high incidences of ESBLs resistance genes (TEM, SHV, OXA, CTX-M and AmpC types) in clinical isolates have been reported from south-western Nigerian. [13, 14] ESBLproducing bacteria are an emerging problem in the community setting in many parts of the world, including Nigeria. [15] The multiple drug resistance of these ESBLs producing Enterobacteriaceae in the community is a source of concern for the treatment of outpatients with infections. [16]

The knowledge of data on surveillance, carriage rate, and spread of ESBLs in community settings will provide information on the distribution and dynamics of ESBLproducing Enterobacteriaceae among asymptomatic carriers. Therefore, this study determined the prevalence of ESBLs-Escherichia coli producing and Klebsiella pneumoniae among asymptomatic participants attending the Medical Centre of the University of Lagos.

### Methods

### *Type of study*

The study was a cross-sectional survey of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* carriage among asymptomatic outpatients scheduled for routine medical

examination between March and September 2017.

### Study population

The participants recruited for the study were all students and staff of the University of Lagos as well as the general public who registered at the Medical Centre for routine medical examination during the period of the study. Only participants who gave informed consent were included in the study.

### Study Centre

This study was conducted at the Medical Centre of the University of Lagos, Akoka, Lagos. This facility offers primary and secondary medical services to students, members of staff of the University of Lagos, and the general public.

### Eligibility

The inclusion criteria include apparently healthy, asymptomatic male and female individuals aged 18 years and above who were scheduled for routine medical examination in the facility. Individuals with a history of antibiotics use within three months before the study and individuals with a history of diarrhoea within the preceding seven days were excluded from the study.

### Sample Collection and Transportation

Participants were given the relevant subject information leaflets to educate them on how to aseptically collect stool and urine samples into sterile containers. The stool and urine samples from each participant were labelled and transported to the laboratory for processing within four hours of collection. questionnaires were used to obtain information on demographic variables, medical history, previous hospital admissions and antibiotic use, and access to potable water.

### Isolation and Identification of bacteria

The urine and stool samples were plated directly on MacConkey agar and were incubated at 37°C for 18-24 hours. After incubation, all lactose fermenting colonies

were further identified using standard methods including Gram staining technique. Oxidase test and other biochemical methods of identification were conducted using the Microbact Identification System [Microbact™ 12A (12E) Oxoid, U.K] according to the manufacturer's instructions.

### Antibiotics Susceptibility Testing

The antibiotic susceptibility of the identified isolates was carried on Mueller-Hinton agar (Biotec, U.K), using the agar disc diffusion method. [17] The suspension of an overnight broth culture of the isolate was adjusted to a turbidity of 0.5 McFarland, with sterile normal saline and was inoculated on freshly prepared dried Muller-Hinton agar plate with sterile swabs. The antibiotic-impregnated disc was aseptically applied on the inoculated plate at a distance of about 20 mm from each other. Similarly, Muller-Hinton agar plates inoculated with the control organisms were set up as controls. All the plates were incubated at 37°C for 24 hours. The diameter of the zone surrounding each antimicrobial agent was measured in millimetre (mm) using a transparent ruler. The results were interpreted as susceptible, intermediate or resistant according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). [17]

### ESBL Detection

The ESBL-producing isolates were detected using a modified double-disk synergy test (DDS20) as described by Garrecet al., [18]. Cefotaxime (30ug) and ceftazidime (30ug) discs were placed 20 mm apart on the plate in a straight line with Amp/Clavulanic acid disc in the middle on a Mueller Hinton agar plate inoculated with 0.5 McFarland standardized inoculum. The plate was incubated at 35°C for 18-24 hours, the zone of inhibition around ceftazidime or cefotaxime and or both that expanded towards Amp/Clavulanic acid disc, with a characteristic champagne cork shape indicates the production of ESBL.

DNA Extraction

DNA was extracted from ESBL-positive isolates using the boiling lysis method. One millilitre of the overnight bacterial culture in Tryptose Soy Broth was centrifuged at 8,000 rpm for two minutes, the sediment was washed with nuclease-free water, homogenized, and heated at 95°C for 15 minutes. The supernatant was used as DNA template for the multiplex PCR using primers already described, [19] to target the ESBL genes: bla<sub>CTX-M</sub>, bla<sub>SHV</sub> and bla<sub>TEM</sub>. The DNA amplification reaction was performed in 20µL final volume containing 2µL of the template DNA, 4µL of PCR mix containing Hot Start Taq polymerase 1.0U, x1 PCR buffer (50mM KCl, 10mM Tris -HCl, pH 8.3), 0.2mM each of dNTP and 1.5mM of MgCl<sub>2</sub>, 0.2uL of the forward and reverse primers (10 picomoles each) and 12.8uL of deionised water.

The thermocycling conditions for all the PCRs were as follows: 95°C for 3 minutes, 95°C for 30 seconds, 55°C for 60 seconds, and 60 seconds at 72°C for 30 cycles, with a final seven minutes extension at 72°C. All the PCRs were performed in the Applied Biosystem Thermal Cycler (Applied Biosystem, USA). The amplified product was loaded in 1.5% agarose gel, electrophoresis in Tris-borate-EDTA buffer containing 10 mg/mL Ethidium bromide staining and was visualized with ultraviolet transilluminator.

### Bacteria control strains

Escherichia coli ATCC 25922 and Klebsiella pneumonia ATCC 700603 (positive), Pseudomonas aeruginosa ATCC 27853 (negative) used as control strains for biochemical identification and antibiotic susceptibility were obtained from the Research Laboratory of the Department of Microbiology, Nigerian Institute of Medical Research, Yaba, Lagos.

### Data Analysis

Data were analysed using Statistical Package for Social Science (SPSS) version 23.0 (Washington, USA). Quantitative data were described as frequencies and percentages. The results were analysed using the Chi-Square test. *P* values less than 0.05 were regarded as statistically significant.

### **Ethical Consideration**

Ethical approval was obtained from the College of Medicine, University of Lagos Health Research Ethics Committee with the approval certificate number CM/HREC/12/16/092. Permission to use the University of Lagos Medical Centre was also obtained while informed consent was obtained from each participant.

### Results

A total of 350 stool and urine specimens were collected from apparently healthy participants on routine medical examination. included members of staff (134; 38.3%), students (170; 48.6%) and members of the general public (46; 13.1%). A total of 366 isolates were recovered; 345 (94.3%) from stools and 21 (5.7%) from urine samples. A participant had bacteria in both stool and urine samples. The isolates identified included Escherichia coli and Klebsiella pnuemoniae from stool samples (256; 69.9% and 89; 24.4% respectively) and urine samples (15; 4.1% and 6; 1.6% respectively). Most of the isolates were resistant to fluoroquinolones, cephalosporins, and aminoglycosides. Although all isolates were resistant to ampicillin, they were highly susceptible to Imipenem (330; 90.6%) and Nitrofurantoin (307; 80.4%) (Table I).

The results of the phenotypic detection of ESBLs showed that 29 (8.3%) of the participants which included staff (9/134; 6.7%), students (13/170; 7.6%) and the general public (7/46; 15.2%) were carriers of ESBL-producing *E. coli* and *K. pneumonia*. Only 1 (4.8%) ESBL producer was detected from the urine and the stool specimens of the same study participant (Table II).

Phenotypically, ESBL was detected in 30 (8.2%) isolates, which included *Escherichia coli* 

(19; 7.0%) and *Klebsiella pneumonia* (11; 12.0%) while only 25 (83.3%) carried the ESBL gene. CTX-M Type II (36.7%) was the predominant gene followed by TEM gene 5 (16.7%) but no SHV gene was detected (Table II). Dual carriage of CTX-M and TEM genes (9; 30%) was observed among the isolates. The ESBL producers were resistant to all the tested

antibiotics except to nitrofurantoin (13; 43.3%), imipenem (12; 40%) and cefotaxime (4; 13.3%) as shown in Table I. The analysis of factors predisposing to ESBL carriage showed that there were no associations between age, sex, antibiotic use, hospitalization and ESBL carriage as shown in Table III.

Table I: Susceptibility of the isolates and the ESBL producers to different antibiotics

Antibiotic discs	Antibiotic susceptibility			ESBL positive			
	Resistance	Intermediate	Susceptibility	Resistance	Intermediate	Susceptibility	
Caftazidime30µg	34 (9.3)	101 (27.6)	231 (63.1)	6 (20.0)	24 (80.0)	0 (0.0)	
Cetriaxone30µg	81 (22.1)	127 (34.7)	158 (43.2)	22 (73.3)	8 (26.7))	0 (0.0)	
Cefuroxime 30µg	113 (30.9)	110 (30.1)	143 (39.1)	16 (53.3)	14 (46.7)	0 (0.0)	
Cefotaxime30µg	15 (4.1)	112 (30.6)	239 (65.3)	17 (56.7)	9 (30.0)	4 (13.3)	
Imipenem10µg	1 (0.3)	35 (9.6)	330 (90.2)	1 (3.3)	17 (53.7)	12 (40.0)	
Aztreonam30µg	104 (28.4)	113 (30.9)	149 (40.7)	14 (46.7)	16 (53.3)	0 (0.0)	
Ciprofloxacin 5µg	71 (19.4)	111 (30.3)	184 (50.3)	23 (76.7)	7 (23.3)	0 (0.0)	
Azithromycin 15μg	108 (29.5)	133 (36.3)	125 (34.2)	19 (63.3)	11 (36.7)	0 (0.0)	
Nitrofurantoin300µg	19 (5.2)	40 (10.9)	307 (83.9)	6 (20.0)	11 (36.7)	13 (43.3)	
Ofloxacin5µg	101 (27.6)	94 (25.7)	171 (46.7)	12 (40.0)	18 (60.0)	0 (0.0)	
Gentamicin 10µg	96 (26.2)	153 (41.8)	117 (32.0)	24 (80.0)	6 (20.0)	0 (0.0)	
Ampicillin 10μg	366 (100)	0 (0.0)	0 (0.0)	30 (100)	0 (0.0)	0 (0.0)	

Table II: Phenotypic and genotypic prevalence of ESBL-producing E. coli and K. pneumoniae in stool and urine samples

Isolates	-	Phenotypic	Positive fo	Positive for ESBL genes			
	n (%)	ESBL	CTX-M	TEM	CTX-M/TEM	SHV	
		Positive					
E. coli	271 (74.0)	19 (7.0)	5 (26.3)	3 (15.8)	7 (36.8)	0	
K. pneumonia	95 (26.0)	11(11.6)	6 (54.5)	2 (18.1)	2 (18.1)	0	
Total	366 (100)	30 (8.2)	11(36.7)	5 (16.7)	9 (30.0)	0	

### Discussion

This study demonstrated the presence of *Escherichia coli* and *Klebsiella pneumoniae* in the stool and urine of apparently healthy participants on a visit to the health facility for routine medical examination. *Escherichia coli* and *Klebsiella pneumoniae* were more prevalent in stool samples (94.3%) than in urine samples (5.7%). Although these bacteria are regarded

as normal commensals of the body, they can cause various types of infections especially urinary tract infection when the immune system of the host becomes weakened or following the occurrence of a chronic or acute microbial infection.

The 100% resistance to ampicillin demonstrated by the isolates in the present study is a source of concern because they were isolated from apparently healthy participants.

This total resistance could be attributed to selective pressure as a result of the availability over the counter and affordability, both of which make the drug liable to abuse. About 90% of ampicillin resistance observed with *E*.

*coli* has been reported to arise from the production of TEM-1 enzymes which is responsible for the increasing rate of ampicillin and penicillin resistance. [20]

Table III: Predisposing factors to ESBL carriage among study participants

Variables	ESBL Carriage				
		Positive	Negative	$\chi^2$	P values
		n (%)	n (%)		
Category	Staff	9 (6.7)	125 (93.3)		
	Student	13 (7.6)	157 (92.4)	3.433	0.23
	Public	7 (15.2)	39 (84.8)		
Sex	Male	11 (8.4)	120 (91.6)		
	Female	18 (8.2)	201 (91.8)	0.003	0.551
Age (years)	<20-25	15 (8.8)	155 (91.2)		
0 0 7	26-30	7 (6.8)	56 (93.2)		
	31-35	5 (8.2)	41 (91.8)	4.591	0.868
	36-40	2 (14.3)	12 (85.7)		
	41-above	0 (0.0)	2 (100.0)		
Hospitalised in the last 6 months	No	28 (8.3)	311(91.7)		
	Yes	1 (9.1)	10 (90.9)	0.010	0.922
Antibiotic use 3-6 months ago	No	24 (1.8)	282 (92.2)		
ŭ	Yes	5 (11.4)	39 (88.6)	0.627	0.428
Availability of water at home	No	5 (12.5)	35 (87.5)		
<b>,</b>	Yes	24 (7.7)	286 (92.3)	1.055	0.304
Availability of water closet toilet at home	No	2 (10.5)	17 (89.5)		
	Yes	27 (8.2)	304 (91.8)	0.133	0.716

A participant carried ESBL producer both in urine and stool specimens. The presence of this organism in the urine and stool of the same individual might be as a result of contamination of the specimens by the subject during specimen collection. Most urinary tract infections are caused by the same bacterial flora found in faeces before the development of urinary tract infections. [21] The prevalence of phenotypic ESBL-producing *Escherichia coli* and *Klebsiella pneumonia* in this study was 8.2%. This is lower than 15.8% reported from

hospital out-patients in the southern part of Nigeria, [12] and 17.2% reported from healthy individuals in south-eastern Nigeria. [14] The low ESBL prevalence rate observed in this study may be attributed to the fact that the participants were apparently healthy individual, with presumably good socioeconomic status, and access to medical services. Similarly, studies elsewhere have reported various prevalence rates of ESBLs among healthy individual; 6.4% in Japan, [22] 6.7% in Cameroon, [23] and 2% in Hungary. [5]

These observed differences suggest that the prevalence of ESBL producer varies markedly between geographic regions. Factors such as poor access to potable water, poverty, high population density, implementation of antibiotic stewardship intervention and proper infection control mechanisms have been deduced to be responsible for the differences in the prevalence rates. [5, 22, 23]

The most predominant ESBL genes detected was the CTX-M type, which was followed by TEM type but no SHV genes were identified in the present study. The predominance of CTX-M family over other types of ESBL genes in this study is consistent with the report in most previous studies. [5, 10] CTX-M type enzymes are acquired from chromosomally encodedlactamases that occur naturally in some Enterobacteriaceae, TEM-type gene is known penicillins hydrolyze and early cephalosporins. [20] The development and spread of ESBLs are probably due to the expanded-spectrum overuse of cephalosporins. [20]

The discordance observed between the ESBL phenotype and genotype results in this study may likely be due to the fact that only three ESBL genes were tested in this study. The observed phenotypes belong to ESBL variants other than the three ESBL genotypes analysed because of the cost and the technicality of running molecular methods. The discrepancy could also arise from false-positive phenotypic results. Although the confirmatory tests for phenotypic determination have been reported to be highly sensitive and specific, compared to genotypic confirmatory tests, however, in several instances, falsely positive or falsely negative phenotypic confirmatory tests results have been identified. [24, 25] Dual carriage of CTX-M and TEM (30.0%) was also observed in the present study; several other studies have reported co-existence of one or more genes in Enterobacteriaceae. [26, 27]

Based on the participants' self-reporting which has potential recall bias, analysis of factors predisposing to the carriage of ESBL showed that there was no significant association with age, sex, prior hospitalization or antibiotic use within the preceding three months. This observation aligns with the report of a study which showed that prior hospitalization or previous use of antimicrobial drugs does not affect faecal carriage of ESBL-producing Enterobacteriaceae in healthy people. [28] Other studies believe that age over 65 years and household contacts of ESBL carrier, [29] hospitalization in the preceding three-months, residence in a hospice, [11] recent antibiotics use, [30] are risk factors associated with ESBLproducers in non-hospitalised patients. These individual and the isolates can serve as a reservoir of resistance genes for pathogens and help in the spread of infections.

### Conclusion

This study reveals carriage of  $bla_{CTX-M}$  as the most predominant genes and dual carriage of  $bla_{CTX-M}$  and  $bla_{TEM}$  genes in the faeces of the asymptomatic participants. Such individuals can serve in the transmission of antibiotic-resistant genes to bacteria in the community. Therefore continuous monitoring of the prevalence, distribution and dynamics of ESBL producer in asymptomatic carriers would provide data for the evaluation, management, and planning; these are important for prevention, control, as well as treatment.

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**Authors' Contributions:** DA-MA conceived the study. DA-MA, OFA and OOA designed the study, OOR and AE collected samples and carried out the laboratory aspects of the study. All the authors participated in data analysis and interpretation of the results. DA-MA drafted the manuscript. All the

authors approved the final version of the manuscript

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