ORIGINAL PAPER

Nig. J. Pharm. Res. 2017, 13 (1), pp 45-52
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

PREVALENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCTION IN CLINICAL *ESCHERICHIA COLI* ISOLATES IN IBADAN METROPOLIS, SOUTH-WEST NIGERIA

* MojirayoT. Durowaiye[1,2 A, D, E, F] and O. Adewuyi[1B, C]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Escherichia coli* is a major extended spectrum beta-lactamase-producing organism. Extended spectrum beta-lactamases (ESBLs) inactivate newer cephalosporins through hydrolysis increasing therapeutic failure and antibiotic resistance worldwide. This prospective experimental study aimed to determine the antibiotic susceptibility profile and ESBL production by clinical *E. coli* isolates.
Methods: Fifty-one clinical *E. coli* isolates were obtained from the microbiology laboratories of University College Hospital, Adeoyo Maternity Hospital, Our Lady of Apostle Hospital, and a private diagnostic laboratory all in Ibadan metropolis. They were identified and confirmed using standard biochemical tests. Kirby-Bauer disc diffusion method was used to determine the antibiogram and interpreted using clinical laboratory standard institute (CLSI) guideline. ESBL production was determined by the double disk synergy test (DDST).
Results: The prevalence of ESBL production was observed to be 78.4%(40). Resistance was common to sulphamethozaxole/trimethoprim 96.0%(49), ceftazidime 94.1%(48), amoxicillin and tetracycline 92.1%(47), fosfomycin 84.3%(43), cefotaxime 76.4%(39), ciprofloxacin 60.7%(31), amoxicillin/clavulanic acid 58.8%(30), and chloramphenicol 50.9%(26). Meropenem was observed to be the most sensitive (100.0%), followed by nitrofurantoin 78.4%(40), and gentamicin 70.5%(36). Multiple antibiotic resistance (MAR) index greater than 0.2 was observed in 98.0%(50) of the isolates.
Conclusion: Majority of the clinical isolates of *E. coli* were ESBL producers which are resistant to frequently used antibiotics.

Keywords: *Escherichia coli*, Extended spectrum beta-lactamase, Antibiotic resistance

1 INTRODUCTION
2

Escherichia coli is one of the most prevalent facultative anaerobic Enterobacteriaceae. Extended-spectrum beta-lactamases (ESBLs) are the beta-lactamases capable of hydrolyzing penicillin, broad-spectrum cephalosporins, and monobactams (Al-Mayahie, 2013). They are generally derived from TEM and SHV-type enzymes but do not affect cefamycins and carbapenems. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species (Rupp and Fey, 2003). Many of these β-lactamases are encoded by transposons, some of which may also carry resistance determinants to several other antibiotics (Rang *et al.*, 2012). ESBL-producing strains of *Escherichia coli* are usually community acquired (Nicolas-Chanoine *et al.*, 2008; Alsterlund *et al*., 2009). In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic options (Al-Mayahie, 2013).

There is an alarming increase in resistance to third generation cephalosporins due to the ESBL producing bacteria. These plasmid mediated enzymes mostly evolved via point mutations of the classical TEM-1 and SHV-1 β-lactamases. Other groups are increasingly prominent, notably the CTX-M types, which evolved via the escape and mutation of chromosomal β-lactamases. ESBL producers are associated with increased morbidity and mortality, especially amongst patients on intensive care and high-dependency units. Accurate laboratory detection of ESBL producers is important to avoid clinical failure due to inappropriate antimicrobial therapy **(**Jarlier *et al*., 1988). In Nigeria, outbreaks of *E. coli* infections with strains resistant to the third-generation cephalosporins have been reported without documentation of ESBL production (Okesola and Makanjuola, 2009). A

study carried out in the university college hospital Ibadan reported 76.9% clinical *E. coli* isolates produced ESBL (Okesola and Fowotade, 2012). However lower ESBL prevalence of 12.8% and 35.0% have also been reported by Mohammed *et al*., 2016 and Aibinu *et al*., 2012 in some parts of the country.

This study is aimed at determining the prevalence of ESBL production and antimicrobial susceptibility profile of *E. coli* isolated from clinical samples from laboratories in Ibadan metropolis.

MATERIALS AND METHODS

Study sites

University College Hospital, Adeoyo Maternity Hospital Yemetu, Our Lady of Apostles Hospital, Oluyoro (OCH/EC/16/01) and Union diagnostic laboratory, all in Ibadan metropolis, Oyo State, Nigeria.

Collection of isolates

Over a period of 5 months (June- September, 2015), fifty-one *Escherichia coli* isolates from clinical samples were collected on sterile agar slants from microbiology laboratories. They were each streaked on agar plates to obtain pure cultures. Thirty-eight (74.5%) from UTI, 5 (9.8%) from wounds, 3 (5.9%) from vagina, 4 (7.8%) from the intestine and 1 (2.0%) from semen. *Escherichia coli* ATCC 35218 and ATCC 25922 were used as positive and negative controls respectively.

Identification and confirmation of isolates

Each isolates was then streaked on MacConkey agar and incubated at 37^{o} C for 24hours. Characteristic *E. coli* colonies were picked and streaked on Eosin Methylene Blue (EMB) agar for further identification. Colonies showing the characteristics morphology of *E. coli* on EMB were picked and further confirmed using indole, methyl red and citrate tests (Cheesbrough, 2000).

Antimicrobial susceptibility testing

Antimicrobial susceptibility pattern to antibiotics was determined according to Kirby-Bauer (1996) method with modifications by Clinical Laboratory Standards Institute (2012). Different classes of antibiotics discs (Oxoid®, UK) used in this study include amoxicillin/clavulanic acid, amoxicillin, cefotaxime, ceftazidime, ciprofloxacin, fosfomycin, chloramphenicol, gentamicin, meropenem, nitrofurantoin, sulphamethoxazole/trimethoprim, and tetracycline.

Extended spectrum βeta lactamase (ESBL) production

ESBL production was determined using the double disc diffusion method. Resistant *E. coli* isolates to cephalosporins were subcultured into 5ml of sterile nutrient broth and incubated overnight.

Three fold serial dilution of the overnight culture which is equivalent to 0.5 McFarland standard (10^{8} /ml) was streaked on Mueller-Hinton agar with the aid of a sterile swab stick. Amoxicillin/clavulanic acid 30µg was placed firmly at the centre of the plate; ceftazidime 30µg, cefotaxime 30µg, cefoxitin 30µg were placed 20mm apart from the center in different angles using a sterile pair of forceps. The plates were then incubated for 18-24 hours and extended spectrum in the zone of inhibition was observed and interpreted accordingly. *Escherichia coli* ATCC 25922 was used as a negative control, and *E. coli* ATCC 35218 as positive control. Isolates positive for ESBL were noted to have zone around the test antibiotics disc increased towards the center disc of amoxicillin-clavulanic acid. The results were further interpreted using standard CLSI (2012) guidelines.

RESULTS

A total of 39 (76.4%) of the *E. coli* strains were ESBL producers. The prevalence of ESBL production in the *E. coli* strains from various clinical sources was 100.0% (intestine), 100.0% (semen), 78.9% (UTI), 60.0% (wound), and 33.3% (vagina) as seen in table 1. The antimicrobial susceptibility profile of the isolates to tested antibiotics showed resistance to Sulphamethoxazole/pyrimethamine 96.0%, ceftazidime 94.1%, amoxicillin and tetracycline 92.1%, fosfomycin 84.3%, cefotaxime 76.4%, ciprofloxacin 60.7%, amoxicillin/clavulanic acid 58.8%, chloramphenicol 50.9%, gentamicin 23.5%, and nitrofurantoin 19.6%. All the isolate were sensitive to meropenem. The multiple antibiotic resistance index (MARI) observed in this study with reference to the tested antibiotics showed that all the isolates have MARI of 0.16 to 0.83 (Tables 2 and 3). Majority of the isolates were resistant to the cephalosporins, it was also observed that none of the ESBL *E. coli* producers was susceptible to more than one of the cephalosporins. Only 19.6% of the ESBL producers were susceptible to any one of the cephalosporins.

Table 1: Prevalence of ESBL production among clinical sources of *Escherichia coli*

Clinical source	Prevalence of ESBL production (%)
Urinary Tract Infection	78.9
Wound	60.0
Vagina	33.3
Intestine	100.0
Semen	100.0

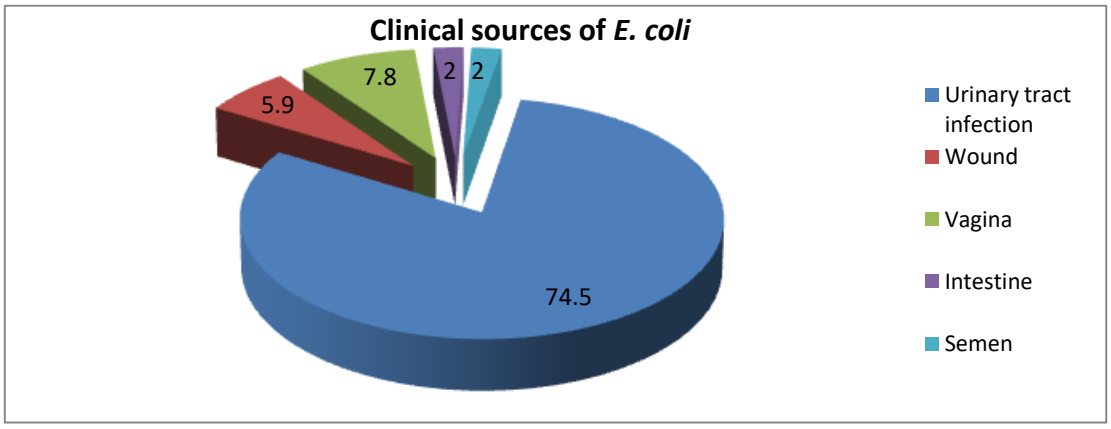

Figure 1: Percentage of clinical sources of *E. coli* isolates

Table 2: Multiple antibiotic resistance (MAR) index and resistance pattern of *E. coli*

Isolate code	Phenotypic resistance pattern	MAR index	Resistance category	ESBL
E1	CIP,AMC,AML,FOS,CAZ,CTX,SXT	0.58	MDR	+
E2	TET,AMC,AML,FOS,CAZ,CTX,SXT	0.58	MDR	+
E3	AMC,AML,FOS,CAZ,GEN,SXT	0.50	MDR	+
E4	TET,AMC,AML,FOS, CAZ,SXT,CHL	0.58	MDR	+
E5	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT	0.67	MDR	+
E6	TET,CAZ	0.17	NMDR	+
E7	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT,CHL	0.75	MDR	+
E8	TET,AMC,AML,FOS,CAZ,CTX,SXT,CHL	0.67	MDR	+
E9	CAZ,CTX,SXT,CHL	0.33	MDR	-
E10	TET,AMC,AML,CAZ,SXT	0.42	MDR	+
E11	TET,AMC,CAZ,SXT	0.33	MDR	+
E12	TET,AMC,AML,CAZ,SXT	0.42	MDR	+
E13	NFT,CIP,TET,AMC,AML,FOS,CAZ,CTX,CHL	0.75	MDR	+
E14	TET,AMC,AML,FOS,CAZ,CTX,GEN,SXT,CHL	0.75	MDR	-
E15	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT	0.67	MDR	+
E16	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT	0.67	MDR	+
E17	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT,CHL	0.75	MDR	-
E18	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT	0.67	MDR	-
E19	TET,AMC,AML,FOS,CAZ, CTX,SXT,CHL	0.67	MDR	+
E20	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT,CHL	0.75	MDR	-
E21	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT	0.67	MDR	-
E22	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT	0.67	MDR	-
E23	TET,AMC,AML,CAZ,SXT	0.42	MDR	+
E24	TET,AML,FOS,SXT	0.33	MDR	+
E25	CIP,TET,AML,FOS,CTX,SXT	0.50	MDR	+
E26	NFT,TET,AML,FOS,CAZ,GEN,SXT,C	0.67	MDR	+
E27	CIP,TET,AML,FOS,CAZ,SXT	0.50	MDR	+
E28	CIP,TET,AML,FOS,CAZ,CTX,SXT,CHL	0.67	MDR	+
E29	NFT,TET,AML,FOS,CAZ,CTX,SXT,	0.58	MDR	+
E30	CIP,TET,AML,FOS,CAZ,CTX,SXT,CHL	0.67	MDR	+
E31	CIP,TET,AML,FOS,CAZ,CTX,SXT,CHL	0.67	MDR	+
E32	NFT,TET,AML,FOS,CAZ	0.42	MDR	+

E33	NFT,CIP,TET,AML,FOS,CAZ,CTX,SXT,CHL	0.75	MDR	+
E34	CIP,TET,AMC,AML,FOS,CAZ,CTX,GEN,SXT,CHL	0.83	MDR	-
E35	CIP,TET,AML,AMC,FOS,CAZ,CTX,GEN,SXT,CHL	0.83	MDR	+
E36	NFT,CIP,TET,AML,FOS,CAZ,CTX,GEN,SXT	0.75	MDR	-
E37	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT,CHL	0.75	MDR	-
E38	NFT,CIP,TET,AML,FOS,CAZ,CTX,SXT,CHL	0.75	MDR	+
E39	TET,AML,FOS,CAZ,SXT,CHL	0.50	MDR	+
E40	NFT,TET,AML,FOS,CAZ,CTX,SXT	0.58	MDR	+
E41	CIP,TET,AML,AMC,FOS,CAZ,CTX,GEN,SXT	0.75	MDR	+
E42	CIP,TET,AMC,AML,FOS,CAZ,CTX,SXT,CHL	0.75	MDR	+
E43	CIP,TET,AML,FOS,CAZ,CTX,GEN,SXT,CHL	0.75	MDR	-
E44	FOS,CAZ,SXT	0.25	MDR	+
E45	CIP,TET,AML,CAZ,FOS,CTX,SXT	0.58	MDR	+
E46	TET,AMC,AML,FOS,CTX,GEN,SXT,CHL	0.67	MDR	+
E47	NFT,CIP,TET,AML,FOS,CAZ,CTX,GEN,SXT,CHL	0.83	MDR	+
E48	NFT,CIP,TET,AMC,AML,FOS,CAZ,CTX,GEN,SXT	0.83	MDR	+
E49	NFT,CIP,TET,AMC,AML,FOS,CAZ,CTX,GEN,SXT	0.83	MDR	+
E50	CIP,TET,AML,FOS,CAZ,CTX,GEN,SXT	0.67	MDR	+
E51	CIP, TET,AMC,AML,FOS,CAZ,CTX,SXT,CHL,	0.75	MDR	+

AMC= Amoxicillin/Clavulanic acid, AML= Amoxicillin, CTX= Cefotaxime, CAZ= Ceftazidime, CIP= Ciprofloxacin, CHL= Chloramphenicol, FOS= Fosfomycin, Gen= Gentamicin, MEM= Meropenem, NFT= Nitrofurantoin, SXT= Sulphamethoxazole/Trimetoprim, TET= Tetracycline, MDR= Multidrug resistant, NMDR= Not multidrug resistant, +=positive, -=negative.

Table 3: Multiple antibiotic resistance index and profile of phenotypic occurrence of *E. coli* isolates

MAR Index	Number of *E. coli* isolates	Percentage (%)
0.16	1	2.0
0.25	1	2.0
0.33	3	6.0
0.41	4	8.0
0.50	4	8.0
0.58	6	12.0
0.66	14	27.0
0.75	13	25.0
0.83	5	10.0
Total	51	100.0

The Multiple Antibiotics Resistance Index (MARI) of the *E. coli* isolates shows that 100% were resistant to 2 or more class of antibiotics

Table 4: Antimicrobial resistance pattern of *E. coli* strains to tested antibiotics

	Percentage of isolates (%)		
Antibiotics	**Resistance**	**Susceptibility**	**Intermediate**
Amoxicillin/clavulanic acid	59.0	25.0	1.0
Amoxicillin	92.0	8.0	-
Cefotaxime	76.0	20.0	4.0
Ceftazidime	94.0	-	6.0
Ciprofloxacin	61.0	35.0	4.0
Chloramphenicol	51.0	45.0	4.0
Fosfomycin	84.0	10.0	6.0
Gentamicin	23.5	70.5	6.0
Meropenem	-	94.0	6.0
Nitrofurantoin	19.6	78.4	2.0
Sulphamethoxazole/trimetoprim	96.0	2.0	2.0
Tetracycline	92.0	8.0	-

13The ESBL production prevalence of 76.4% observed in this study agrees with the findings of Okesola and Fowotade (2012) where 76.9% was reported in some Enterobacteriaceae, and 63.2% by Olowo *et al*., 2015 both in south western Nigeria. Igwe *et al*., 2014 also reported an ESBL prevalence of 70.0 % among *E. coli* isolates in the northwest, while 52.5% was reported in the eastern region by Azekhueme *et al*., 2015. Majority of the *E. coli* isolates 38 (74.8%) were from patients with urinary tract infection (UTI) and most of them 30 (78.9%) were ESBL producers. A study by Ejaz *et al*., 2011 showed that the ESBL producing bacteria are increasingly causing UTIs both in hospitalized and outpatients, which is making therapy of UTI difficult and promoting greater use of expensive broad spectrum antibiotics, such as carbapenems. This confirms the study done by Paterson in 2005 that carbapenems are considered the first choice for treatment of patients infected with ESBL-producing Enterobacteriaceae. Multidrug resistance as defined by Subramani and Vignesh, 2012 was common.

CONCLUSION

A high prevalence of ESBL production (76.4%) was observed among the clinical isolates of *E. coli*, and majority of them (98.0%) were multidrug resistant, having a MAR index ≥0.2. Meropenem (a carbapenem) is highly effective against ESBL producing *E. coli*.

RECOMMENDATIONS

Rational use of antibiotics should be encouraged. Patients to whom antibiotics are prescribed should be monitored closely to ensure strict adherence to dosage regimen. There should be increased awareness on the detriments of antibiotics misuse.

.

REFERENCES

Aibinu, I., Odugbemi, T., Koenig, W., and Ghebremedhin, W. (2012). Sequence Type ST131 and ST10 Complex (ST617) predominant among CTX-M-15-producing *Escherichia coli* isolates from Nigeria. Clinical Microbiology and Infection, Volume 18: Number 3, E49-E51.

Al-Mayahie, S.M.G. (2013). Phenotypic and genotypic comparison of ESBL production by vaginal *Escherichia coli* isolates from pregnant and non-pregnant women. Annals of Clinical Microbiology and Antimicrobials, 12:77-82.

Alsterlund, R., Carlsson, B., Gezelius, L., Haeggman, S., Olsson-Liljequist, B. (2009). Multiresistant CTX-M-15 ESBL-producing *Escherichia coli* in southern Sweden: Description of an outbreak. Scandinavian Journal of Infectious Diseases; 41(6-7):410-5.

Azekhueme, I., Moses, A.E., and Abbey, S.D. (2015). Extended Spectrum Beta-Lactamases in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from University of Uyo Teaching Hospital, Uyo-Nigeria. Journal of Advances in Medical and Pharmaceutical Sciences 2(3): 117-125.

Clinical Laboratory Standards Institute (2012). Performance standards for antimicrobial susceptibility testing; twenty-second information supplement. M100-S22. Vol 32, No 3.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries (Part II). Cambridge University Press, U.K. pp. 134-143.

Ejaz, H., Ikram-ul-Haq., Zafar, A., Mahmood, S., and Javed, M.M. (2011). Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumonia.* African Journal of Biotechnology Vol. 10 (73), pp. 16661-16666.

Igwe, J.C., Onaolapo, J.A., Kachallah, M., Nworie, A., Oladipo, H.O., Ojiego, B.O., Enose, O.D., Adeboye, S.E., Durowaiye, M.T., Akpa, A.U., Ibanga, I.A. (2014). Molecular characterization of extended spectrum β-Lactamase genes in clinical *Escherichia coli* isolates. Journal of Biomedical Science and Engineering, Vol. 7, 276-285.

Jarlier, V., Nicholas, M.H., Fournier, G. and Philippon, A. (1988). Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Reviews of Infectious Diseases 10, 867-78.

Kirby-Bauer (1996). Antimicrobial sensitivity testing by agar diffusion method. Journal of Clinical Pathology.44: 493.

Mohammed, Y., Gadzama, G.B., Zailani, S.B., and Aboderin, A.O. (2016). Characterization of Extended-Spectrum Beta-lactamase from *Escherichia coli* and *Klebsiella* species from North Eastern Nigeria. Journal of Clinical and Diagnostic Research. Vol-10(2): DC07-DC10.

Nicolas-Chanoine, M.H., Blanco, J., Leflon-Guibou, V., Demarty, R, Alonso, M.P., Canica, M.M. (2008). Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. Journal of Antimicrobial Chemotheraphy; 61(2):273-81.

Okesola, A.O., and Makanjuola, O. (2009). Resistance to third generation cephalosporins and other antibiotics by Enterobacteriaceae in western Nigeria. American Journal of Medical Sciences.; 5(1): 17-20.

Okesola, A.O., and Fowotade, A., (2012). Extended spectrum beta-lactamase production among clinical isolates of *Escherichia coli*. International Research Journal of Microbiology, 3:140-143.

Paterson, D.L., and Bonomo, R.A. (2005). Extended spectrum β-lactamases: a clinical update. Clinical Microbial Review. 18 : 657-86.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower R.J. and Henderson G. (2012). Rang and Dale's pharmacology. 7th edition. Elsevier Churchill Edinburgh, London.609-619.

Rupp, M.E, and Fey, P.D. (2003). Extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. Drugs. 63(4):353-65.

Subramani, S., Vignesh, S. (2012). MAR Index study and MDR character analysis of a few golden *Staphylococcus* isolates. Asian Journal of Pharmacy and Life Sciences. 2 (2) 151-153.

Address for correspondence:

Durowaiye, M. T
Department of Pharmaceutical Microbiology,
University of Ibadan, Ibadan, Nigeria
E-mail: mojihambolu@yahoo.com

Conflict of Interest: None declared
Received: 7 February, 2017
Accepted: 25 April 2017