Antibiotic susceptibility of extended spectrum beta lactamase producing *Escherichia coli* of poultry origin

M. I. Mbah[1]* and C. O. Anyamene[2]
[1]Department of Medical Laboratory science.Taraba State University,Jalingo

[2]Department of Applied Microbiology and Brewing, NnamdiAzikiwe University, Awka.

***Corresponding author E mail** :mdduruoha@gmail.com

ABSTRACT

Threat to life caused by antibiotic resistance bacteria led to this study on the antimicrobial susceptibility of extended spectrum beta lactamase(ESBL) producing *Escherichia coli* of poultry origin. Two hundred and sixty one isolates of *E. coli* were obtained from broilers,layers and cockerels farms.The isolates were tested for beta lactamase production using acidimetric test .The betalactamase producing strains were further tested for the ability to secrete ESBL using double disk synergy test(DDST).The antibiotic susceptiblity profile and the plasmid sizes of the ESBL producing E. *coli* were determined.They were also subjected to acridine orange for curing.The results obtained showed that the occurrence of *E.coli* in layers', broilers and cockerels farms were respectively 35.2%,37.2% a and 26.8% .The occurrence of the ESBL producers was18.8%.The antibiotic susceptibility profile showed that the proportion of the ESBL producing *E.coli* resistance to ampicilin,ceftazidime and ceftriaxone was absolute while ciprofloxacin,chloramphenicol,tetracycline,gentamicin was 71.2%,48.1%,59.6%,25.0% respectively.All the isolates investigated harboured high molecular (23130bp) plasmids while the curing rate was 15.2%.Chicken farms harbor ESBL producing *E. coli* .

Key words: antibiotic resistance **,**third generation cephalosporins ,chickens,curing, *Escherichia coli*, extended spectrum beta lactamase. ,

INTRODUCTION

Extended spectrum beta lactamase(ESBL),an enzyme, secreted by bacteria is known for hydrolyzing third generation cephalosporins (1).Third generation cephalosporins are particularly effective against Gram negative bacteria.Third generation cephalosporins distinguishes itself from other cephalosporins(except cefoperazone) by its ability to reach the central nervous system and to appear in the spinal fluid in sufficient concentration to treat meningitis caused by Gram negative rods (2).ESBL can be secreted by bacteria like *E. coli* and this characteristic makes the strains that produce it to be antibiotic resistant.Antibiotic resistance can either be plasmid or chromosomally mediated.The expression of chromosomal beta lactamase can either be induced or stably depressed by exposure to beta lactam drugs (3).

The plasmid mediated extended spectrum beta lactamase are of increasing concern.This is because the ESBL genes located on plasmids can easily be transferred between and within bacterial species.Thereby making once antibiotic susceptible bacteria to become antibiotic resistance.Plasmid genes for antimicrobial resistance often control the formation of enzymes capable of destroying the antimicrobial drugs .Plasmids code for enzymes that destroy chloramphenicol(acetylferase),for enzymes that acetylate,adenylate or phosphorylate various aminoglycosides,for enzymes that determine the active transport of tetracycline across the cell membrane (2).

Antibiotic resistance is a menace to our generation and calls for attention from all and sundry.It is threatening the life of all;old and young,poor and rich.It has caused a lot of havoc already and if actions are not urgently taking,more harm will be done.Treatment failures for patients with blood infections caused by bacteria that produce ESBL is almost twice as high as that of the non ESBL producing bacteria (4).Also,the achievements of modern medicine are put at risk by antibiotic resistance.It is putting at risk the ability to treat common infections in the community and hospitals. ESBL producing Gram negative bacteria in which *E. coli* is one of the chief culprits limit therapeutic options as a result of their multidrug resistances (5),(6).

ESBL producing *E. coli* can be found In a variety of places.It has been isolated from chicken feeds (7) ,surface water adjacent to farms (8),broiler farmers(9).This study is therefore to investigate the antimicrobial susceptibility of ESBL producing *E. coli* of poultry origin.

MATERIALS AND METHODS

Study Area

Jalingo, the headquarter of Jalingo local government area and the capital of Taraba state in Nigeria, located between latitude 8^0 47' North and $90^0$1' 'North; longitude $11^0$9',East and $11^0$30' East is the study area.

Collection of Samples

Layers'.broilers' and cockerels' cxhicken farms'were used for this study. The samples included chickens, chicken environment/environ and rearers'stool A swab stick was soaked in sterile distilled water and was inserted into the cloaca of each of the randomly selected chickens in each of the poultry farms . While still in the cloaca, the swab stick was rotated three times before it was finally put back into its container.Also,swab sticks were soaked in sterile distilled water and used to swab strategic areas on the floor of the poultry . Each swab stick was then put back into its container.A sterile wide mouthed container was used to collect the stool specimen of each of the chicken rearers .

Bacterial Isolation and Identification

Samples collected were cultured within 2 hours of collection on MacConkay agar and EMB agar (Oxoid CM 516, UK) and incubated at 37°C for 18–24 hours. *E. coli.* was identified using standard microbiological techniques (10).

ESBL detection

Isolates were tested for beta-lactamase production using acidimetric method as previously described earlier by the procedure outlined by Cheesbrough (10).All positive *β*-lactamase isolates were screened for ESBL production by double disk synergy test according to (11). Four milliliter of 0.5 Mc Farland equivalent standard of the test organisms were spread on the surface of a sterile Mueller Hinton agar plate using a sterile swab stick.After 20 min , Augmentin disc (30µg) (Amoxicillin 20µg/clavulanic acid 10µg combination) was placed 15mm apart from the center of ceftriaxone disc(30µg) and ceftazidime disc(30µg) .This was incubated for 18 hours at 37^{o}C.

Antimicrobial Susceptibility Testing

This test was done using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar as described by (12). Briefly,a suspension was made from a 24 h growth of the organism in saline to match the 0.5 McFarland turbidity standard. This was spread on the entire surface of a Mueller-Hinton agar plate using a sterile swab stick. The following antibiotic discs were used: ceftazidime (30 µg), ceftriaxone (30 µg), ampicillin (10 µg), nitrofurantoin (300 µg), gentamicin (10 µg),chloramphenicol(30 µg),tetracycline(30 µg) and ciprofloxacin (5 µg). The Mueller-Hinton agar plate was then incubated at 35°C for 18–24 h, after which the diameter of the zones of growth inhibition around the discs was measured with a ruler. The results were further interpreted using the Performance Standards for antimicrobial susceptibility Testing (12).

Plasmid Profile of Extended Spectrum Betalactamse (ESBL) Positive Isolates

Extraction was done as previously described (13). Extracted plasmid DNA was loaded unto 0.8% Agarose gel. The resulting gel electrophoresis was visualized in a UV Trans-illuminator and molecular weight distances were determined according to)(14).

Plasmid Curing

ESBL positive isolates were selected and subjected to acridine orange as described by (15). Each tested organism was grown in a solution of 5ml double strength nutrient broth supplemented with *0.1ng/ml* of acridine orange and incubated at 37^{0}C for 24 hours. After incubation the test organisms were retested for ESBL production using double disk synergy test.

RESULTS

Two hundred and sixty one isolates of *E.coli* was obtained from chickens ,chickens environment and chicken rearers.This is presented in in table 1 below.

Table I: Occurrence of *E. coli* in Chicken Farms

farms	sources	No of Isolates	Frequency (%)
Layers	chickens	79	30.3
	Environ	9	3.4
	Rearers	4	1.5
Broilers	Chickens	69	26.4
	Environ	28	10.7
	Rearers	2	0.8
Cockerels	Chickens	50	19.2
	Environs	16	6.1
	Rearers	4	1.5
total		261	100

Clavulanic acid(in disk 4) inhibited the action of the ESBL produced by *E.coli* thereby making the zone of inhibition around disk 1 and 2 on the area closer to the disk 4(which contained clavulanic acid) to augment towards disk 4 as shown in plate 1.

1 – Ceftazidime

2 – Ceftriaxone

4 – Augmentin

Plate I: Synergy of clavulanic acid containing disk(disk 4) with ceftazidime(disk 1) and ceftriaxone(disk 2) in double disk synergy test (DDST)

A total of 49(18.8%) ESBL producing *E.coli* was obtained; 3.8 %, 6.9 % and1.5 % were obtained from the chickens reared in layers,broilers and cockerels farms respectively as presented in table 2 below.

Table 2: Occurrence of ESBL positive *E. coli* in chicken Farms

farms	sources	No of Isolates	ESBL positive	No of *E.coli*	Frequency(%)
Layers	chickens	79	10		3.8
	Environ	9	3		1.1

	Rearers	4	0	0
Broilers	Chickens	69	18	6.9
	Environ	28	8	3.1
	Reaerers	2	2	0.8
Cockerels	Chickens	50	4	1.5
	Environs	16	2	0.8
	Rearers	4	2	0.8
total		261	49	18.8

The ESBL producing *E. coli* isolates were 100% resistant to ampicillin, ceftriaxone and ceftazidime.They were also 48.1%,71.2%,1.9%,25.0% and 59.6% resistant to chloramphenicol, ciprofloxacin, nitrofurantoin, gentamincin and tetracycline respectively as presented in table 3 below

Table 3: Antibiotic Susceptibility Profile of ESBL Positive *E. coli*

Antibiotics	(Ug/Disk)	S	%	I	%	R	%
Ampicillin	10	0	0	0	0	52	100
Chloramphenicol	30	15	28.8	12	23.0	25	48.1
Ciprofloxacin	5	13	25.0	2	3.8	37	71.2
Nitrofurantoin	300	46	88.5	4	7.7	1	1.9
Gentamicin	10	35	67.3	4	7.7	13	25.0

Tetracycline	30	12	23.1	9	17.3	31	59.6
Ceftriaxone	30	0	0	0	0	52	100
Ceftazidime	30	0	0	0	0	52	100

S-Sensitive, I-Intermediate, R-Resistant

All the Fifteen isolates of ESBL producing *E.coli* randomly selected harboured only one plasmid each of size 23130bp.This is presented in table 4 below.

Table 4: Plasmid sizes of ESBL Positive Isolates from different sources

Source	**Number of Isolates of *E. coli***	**Number of plasmid**	**Number of isolates 23130bp**
Chickens	5	1	5
Chickens rearers	5	1	5
Chickens' environment	5	1	5

Fifty two ESBL producing isolates were subjected to acridne orange and only 8(15.2%) were cured.This is presented in table 5.

Table 5: Plasmid Curing Rate of the ESBL positive *E.coli* isolated from chicken farms

Farms	Number of ESBL Positive isolates	Number of Isolates Cured	Frequency of Isolates Cured (%)
Layers farm	12	4	7.6
Broilers farm	29	4	7.6
Cockerels farm	11	0	0.0
Total	**52**	**08**	**15.2**

DISCUSSIONS

Food producing animals are known to be reservoirs for ESBL producing strains especially *E. coli* (16),(17).In this study,261 *E.coli* isolates were obtained from all the farms.Table 1 shows the occurrence of *E.coli* in layers,broilers and cockerels farms and the findings revealed that a high occurrence of *E.coli* was obtained fom the chickens and this agrees with a previous report by (18).The high occurrence of *E.coli* in chickens is because cloacal swabs was the sample and *E.coli* are highly prevalent in the gastrointestinal tract.Table 1 also revealed that the occurrences of *E.coli* in layers,broilers and cockerels farms were respectively 35.2%,37.2% and 26.8%

Figures 1 shows the synergy of clavulanic acid containing disc with ceftazidime and ceftriaxone in double disk synergy test (DDST).The observations made from Fig.1 revealed that the enzyme, ESBL, was inhibited by clavulanic acid which is a componenent of the antibiotic disc 4(augmentin).This made the zone of growth inhibition around antibiotic disc 1(ceftazidime) and 2(ceftriaxone) to augment towards antibiotic disc 4.The synergy observed in Fig 1 supports a previous report by (7).

Table 2 shows the occurrence of ESBL positive *E,coli* in chcken farms and the findings revealed that the occurrence,18.8%,obtained in this study is lower than 84.5% reported by (19) in Netherland.It is also higher than 15.6% reported by (20).Also,the observations from Table 2

revealed that the broilers' farm had the highest(10.8%) occurrence while the cockerels farm had the lowest(3.1%).This is because of the differences in the management practices in the various farms.In broilers farms the chickens drinking water was not portable water also ,a mixture of 'Home made feed' and' factory made feed' were used for the chickens.Aseptic techniques were not observed in preparing the 'Home made ration of the mixture.Therefore ,there was a high probability of contamination of the feed with ESBL producing *E.coli* which eventually gets to the chickens

Table 3 shows the antibiotic susceptibility profile of ESBL positive *E.coli* and the observations made revealed that this strain of bacteria is resistant to several antibiotics.The bacteria showed absolute resistance to ampicllin and this is as a result of high use of ampicillin in poultry production in Nigeria (20).They also showed absolute resistance to ceftriaxone and ceftazidime and this is because of high use of third generation cephalosporin in clinical practice. High resistance(59.6%) to tetracycline was revealed which is lower than the high resistance (77.8%) resistance reported by (22) .Also,high resistance(71.2%) to ciprofloxacin agrees with 70.3% high resistance reported by (23).Also 48.1% resistance was recorded on chloramphenicol and this is higher than 25% reported by Tadesse *et al*.,(2018)(24) but lower than 100% reported by(25). High resistance to these antibiotics(tetracycline,ciprofloxacin and chloramphenicol) is because of the abuse of these antibiotics in self medication and also their therapeiutic use in animals and widespread addition of it to the animal feed(26),.The multidrug resistance observed agrees with report by (27) and this is because plasmids responsible for ESBL production carry resistance to several antibiotic agents(6).

Table 4 shows the plasmid sizes of ESBL positive *E.coli* isolated from chicken farms.It revealed that all the ESBL producing *E. coli* harboured heavy molecular plasmids(23130bp). A study by (23) on clinical samples reported that the ESBL producing *E. coli* harbored heavy molecular plasmids(24.3Kbp).

Table 5 shows the plasmid curing rate of the ESBL positive *E. coli* isolated from chicken farms and the observations made revealed that 15.2% of the isolates were cured and this is lower than 100% reported by (15) .

CONCLUSION

Chicken farms are reservoirs of ESBL producing *E. coli* .The *E.coli* are multidrug resistant Therefore,the need for all to put hands on deck to eliminate this organism.

Conflict of Interest :The authors declare no conflict of interest

REFERENCES

1.Philippon, A., Labia R.,and Jacoby, G. (1989) Extended – spectrum beta – Lactamases.*Journal of Antimicrobial Agents and Chemotherapy.*33:1131 – 1136.

2.Brooks, G.F, Butel, J.S. and Morse, S.A (1998). Medial Microbiology (21st edn). Lange, Stamford Plaza, Stamford Conneticut, Pp145-176.

3.Dever, L.A.I and Dermody, T.S. (1991), Mechanisms of bacterial resistance to antibi otics.A*rchives of internal medicine* 151: 886 – 895.

4.Tumbarello, M., Spanu, T., Sanguinetti, M., Citton, R., Montouri, E., Leone F., Fadda, G. and Cauda, R. (2006). Blood stream infections caused by extended spectrum β-lactamase – producing *Klebsiella pneumoniae:* Risk factors, molecular epidemiology and clinical outcome. *Journal of antimicrobial agents and chemotherapy. 50(2):498 – 504.*

5.Anago, E., Ayi-Fanou, L.,Akpovi, D.C., Hounkpe, W.B., Tchibozo, M.A., Bankole, S.H. and Sanni, A. (2015). Antibiotic resistance and genotype of beta lactamase producing Escherichia coli in Nosocomial infections in cotonou, Benin. *Annals of clinical microbiology and antimicrobials.14:5–16*

6.Nathisuwan ,S.,Burgess, D.S.and Lewis II,J.S (2001). ESBLS: Epidemiology detection and treatment. *Journal of Phamacotherapy,* 21(8): 920 – 928.

7.Oyinloye JMA Jr. & Ezekiel C.N.(2011). Extended spectrum beta-lactamase (ESBL) – producing multidrug resistant enterobacteria from commercial poultry feeds in Nigeria. *Journal of Annals of Biological Research* 2(2): 250 – 254.

8.Blaak, H., Van Hoek, A.H.A.M., Hamidjaja, R.A., Vander Plaats, R.Q.J., Kerkhof-der Heer, L., De Roda, H.A.M. (2015). Distribution, numbers and diversity of ESBLproducing *E. coli* in the poultry farm environment.*PLOS ONE 10(8):e135402 doi:10.137/journal.pone.0135402*

9. Dahms C., Hubner N.O. and Kossow.A,Mellimann A.,DittmannK.and KramerA.. (201 5). Occurrence of ESBL producing *E.coli* in livestock and farm workers in Mecklenbur Western Pomerania, Germany.*PLOS One* 10(11): e0143 326.https://doi.org/10.1371/journal pone0143326.

10.Cheesbrough M. (2010). Antimocrobial Susceptibility Tests. District Laboratory Practice inTropical Countries (Vol. II) Tropical Health Technology ELBS, London, pp 36 – 72.

11.Liofilchem (2014). ESBL Disc Tests. Rev. 2/19.06 via Scoziazonaind.le,64026*Roseto deg* Abru zzi (Te) Italy. www.liofilchem.net.

12.Clinical Laboratory Standards Institute (CLSI)(2012).Methods for Dilution Antimicrobial Susc eptibility Testing for Bacteria That Grows Aerobically. Ninth Edition (M07-A9), Clinical Laboratory Standards Institute, Wayne, Pa, USA.

13.Akinniyi, A. P., Afolabi, O.,Iwalokun, B. A. , Oluwaseun, E. and Onagbesan, K. O. .(2011) "Clonal dissemination of blaTEM β-lactamase strains among enteric isolates in Abeokuta, Nigeria," *Research Journal of Microbiology* 6(12) : 919–925.

14.Kim, Y.K., Pai, H., Lee, H.J., Park, S.E., Choi, E.H., (2002). Blood stream infections by *ESBL* producing *Escherichia coli and Klebsiella pneumoniae* in children: Epidemiology and clinical outcome. *Journal of antimicrobial agents and chemotherapy.46:1481 – 1491*.

15. Iroha I.R, Amadi, E.S.,Oji,A.E,Nwuzo,A.C and Ejike Ugwu,P.C(2010).Detection of plasmid borne extended spectrum beta lactamase enzymes from blood and urine isolates of Gram Negative bacteria from University teacing hospital in Nigeria.*C urrent Research in bacteriology*.32:77-83

16.Carotolli, A.(2008). Animal reservoirs for extended spectrum beta lactamase producers.*Journal of clinical microbiology infections.1:1851 – 1883.*

17.Gao L., Hu, J., Zhang, X., Ma, R., Gao, J., Li, S., Zhao, M., Miao, Z. and Chai, T. (2014). Dissemination of ESBL – producing *Escherichia coli* of chicken origin to the nearby river water. *Journal of molecular microbiology and biotechnology.24:279– 285.*

18.Dadheech, T., Vijas, R. and Rastagi, V. (2016).Prevalence, bacteriology, pathogenesis and isolation of E. coli in sick layer chickens in Ajmer Region of India.*International Journal of current Microbiology and Applied Sciences 5(3): 129-136.*

19.Overdevest, I., Willemsen, I., Rijinsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M.,Savelkoul, P, Vandenbroucke, G., Zwaluw,K., Huijsdens, X. and Kluytmans, J. (2011).Extended spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands.*Journal of emerging infectious diseases 17(7):1216 – 1222.*

20.Chah, K.F. & Oboegbulem, S.I. (2007). Extended spectrum beta-lactamase production among ampicillin-resistant *Escherichia coli* strains from chicken in Enugu State, Nigeria. *Brazilian Journal of Microbiology* 38(4): 17-20.

21.Grave, K., Torren-Edo, J. and Mackay, D. (2010). Comparison of the sales of veterinary antimicrobial agents between 10 European countries. *Journal of antimicrobial chemotherapy.65:2037 – 2040*

22..Gundogan, N. and Avci, E. (2003). Prevalence and antibiotic resistance of extende spectrum beta-Lactamase (ESBL) producing *Escherichia coli and klebsiella* specie isolated from foods of animal origin in turkey. *African journal of microbiology research. 7(31): 4059 – 4064.*

23.Folasage A. A., Babatunde O. M., Akinniyi A., Akinbo,J., Ogiogwa J.I Aboderin B.W.1 and. Agunlejika,R.A.(2014).A multicenter study of Beta Lactamaseresistant *Escherichi a coli klebsiella pneumoniae* reveals high level chromosome mediated extended spectrum B-Lactamase resistanc in Ogun State,Nigeria. *Journal of interdisplinary perspectives of infectious diseases 2(2014):2014 Article ID819896, 7pages.http://doi.o rg/10.1155/2014/819896*

24.Tadesse,D.A.,Mukherjee,C.S.,Hsu,C.,Jones,.S,B.,Gaines,S.A.Kabera,C.mLoneragan,G.H.,Torr e nce,M.mHarhay,D.M.,McDemott,P.F. and Zhao..S. (2018;)Whole Genome Sequence Analysis of CTX-M containing *Escherichia coli* isolates from retail meats and cattles in the United State.*Microbial Drug Resistance*. 24(7):939-948.

25.Nawfia,I.N.,Ohanu M.E.,Ebede,S.O. *and* Ozumba,U.C.(2019).Molecular detection and antibiotic resistance pattern of extended spectrum betalactamase

producing *Escherichia coli* in a tertiary hospital in Enugu, Nigeria.*Annals of clinical microbiology and Antimicrobials* .18(41): http//doi.org/101186/s12941-019-0342-9.

26.Nakamura,T.,Komatsu,M.,Yamasaki,K.andYamamoto,Y.(2012).Epidemiology of *Escherichia coli,Klebsiella* species and Proteus mirabilisstrains producing Extended Spectrum Lactamases from clinical samples in the Kinki Region of Japan.*American Journal of clinical pathology*.137(4):620-626.

27.Gundogan, N. and Avci, E. (2003). Prevalence and antibiotic resistance of extende spectrum beta-Lactamase (ESBL) producing *Escherichia coli and klebsiella* specie isolated from foods of animal origin in turkey. *African journal of microbiology research. 7(31): 4059 – 4064.*