FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 3 No. 4, December, 2019, pp 434 –439

PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES IN *ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE* ISOLATED FROM PATIENTS AT THE FEDERAL TEACHING HOSPITAL GOMBE, GOMBE STATE, NIGERIA

***[1]Yarima, A., [2]Salihu, M. K. and [3]Gurama, A. G.**

[1]Department of plant Science, Bio resource Development Center Michika, National Biotechnology Development Agency.
[2]Department of Microbiology, Federal University Dutsin-Ma, Katsina State, Nigeria.
[3]Department of Pharmaceutical Microbiology and Biotechnology, Gombe State University.

*Corresponding author's email: adamuyerima08@gmail.com, +2347067907982

ABSTRACT
Klebsiella pneumoniae and Escherichia coli are the major bacterial pathogens being isolated and reported from urine samples worldwide as Extended Spectrum Beta Lactamases (ESBLs) producers. This study was conducted to determine the prevalence of ESBLs producing *K. pneumoniae* and *E. coli* from urinary clinical isolates in Federal Teaching Hospital Gombe. A total of two hundred and nine (209) bacterial isolates comprising 100 *E. coli* and 109 *K. pneumonia* were collected and recovered on MacConkey agar at 35ºC. The isolates were further identified as members of *Enterobacteriaceae* family using conventional biochemical test. Preliminary screening for ESBL production was carried out using antibiotics disk of ceftadizime (30µg), ceftriazone (30µg) and cefpodozime (10µg) placed on to a surface of Muller-Hinton agar plates, separated from each other by a distance not less than 20mm apart, zones of growth inhibition were measured to the nearest mm. The screened isolates were further confirmed as ESBL producers using Double Disks Synergy Test (DDST). The percentage distribution of ESBL production was 54.13%, 40% and Non ESBL was 45.87% and 60% for *K. pneumoniae and E. coli* respectively. Majority of ESBL-producing isolates were from young patients, particularly women below 30 years of age. In conclusion, the findings of this study showed an alarming ESBL production in *E. coli* and *K. pneumoniae* was observed and recorded among patients receiving health care in FTH Gombe.

Keyword: Extended Spectrum Beta Lactamase, *E. coli, K. pneumoniae*, Urinary clinical isolates.

INTRODUCTION

Enterobacteriaceae especially *Escherichia coli* and *Klebsiella pneumoniae* producing Extended Spectrum Beta Lactamases (ESBLs) are opportunistic pathogens that emerged as a major contributory factors to nosocomial and urinary tract infections. These infections causing organisms are treated with a variety of antimicrobial agents comprising the beta lactams, beta lactam/beta lactamase inhibitors, flouroquinolones and carbapenems (Hoban *et al.,* 2011). The beta lactams antibiotics were however resisted by an enzyme, beta lactamase, which was first identified in *E. coli*, even before the first beta lactam antibiotics (penicillin) was developed (Fouzia and Damle, 2015). Because of their increased resistance against the oxyimino-cephalosporins, these enzymes (beta lactamases) were called Extended Spectrum Beta Lactamases (Bradford, 2001 and Fauzia and Damle, 2015). Hoban *et al.* (2011) reported that majority of the ESBL producing *E. coli* and *K. pneumoniae* are resistant to most of these antibiotics used against them. Production of beta-lactamase enzymes by bacteria remains the major mechanism employed for resistance (Leylabadlo *et al.*, 2016). The resistance developed in one part of the country or indeed in the world can be disseminated readily (Greenwood, 1998). According to WHO. (2012) unnecessary use of antibiotics favours the spread of resistant bacteria and as such, it is time to take much stronger action worldwide to avert a situation that entails an ever increasing health and economic burden of antimicrobial resistance. ESBL is an enzyme produced by plasmid-mediated bacteria responsible for the increase resistances worldwide and this resulted to challenge in public health. *Klebsiella pneumonia* and *Escherichia coli* remain the major ESBL-producing organisms isolated worldwide, but these enzymes have also been identified in several other members of the *Enterobacteriaceae* family and in certain non fermentors (Johann *et al.,* 2008). These beta lactamases are known to inactivate beta lactam ring in the antibiotic thereby rendering them ineffective. This study investigates the prevalence of ESBLs among urinary clinical samples from FTH Gombe

MATERIALS AND METHODS

Study area

Federal Teaching Hospital (FTH) Gombe is a tertiary health care institution with 300-bed capacity that was established in 1996 and is located along Ashaka road within Gombe metropolis, the capital city of Gombe State.

Ethical Approval

This research was conducted in accordance with the ethical principles on clinical specimens as approved with approval no: NHREC/25/2013 by the ethical committee of the Federal Teaching Hospital Gombe.

Clinical bacterial isolates

Two hundred and nine (209) urinary clinical isolates were taken from the Department of Medical Microbiology to Microbiology Laboratory FTH Gombe. The isolates were

collected weekly and recovered on MacConkey agar plates at 35°C for 18 hours. Suspected bacterial isolates were further analyzed using biochemical test as described by Cheesbrough. (2006).

Biochemical confirmation of the isolates
The bacterial isolates were identified as members of the *Enterobacteriaceae* through the following tests

Indole test
A loopful of an overnight culture of the test organisms were inoculated into a peptone water and incubated at 37^0C for 24hrs. Indole production was detected by adding 0.5ml of Kovac's reagent. Development of red coloured ring over the surface in the broth within 10 minutes indicates positive test for indole as described by Cheesbrough, 2006.

Citrate test
A loopful of an overnight culture of the test organisms were inoculated into a tube containing prepared Simmon's citrate agar. Sterile straight wire-loop was used to streak the slope with the test organism and then the butt was stabbed, the tube was incubated at 37°C for 24hours. Change of colour from light green to bright blue in the medium indicated positive citrate test (Cheesbrough, 2006).

Urease test
A loopful of an overnight culture of the test organisms were inoculated into a tube containing 3ml of sterile urea agar as described by Cheesbrough, (2006). Sterile wire loop was used to streak the slope with the test organisms and stabbed the butt. The tube was incubated at 35°C for 18 hours after which the colour of the medium changed from yellow to pink red.

Kligler iron agar test
A loopful of overnight culture of the test organisms were streak into a test tube containing Kligler Iron agar, using sterile wire loop at the slope of the tube and the butt was stabbed. The inoculated medium was incubated at 35°C for 18 hours. This medium allowed the determination of four biochemical properties of the unknown organism:

a. **Lactose (+) or (-):** Yellow slant were observed in the tubes hence, lactose (+) positive
b. **Glucose (+)** or **(-):**Yellow butt were observed in the tubes hence, glucose (+) positive
c. **Hydrogen Sulphide (H_2S) (+) or (-).** No black spots were observed in the tubes, hence H_2S (-) negative.
d. **Gas formation (+) or (-)**.Cracks/bubbles were observed in the agar which indicated gas production (+)**.**

Motility test

The motility can be determined by observing cells in a liquid environment. From the peptone water, a suspension of the isolates grown were placed on grease free, clean glass slide and then covered with a cover glass. This was then scrutinized using a microscope.

Phenotypic detection of ESBL production

Screening for ESBL production

The procedure of Samir *et al.* (2016) was employed:

A loopful of an overnight culture of the test organism was used to make a suspension of the test isolates in a normal saline to match the density of a McFarland 0.5 turbidity standard. The test organisms were swabbed on to a surface of Muller-Hinton agar plates using sterile swab stick. The antibiotic discs of cefpodozime (10µg), ceftriaxone (30µg) and ceftadizime (30µg) were placed on the surface of Muller Hinton agar using sterile forceps, leaving 15 mm away from the edge of the petri dish, the sensitivity discs were separated from each other by a distance not less than 20mm to avoid overlapping zones of inhibition. The plates were then incubated at 37°C for 18 hours. Zones of growth inhibition around the antibiotic disks were measured to the nearest mm, when a diameter zone of ≤22mm for ceftadizime, ≤25mm for ceftriazone and ≤17mm for cefpodozime were recorded, the isolates were recorded as ESBL producers and were confirmed using double disk synergy test (CLSI, 2015). *E. coli* (ATCC-25922) and *K. pneumoniae* (ATCC-700603) were used as reference strains.

Confirmatory test using Double Disk Synergy Test (DDST).

This test relies on the action of beta lactamase inhibitors such as clavulanic acid to enhance the inhibition zones. In this assay, the test organisms were swabbed onto a Mueller-Hinton agar plate with a suspension (adjusted to 0.5 McFarland turbidity standards) made from an overnight agar plate of the test strain as described by Rahman *et al.* 2014. A susceptibility disk containing amoxicillin-clavulanate (20/10µg) was placed in the center of the plate, and disks of ceftriazone (30µg) and ceftadizime (30µg) were placed around it at a distance of 15mm apart. Plates were incubated overnight at 37°C for 18 hours. An increase in the zone of inhibition towards the centrally place disk was considered positive for ESBL production (CLSI, 2014).

RESULTS

Biochemical identification of the isolates

The result of the biochemical identification of the isolates is presented in Table 1. When subjected to biochemical test, the isolates, depending on the test type, colour change was observed and recorded below:

Table 1: Biochemical identification of the isolates

Isolates	Number tested	Urea	Indo	Cit	Mot	KIA			
						B	S	Gas	H_2S
Escherichia coli	100	-	+	-	+	Y	Y	+	-
Klebsiella pneumoniae	109	+	-	+	-	Y	Y	+	-

KEY:Urea=Urease test, **Ind**=Idole test, **Cit**=Citrate test, **Mot**=Motility test, **KIA**=Kligler Iron agar test, **B**=Butt, **S**=Slope, **Gas**=Gas production, **H_2S**=Hydrogen Sulphide production, **Y**=yellow, +=positive and **-** =negative

Distribution of Extended Spectrum Beta Lactamase (ESBL) among clinical isolates

The result of the distribution of Extended Spectrum Beta Lactamase (ESBLs) among *K. pneumoniae* and *E. coli* isolates is presented in Table 2. Out of the two hundred and nine (209) isolates screened, comprising one hundred (100) *E. coli* and one hundred and nine (109) *K. pneumoniae*. The percentage distribution of ESBL production was 54.13%, 40% and Non ESBL production was 45.89% and 60% for *K. pneumoniae and E. coli* respectively.

Table 2: Distribution of Extended Spectrum Beta Lactamases among *K. pneumoniae* and *Escherichia coli* isolates

Isolates	*K. pneumoniae*	*E. coli*
Number of isolates screened	109	100
Preliminary screening	63 (57.79%)	41 (41%)
Confirmatory test using Double Disk Synergy (DDS)	59 (54.13%)	40 (40%)
ESBL	59 (54.13%)	40 (40%)
NON ESBL	50 (45.87%)	60 (60%)

Prevalence of ESBL production according to age and sex of the patients

The highest (10.9% in males and 13.0% in females) prevalence of ESBL based on age group was recorded in 00-10 in *E. coli* and 21-30 (8.9% in males and 24.4% in females) age group in *K. pneumoniae*, followed by age group of 31-40 (0.0% in males and 27.3% in females) in *E. coli* and 00-10 (8.7% in males and 19.6% in females) in *K. pneumoniae.* While the least prevalence was recorded in 51-60 (7.1% in both males and females) age group in *E. coli* and 71-80 (20.0% in males and 6.7% in females) age group in *K. pneumoniae.*

Table 3 Prevalence of ESBL and Non ESBL production according to age and sex of the patient

Demographic Variance		*Escherichia coli*				*Klepsiella pneumoniae*			
Number Screened		100				109			
		ESBL Positive		Non ESBL		ESBL Positive		Non ESBL	
Age group (Years)	Number of isolates								
		M	F	M	F	M	F	M	F
00-10	46	5 (10.9%)	6 (13.0%)	3 (6.5%)	9 (19.6%)	4 (8.7%)	9 (19.6%)	2 (4.3%)	8 (17.4%)
11-20	27	1(3.7%)	2 (7.4%)	2 (7.4%)	9 (33.3%)	2 (7.4%)	6 (22.2%)	1 (3.7%)	4 (14.8%)
21-30	45	1 (2.2%)	6 (13.3%)	2 (4.4%)	10 (22.2%)	4 (8.9%)	11 (24.4%)	3 (6.7%)	8 (17.8%)
31-40	33	0 (0.0%)	9 (27.3%)	1 (3.0%)	4 (12.1%)	4(12.1%)	5 (15.2%)	4(12.1%)	6 (%18.2)
41-50	12	0 (0.0%)	2 (16.7%)	1 (8.3%)	2 (16.7%)	2 (16.7%)	0 (0.0%)	2 (16.7%)	3 (25%)
51-60	14	1 (7.1%)	1(7.1%)	3 (21.4%)	2 (14.3%)	0 (0.0%)	2 (14.3%)	3 (21.4%)	2 (14.3%)
61-70	16	2 (12.5%)	0 (0.0%)	3 (18.8%)	3 (18.8%)	5 (31.3%)	0 (0.0%)	1 (6.3%)	2 (12.5%)
71-80	15	3 (20.0%)	1 (6.7%)	4 (26.7%)	2 (13.3%)	3 (20.0%)	1(6.7%)	1(6.7%)	0 (0.0%)
>80	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)

Values before parentheses are number of ESBL observed while those within are percentage
ESBL=Extended Spectrum Beta Lactamases, **M**=male, **F**=female

DISCUSSION

There has been an alarming rate of resistance in gram negative bacteria (Bush, 2010) and the problem of ESBL is becoming a global concern. Studies conducted in different countries shows a widespread prevalence of ESBL (Rupp and Fey, 2003). According to FMAEH. (2017) the resistance among bacteria is accelerated by selective pressure from inappropriate antimicrobial use while the spread of resistant organisms from one person to another is promoted by poor infection prevention and control. Production of beta lactamase is the most common mechanism employed by the bacteria to acquire resistance.

The observed prevalence of ESBL production in the present study among *K. pneumoniae* (54.1%) and *E. coli* (40%) was similar to the prevalence reported in Egypt by Elsayed. (2015) whose result showed 54.5% prevalence in *Klebsiella pneumoniae* and 41.5% in *Escherichia coli.* Similarly, another result that buttressed our finding is that of Kaur and Aggarwal. (2013) where ESBLs prevalence was 52.27% in *K. pneumoniae* and 46.43% in *E. coli* in India. ESBL prevalence of 55% in *K. pneumoniae* was reported in Saudi Arabia by Al-Agamy*et al.* (2009). However, higher results was reported by Badri *et al*. (2017) in Aljazira state of Sudan in which 91% prevalence in *E. coli* and 89.2% in *K. Pneumoniae was observed,* much lower compares to our study also reported 17.07% prevalence of ESBL in *E. coli* and 11.11% in *Salmonella typhi* in Kaduna by Yusha'u and Umar, 2016.

Out of the total sampled population 136/209 (65.09%) were female, whereas 73/209 (34.9%) were males. Highest ESBL prevalence based on age group was recorded in 00-10 in *E. coli* and 21-30 age group in *K. pneumoniae,* followed by age group of 31-40 in *E. coli* and 00-10 in *K. pneumoniae.* While the least prevalence was recorded in 51-60 age group in *E. coli* and 71-80 age group in *K. pneumoniae.* This distribution shows that, majority of ESBL-producing isolates were from young patients, particularly women age below 30.This finding agrees with the result of Aiyegoro *et al*. (2007) whose result revealed that female were more infected than males. Therefore, it has been established that young women have a higher prevalence of ESBL isolated from patients with UTI than men in FTH Gombe.

Variation exists between the present finding and several other studies indicated that the prevalence and type of ESBL varies from one geographical region to another and may be related to the use and or misuse of antibiotics (Nwosu *et al.,* 2014). It might also be attributed to different methods employed in detecting the ESBLs. High prevalence of ESBL reported in this study might be probably associated to high resistance to most of the drugs usually prescribed for urinary tract infections, indiscriminate consumptions of antibiotics, prolong hospitalization, incomplete antibiotic treatment, misuse, and the use of antimicrobials in animal husbandry Tajick. (2006).

CONCLUSION

Considering various findings in the present study, it can be concluded that the prevalence of Extended Spectrum Beta Lactamases prevalence among pathogenic *Klebsiella pneumoniae* and *Escherichia coli* in Federal Teaching Hospital Gombe is high. This high prevalence of ESBL producers in Gombe requires routine, reliable, rapid and sensitive diagnostic system such as polymerase chain reaction (PCR) to ensure quality health.

ACKNOWLEDGEMENT

The authors thank the management of Federal Teaching Hospital Gombe for providing the necessary facilities to conduct this study.

REFERENCES

Aiyegoro, O. A; Igbinosa, O. O; Ogunmwonyi, I. N; Odjadjare, E. E; Igbinosa, O. E. and Okoh, A. I. (2007). Incidence of urinary tract infections (UTI) among children and adolescents in Ile-Ife, Nigeria. *African Journal of Microbiology Research* 13-19.

Al-Agamy M. H. M; Atef, M. Shibl; Abdelkader, F. Tawfik. (2009). Prevalence and molecular characterization of extended spectrum beta lactamase producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. *Annals of Saudi Medicine.* 29(4): 253-257.

Badri, A. M; Ibrahim, I. T; Mohamed, S. G; Garbi, M. G I; Kabbashi, A. S. and Arbab, M. H. (2017) Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Raw Milk Samples in Al Jazirah State, Sudan. *Mol Biol* 7: 201. doi: 10.4172/2168-9547.1000201.

Bradford P.A (2001) Extended- Spectrum Beta Lactamases in the 21st Century: Characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews* 14: 933-951.

Bush, K., Jacoby, G. A. (2010). An updated functional classification of-lactamases. *Antimicrobial Agents and Chemotherapy*. 54 (3): 969– 976.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries, Part-2 (2nd Edition) New York. Cambridge University. Pp 62-70.

Clinical and Laboratory Standards Institute, (2014). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fourth Informational Supplement M100-S24. Wayne, PA, USA.

Clinical and Laboratory Standards Institute, (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement M100-S25. Wayne, PA. USA.

Elsayed, N; Awad, A; Omar, M; and Desouki, D. (2015). Rapid Simultaneous detection of AmpC and ESBLs among *Enterobacteriaceae* using Mast D68C detection set and possible therapeutic options. *Egyptian Journal of Medical Microbiology*. 24 (3): 1-12.

Federal Ministries of Agriculture, Environment and Health (FMAEH). (2017). Antimicrobial Use and Resistance in Nigeria. Situation analysis and recommendations.

Fouzia, B and Damle, A. S. (2015) Prevalence and Characterization of Extended Spectrum Beta-Lactamase Production in Clinical Isolates of *Klebsiella pneumonia*. *Journal of Medical Microbiology and Diagnosis* 4 (2): 1000182.

Hoban, D. J; Nicolle, L. E; Hawser, S; Bouchillon, S. and Badal. R. (2011). Antibiotic susceptibility of global inpatient Urinary Tracts isolates of *Escherichia coli*: Result from the Study for Antimicrobial Resistance Trends (SMART)

program: 2009-2010. *Diagnostic microbial infectious diseases* 70 (4): 507-511.

Johann, D. D; Pitout, Kevin; and Laupland. B. (2008). Extended Spectrum beta-Lactamase-Producing *Enterobacteriaceae*: An emerging public-health concern. *The Lancet Infectious Diseases* .8 (3):159-166.

Kaur, M and Aggarwal, A. (2013). Occurrence of the CTX-M, SHV and the TEM Genes among the Extended Spectrum beta-Lactamase Producing Isolates of *Enterobacteriaceae* in a Tertiary Care Hospital of North India. *Journal of Clinical and Diagnostic Research* 7(4): 642-645.

Leylabadlo, H. E., Kafil, H. S., Yousefi, M., Aghazadeh, M., and Asgharzadeh, M. (2016). Persistent infection with metallo-beta-lactamase and extended spectrum beta-lactamase producer *Morganella morganii* in a patient with urinary tract infection after kidney transplantation. *Journal of Natural Science, Biology and Medicine;* 7 (2): 179-181.

Nwosu1, I. L; Amadi, E. S; Nwanyanwu, C. E; Chikwendu, C. I. and Madu, C. L. (2014). The prevalence of extended spectrum beta-lactamases (ESBLs) among *Escherichia coli* and *Klebsiella species* urinary isolates from Abia state university teaching hospital (ABSUTH) aba, Abia State Nigeria. *International Journal of Microbiology and Mycology*. 2 (3): 20-28.

Rahman, M; Mujibur Rahman, M. and Akhter, J. W. (2014). Clinical Laboratory and Molecular Detection of Extended Spectrum beta lactamases: A Review Update. *Bangladesh Journal of Infectious Diseases*, 1(1): 12-17.

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):353–365.

Samir, I; Abdelhady, N; ElSheshtawy, M. and Hamdi, N, A. (2016). Rapid detection of extended spectrum β-lactamases production in *Escherichia coli*. *International Journal of Applied Microbiology and Biotechnology Research.* 4:109-116.

WHO, (2012). The evolving threat of antimicrobial resistance Options for action. GPS Publishing, France.

Yusha'u, M. and Umar, M. I (2016). Occurrence of Extended Spectrum Beta-Lactamases among Bacterial Isolates from Meat Products Sold within Kaduna Metropolis, Nigeria. *International Journal of Advances in Chemical Engineering and Biological Sciences* (IJACEBS) 3 (2): 2349-1515.