



Topic: Prevalence and Antibiogram of Extended Spectrum β -Lactamase (ESBL)-Producing *Enterobacteriaceae* in Asymptomatic Individuals

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ABSTRACT: Bacterial organisms producing extended-spectrum beta-lactamases (ESBLs) are becoming a major problem in infectious disease units globally; and this is due in part to the multidrug resistance nature of these pathogens – which makes it difficult to select antibiotics for the treatment of infections that they cause. This study was carried out to determine the antibiotic susceptibility pattern and ESBL production among fecal isolates of *Escherichia coli* and *Klebsiella pneumoniae* in asymptomatic healthy individuals in the community. A total of 192 fecal samples collected between September 2011 and June 2012 were bacteriologically cultured onto Eosin Methylene Blue (EMB) agar plates supplemented with 1 μ g/ml of either ceftazidime or cefotaxime. Positive cultures were screened for antimicrobial susceptibility using the Kirby – Bauer sensitivity testing method. All the recovered test isolates were identified based on standard biochemical/microbiological techniques. Presumptive ESBL producing isolates were phenotypically confirmed by the double disc synergy test (DDST) method. Eight (17.02 %) isolates were found to be ESBL producers. Of these, 5 (62.5 %) were *Escherichia coli* and 3 (37.5 %) of the isolates were *Klebsiella pneumoniae*. The *E. coli* and *Klebsiella pneumoniae* showed high resistance to the tested antibiotics especially to the third generation cephalosporins, amoxicillin/clavulanic acid, ticarcillin and sulphamethoxazole/trimethoprim. However, none of the isolates was resistant to imipenem, a carbapenem. Conclusively, our findings suggest that asymptomatic healthy individuals could serve as potential reservoir of ESBL-producing bacteria in the community.

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INTRODUCTION

Resistance to β -lactam antimicrobial drugs among pathogenic Gram-negative bacteria is usually mediated by several genetic/enzymatic factors of the pathogens including the production of extended-spectrum β -lactamases (ESBLs). ESBLs are a major group of enzymes that commonly mediate resistance to β -lactam antimicrobial drugs (including the cephalosporins) in Gram-negative bacteria, and these multidrug resistance

factors are most commonly found in *Escherichia coli* and *Klebsiella pneumoniae* (Kader and Kumar, 2004). ESBL-producing organisms have been widely reported in many countries and multidrug resistance is increasingly seen in many Gram-negative bacteria as a result of the widespread use and misuse of various antibiotics (Livermore, 2003; Waterer and wunderink, 2001). Extended-spectrum β -lactamases (ESBLs) have the ability to hydrolyze penicillins, 3rd generation

cephalosporins and monobactams but bacterial pathogens producing ESBLs are highly susceptible *in vitro* to β -lactamase inhibitors such as clavulanic acid (Livermore, 2003). These enzymes are encoded by transferable conjugative plasmids, which often code resistance determinants to other classes of antimicrobial agents such as the fluoroquinolones and aminoglycosides. And these transferable conjugative plasmids present in ESBL-producing bacteria are also responsible for the dissemination of resistance to other Gram-negative bacteria in both hospital and non-hospital environments (Bradford, 2001). The first plasmid mediated β -lactamase in Gram-negative bacteria, TEM-1, was described in the early 1960s (Turner, 2005). Afterwards it was detected from *Klebsiella* in Europe 1980, in Germany 1983, and in France 1985 (Perez *et al.*, 2007). It has become very important to study the prevalence of ESBL-producing organisms because of the increasing antimicrobial resistance and the decreasing number of new drugs available against such microbes (Kader *et al.*, 2004). Bacterial infections caused by ESBL-producing bacteria are an emerging problem in the community setting in many parts of the world including Nigeria (Ejikeugwu *et al.*, 2013; Iroha *et al.*, 2010; Colodner *et al.*, 2004). Several reports have addressed fecal carriage of these organisms during nosocomial outbreaks (Lucet *et al.*, 1996; Moland *et al.*, 2003). Although carriers of ESBL producers are expected to be present in general practice, their occurrence has rarely been reported and there are few studies conducted in the community in Nigeria as per the issue.

MATERIALS AND METHODS

Study Population: The study was carried out in Owerri, Imo State, located in the Eastern part of Nigeria. A total of 192 people living in two remote villages in Owerri town consented for the study verbally. The participants were asked to fill out a questionnaire. All the participants were screened for medical history. Exclusion criteria included any antibiotic treatment in the 3 months prior to specimen collection and confirmed diagnosis of digestive tract diseases. Age ranged from 1-80 years (117 females and 75 males; of which children were 89 and adults 103). Stool samples were collected aseptically and seeded immediately onto Eosin Methylene Blue (EMB) agar plates. One of the EMB agar plates was supplemented with 1 μ g/ml of ceftazidime while the other was supplemented with 1 μ g/ml cefotaxime.

Bacterial isolates: A total of 47 non replicate isolates were collected between September 2011 and June 2012, from 192 stool samples of asymptomatic healthy individuals bacteriologically analyzed in this study. All the bacterial isolates were identified by standard

microbiology identification techniques (Cheesbrough, 2006).

Susceptibility studies: Susceptibility to antimicrobial agents was determined by the Kirby-Bauer Disc Diffusion method on Muller-Hinton agar (Oxoid, England) plates as described by the Clinical Laboratory Standard Institute (CLSI) (CLSI, 2010). The antibiotic discs used included amoxicillin/clavulanic acid (AMC 20/10 μ g), ampicillin (AMP 10 μ g), cefepime (FEP 30 μ g), ceftriaxone (CRO 30 μ g), imipenem (IPM 10 μ g), nalidixic acid (NA 30 μ g), ofloxacin (OFX 30 μ g), ticarcillin (TIC 30 μ g), sulphamethoxazole/trimethoprim (SXT 25 μ g), ceftazidime (CAZ 30 μ g), cefotaxime (CTX 30 μ g); and these antibiotic discs were procured from Oxoid, England.

Detection of extended spectrum- β -lactamase (ESBL) Enzymes: ESBL production in the test bacterial isolates was determined by the disk diffusion method as was previously described (Ejikeugwu *et al.*, 2013; Iroha *et al.*, 2010; Ramalivhana *et al.*, 2010). Mueller Hinton agar plates were prepared and inoculated with inoculums (equivalent to 0.5 McFarland turbidity standards) of the test isolates. Thirty microgram's disc each of cefotaxime (30 μ g) and ceftazidime antibiotics were placed on the agar at a distance of 15 mm center to center from a central combination disc of augmentin (comprising of amoxicillin 20 μ g and clavulanic acid 10 μ g) in triplicates. A clear extension of the edges of the inhibition zone of any of the antibiotics towards the disc containing clavulanic acid was regarded as a phenotypic confirmation of the presence of ESBL (Ramalivhana *et al.*, 2010). A ≥ 5 mm increase in the inhibition zone diameter for either of the cephalosporins (ceftazidime or cefotaxime) tested in combination with amoxicillin-clavulanic acid versus its zone when tested alone confirms ESBL production phenotypically (Ejikeugwu *et al.*, 2013).

Control organism: *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 (Oxoid, UK) were used as positive control strains for antimicrobial susceptibility studies.

RESULTS

Bacterial isolates other than *E. coli* and *K. pneumoniae*, which grew on the eosin Methylene Blue agar, were disregarded. Of the 192 stool samples tested, 47 (24.47 %) isolates that were resistant to CTX and/or CAZ were obtained; 23 *Escherichia coli* and 24 *Klebsiella pneumoniae*.

Eight (8) (17.02 %) out of the 47 isolates were found to be ESBL positive with DDST (3 from children and 5

from adults). Of the total ESBL isolates, five (62.5 %) were from *Escherichia coli* and three (37.5 %) from *Klebsiella pneumoniae* (Table 1).

Table 1: Occurrence of ESBL positive bacteria and non ESBL producing bacteria from fecal samples

Bacteria	ESBL positive n (%)	ESBL negative n (%)	Total
<i>Escherichia coli</i>	5 (62.5)	18 (46.1)	23
<i>Klebsiella pneumoniae</i>	3 (37.5)	21 (53.8)	24
Total	8	39	47

n=number of organisms, %=percentage

All the isolated organisms in this study were 100 % susceptible to imipenem and 73-75 % was susceptible to ofloxacin. The antibiotic susceptibility pattern of the isolates is demonstrated in Table 2. Resistance pattern of *E. coli* and *K. pneumoniae* revealed that 90-100 % were resistant to ampicillin, ceftazidime, cefotaxime, ticarcillin and sulphamethoxazole/trimethoprim, 87-95 % to ceftriaxone.

Table 2: Antimicrobial susceptibility pattern of test isolates

Antibiotics (μ g)	% susceptibility of <i>E. coli</i> (n=23)		% susceptibility of <i>K. pneumoniae</i> (n=24)	
	Resistant	Susceptible	Resistant	Susceptible
AMC (30)	19 (82.6)	4 (17.3)	24 (100)	0 (0)
AMP (10)	23 (100)	0 (0)	24 (100)	0 (0)
FEP (30)	16 (69.5)	7 (30.4)	14 (58.3)	10 (41.6)
CRO (30)	22 (95.65)	1 (4.34)	21 (87.5)	3 (12.5)
IMP (10)	0 (0)	23 (100)	0 (0)	24 (100)
NA (30)	19 (82.6)	4 (17.39)	23 (95.8)	1 (4.1)
CAZ (30)	23 (100)	0 (0)	24 (100)	0 (0)
CTX (300)	23 (100)	0 (0)	24 (100)	0 (0)
OFX (5)	6 (26.08)	17 (73.9)	6 (25.0)	18 (75.0)
TIC (75)	23 (100)	0 (0)	24 (100)	0 (0)
SXT (25)	23 (100)	0 (0)	24 (100)	0 (0)

DISCUSSION

Our ability to promptly and accurately detect ESBL-producing bacteria from both clinical and environmental samples is crucial to the control of the development and spread of drug-resistant pathogens. This study demonstrates the presence of ESBL in fecal strains of *E. coli* and *K. pneumoniae* from healthy individuals in Owerri metropolis, Southeastern Nigeria. Despite normally living harmlessly in the gut as part of the body's normal microflora, *E. coli* and *K. pneumoniae* can cause various types of infections, especially urinary tract infection when the host's immune system becomes

weakened or following the occurrence of a chronic or acute microbial infection. In a study published from Saudi Arabia, it was observed that 10.2 % of the uropathogens isolated from healthy individuals were ESBL-producing *E. coli* (Kader and Kamath, 2009). In another study, also in Saudi Arabia, it was reported that > 12 % of the Gram-negative uropathogens isolated from community patients were ESBL-producers (Kader and Kumar, 2005). Some reports from Europe also suggest that infections caused by ESBL-producing organisms are emerging among community patients; and these individuals not only serve as reservoirs of the pathogens

in the community – but they also act as potential source of contamination especially to susceptible members of the community or population (Woodford *et al.*, 2004). The presence of ESBL-producing organisms in the gut not only contributes to difficulty in the treatment of extraintestinal infections, but these organisms can also mediate the transfer of antibiotic-resistance determinants to other organisms within the gastrointestinal tract (Moland *et al.*, 2003). Their presence increases the risk of transmission to other individuals as a result of human-to-human transmission or through the environment (Woodford *et al.*, 2004). In Serbia, it has been reported that 65-92 % of commensal *Enterobacteriaceae* and other organisms isolated from feces are resistant to commonly used antibiotics such as ampicillin, sulphamethoxazole/trimethoprim and fluoroquinolones (Irina *et al.*, 2007). In this study, the *E. coli* and *K. pneumoniae* isolates recovered from the fecal samples of asymptomatic individuals in Owerri metropolis, Southeastern Nigeria were found to be highly resistant to ampicillin, sulphamethoxazole-trimethoprim, and ticarcillin (as shown in Table 2). This high resistance of the test isolates to ampicillin, sulphamethoxazole-trimethoprim and ticarcillin is in agreement with other studies where higher levels of *Enterobacteriaceae* including *E. coli* and *K. pneumoniae* to some first line antibiotics were reported (Irina *et al.*, 2007; Brinas *et al.*, 2003). Carbapenems are the drugs of choice for many infections caused by Gram positive and Gram negative bacteria including those infections caused by ESBL-producing bacteria (Ullah *et al.*, 2009). In this study, imipenem demonstrated 100 % sensitivity against all the test isolates (as depicted in Tables 2). These findings were similar to the studies conducted in Saudi Arabia (Kader and Kamath, 2009), and Turkey (Kiremitçi *et al.*, 2011; Ozlem Kurt azap *et al.*, 2007) where imipenem showed good antimicrobial activity against the test bacteria. Previous studies from Nigeria have also reported ESBL production from humans; and the rate usually varies from 6 % to 87 % (Yushua *et al.*, 2010; Iroha *et al.*, 2010; Akujobi and Ewuru, 2010; Aibinu *et al.*, 2003). ESBL prevalence in other parts of the world have also been observed in asymptomatic/healthy humans (Geser *et al.*, 2012; Kiremitçi *et al.*, 2011; Rajesh *et al.*, 2010; Kader and Kamath, 2009; Ozlem Kurt azap *et al.*, 2007). The prevalence of ESBL positive bacteria in this study was found to be 17.02 %, which was lower compared to a similar study carried out in Thailand (52.8 %) (Tadahiro *et al.*, 2010), in Turkey (47.3 %) (Ozlem Kurt azap *et al.*, 2007). However, the prevalence of ESBL bacteria in our study is far higher than the prevalence reported in Cameroon from healthy individuals (6.7 %) (Lonchel *et al.*, 2012). The spread of ESBL-producing organisms to the community could be related to previous hospital acquisition as some hospitalized patients continue to carry ESBL-producing

bacteria over prolonged periods, which may contribute to their extra hospital propagation (Colodner *et al.*, 2004). Their emergence in the community could also be caused by the overuse and/or misuse of antibiotics in the community. Antibiotic use creates a selective pressure on host bacteria in the large bowel, leading to the emergence of antimicrobial-resistant organisms. This may cause an increase in the number of carriers harboring resistant bacteria (Woodford *et al.*, 2004). The increasing prevalence of ESBL producing isolates and emergence of extensively resistant isolates to third generation cephalosporins and other antimicrobial agents is alarming. This development warrants global public health programs to enhance effective use of antibiotics in both the community and hospital environments.

REFERENCES

- [1] Adelakun E.A, Finbar E.A, Agina S.E and Makinde A.A (2001). Antimicrobial activity of *Boswellia dalzielii* stem bark. *Fitoterapia* 72(7), 822-824.
- [2] Aibinu I.E, Ohaegbulam V.C, Adenipekun E.A, Ogunsola F.T, Odugbemi T.O and Mee B.J (2003). Extended spectrum beta lactamase enzymes in clinical isolates of *Enterobacter* species from Lagos, Nigeria. *Journal of Clinical Microbiology*, 41:2197-2200.
- [3] Akerele O.S, Obasuyi O, Ebomoyi M.I, Oboki I.E and Umumarongie O.H (2007). Antimicrobial activity of the Ethanol extract and fractions of the seeds of *Garciniakola* Heckel (Guttiferae). *Afr. J Biotechnol.* 7(2): 169-172.
- [4] Akinyanju J.A, Owoyale J.A and Okonla E.O (1986). Antimicrobial effects of leaf extract of *Acalyphatorta* In: Safowora, A. (ed). *The state of medicinal plants Research in Nigeria*, Ibadan University Press, Ibadan, Nigeria pp. 247-253.
- [5] Akujobi C.N and Ewuru C.P (2010). Detection of Extended Spectrum beta-lactamases in gram negative bacilli from clinical specimens in a teaching hospital in South Eastern Nigeria. *Niger Med J* 51:141-146.
- [6] Bradford P.A (2001). Extended-Spectrum β -Lactamases in the 21st Century:Characterization, Epidemiology, and Detection of This Important Resistance Threat, *Clinical Microbiology Review*, 14(4):933-951.
- [7] Braga L.C, Leite A, Xavier K.G, Takahashi J.A, Bemquerer M.P, Chartone-Souza E and Nascimento A.M (2005). Synergic interaction between pomegranate extracts and antibiotics against *Staphylococcus aureus*. *Can. J. Microbiol.* 51, 541-547.
- [8] Chessbrough M (2006). *District laboratory practice in tropical countries, Part-2*, NewYork, USA: Cambridge University. pp. 184- 186.

- [9] Clinical and Laboratory Standards Institute (2010). Performance standards for antimicrobial susceptibility testing; Seventeenth information supplement. Wayne, PA: CLSI.
- [10] Colodner R, Rock W, Chazan B, Keller N, Coy N, Sakran W and Raz R (2004). Risk factors for the development of Extended spectrum beta lactamase producing bacteria in non hospitalized patients. *European Journal of Clinical Microbiology and Infectious diseases* 23: 163-167.
- [11] Ejikeugwu C, Iroha I, Adikwu M and Esimone C (2013). Susceptibility and Detection of Extended Spectrum β -Lactamase Enzymes from Otitis Media Pathogens. *American Journal of Infectious Diseases*. 9(1):24-29.
- [12] Esimone C.O, Adikwu M.U and Okonta J.M (1998). Preliminary antimicrobial Screening of ethanolic extract from Lichen usnea subflandans. *J. Pharm. Res. Dev.*, 3(2): 99-101.
- [13] Esimone C.O, Nworu C.S, Onuigbo E.B, Omeje J.U, Nsirim K.L, Ogbu J.C, Ngwu M.I and Chah K.F(2009). Anti-mycobacterial activity of root and leaf extracts of *Anthocleista djalonensis* (Loganiaceae) and *Diospyros mespiliformis* (Ebenaceae). *Int. J.Green Pharm.*3: 201-205
- [14] Gomber C and Sanjai S (2007). Anti-Staphylococcal potential of *Callistemon rigidus*. *Central European Journal of Medicine*, vol 2(1):79-88.
- [15] Iroha I.R, Amadi E.S, Oji A.E, Nwuzo A.C and Ejikeugwu P.C (2010). Detection of plasmid borne extended spectrum beta lactamase enzymes from blood and urine isolates of Gram negative bacteria from a university teaching hospital in Nigeria. *Current Research in Bacteriology* 3(2):77-83.
- [16] Kader A and Kumar A (2004). Prevalence of extended spectrum betalactamase among multidrug resistant gram negative isolates from a general hospital in Saudi Arabia. *Saudi Med J*, 25:570 - 574.
- [17] Kader AA and Kumar A (2005). Extended-spectrum beta-lactamases in urinary isolates of *Escherichia coli*, *Klebsiella pneumoniae* and other Gram-negative bacteria in a hospital in Eastern Province, Saudi Arabia. *Saudi Med J*; 26:956-959.
- [18] [Kader A.A](#) and [Kamath K.A](#) (2009). Faecal carriage of extended-spectrum beta-lactamase-producing bacteria in the community. [*East Mediterr Health Journal*](#). 15(6):1365-70.
- [19] Kayode J, Jose R.A and Ige O.E (2009). Conservation and Biodiversity Erosion in Ondo State, Nigeria: (4). Assessing Botanicals Used in the Cure of Sexually Transmitted Diseases in Owo Region. *Ethnobotanical Leaflets*, 13: 734-38.
- [20] Kiremitçi A, Ener Ç.D, Zeynel A, Yargıç G.D, Neslihan T, Aşkın D.A and Mehmet A.A (2011). Prevalence and Risk of Fecal Carriage of Extended-Spectrum β -lactamase (ESBL) Producing Enterobacteriaceae in Hospitalized and Ambulatory Children. *J Pediatr Inf*; 5: 54-8
- [21] Livermore D.M (2003). Bacterial resistance: origins, epidemiology, and impact, *Clin Infect Dis*, 15(36):111-23.
- [22] Lonche C.M, Cécile M, Joseph G.P, Raphaël B, Marie-Claire O.A, Pierrette M and Patrick D.M (2012). Proportion of extended-spectrum β -lactamase producing Enterobacteriaceae in community setting in Ngaoundere, Cameroon, *BMC Infectious Diseases* 12:53.
- [23] Lucet JC, Chevret S, Decre DI (1996). Outbreak of multiply resistant Enterobacteriaceae in an intensive care unit epidemiology and risk factors for acquisition. *Clin Infect Dis*, 22:430-436.
- [24] Mohamed SH., Hansi P and Kavitha S.S. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medical plants. *International Journal of Pharma Science and Research (IJPSR)*, 1(10): 430-434. Moland SE, Jenifer AB, Jason O,
- [25] Mark DR, Nancy DH and Kenneth ST (2002). Occurrence of newer beta-lactamases in *Klebsiella pneumoniae* isolates from 24 U.S. hospitals. *Antimicrobial Agents and Chemotherapy*, 46:3837-42.
- [26] Ngassoum M.B, Jirovetz L, Buchbauer G and Fleischhacker W (2001). Investigation of essential oil of *Plectranthus glandulosus* Hook f. (Lamiaceae) from Cameroon. *Journal of oil Research* 13, 73-75.
- [27] Ogueke C.C, Ogbulie J.N and Nsoku H.O (2006). Antimicrobial properties and preliminary phytochemical analysis of ethanolic extracts of *Aistonia bonnie*. *Niger. S. Microbial*. 20(2): 896-899.
- [28] Özlem K.A, Hande A, Sedef Ö.K and Turhan T (2007). Risk Factors for Fecal Carriage of Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* spp. in the Community. *Turk J Med Sci*; 37 (1): 31-38.
- [29] Parekh and Chanal S.V (2007). In Vitro antimicrobial activity and phytochemical analysis of some Indian Medicinal Plants. *Turk. J Biol*, 31:53-58.
- [30] Paterson D.L and Bonomo R.A (2005). Extended-Spectrum β -Lactamases: a Clinical Update. *Clinical Microbiology Reviews*. 18:657-686.
- [31] Rajesh K.R, Mathavi S.K and Indra P (2010). Detection of extended spectrum beta-lactamase

- producing gram negative bacilli in urinary isolates. *Int J Biol Med Res.* 1(4):130-132.
- [32] Ramalivhana J. N, Obi C. L and Moyo S. R (2010). Prevalence of extended-spectrum beta-Lactamases producing *Aeromonas hydrophila* isolated from stool samples collected in the Limpopo province, South Africa. *African Journal of Microbiology Research*, 4(12):1203-1208.
- [33] Singh B and Bhat T.K (2003). Potential therapeutic applications of some anti-nutritional plant secondary metabolites. *J. Agric. Food Chem.* 51:5579-5597
- [34] Tadahiro S, Itaru H, Marie N, Tatsuya N, Chalit K, Wanna M, Teera K, Surapol S, Somchit P and Yoshimasa Y (2010). High prevalence of CTX-M β -lactamase-producing Enterobacteriaceae in stool specimens obtained from healthy individuals in Thailand. *J Antimicrob Chemother*, 65: 666–668.
- [35] Turner P.J (2005). Extended Spectrum β -lactamases. *Clinical Infectious Disease*, 41(4). 273-5.
- [36] Ullah F, Malik S.A, Ahmed J (2009). Antimicrobial susceptibility pattern and ESBL prevalence in *Klebsiella pneumoniae* from urinary tract infections in the North West of Pakistan, *African Journal of Microbiology*, 3(11):670-680.
- [37] Waterer GW and Wunderink RG. Increasing threat of Gram-negative bacteria. *Critical Care Medicine*, 2001, 29:75–81.
- [38] Woodford N, Ward M.E, Kaufmann M.E, Turton J, Fagan E.J, James D, Johnson A.P, Pike R and Warner M (2004). Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β -lactamases in the UK. *J Antimicrob Chemother* 54:735–743.
- [39] Yusha'u MM and Dabo N.T (2004). Sensitivity of *A. fumigatus* to ketoconazole and leaf extract of *A. godseffiana*. *Best Journal* 1(2): 103-105.
- [40] Yusha.u M.M, Kumurya A.S and Suleiman L (2010). Prevalence of Extended spectrum β -lactamases among Enterobacteriaceae in Murtala Mohammed specialist hospital, Kano, Nigeria, *Journal of Pure and Applied Sciences*, 3(1):169-172.

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