EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING *Escherichia coli* AND *Klebsiella pneumoniae* FROM CHICKEN FARMS

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Abstract: Extended spectrum beta lactamase (ESBL) is an enzyme that is capable of hydrolyzing third generation cephalosporin. It is a threat to public health, This study is to investigate the occurrence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* in chicken farms and ways of remedying it. Four chicken farms made up of broilers and cockerels farms were used. One hundred and ninety eight *Escherichia coli* and six *Klebsiella pneumoniae* isolates were used for this study. The isolates were screened for beta lactamase production using acidimetric method. The beta lactamase producing isolates were further confirmed for ESBL production using the double disk synergy test. The antimicrobial susceptibility profile was determined using the Kirbry-bauer disk diffusion method and finally, the isolates were subjected to 0.lng/ml of acridine orange for curing. Results revealed that the occurrence rates of *Escherichia coli* and *K. pneumoniae* were 97.1 and 2.9%, respectively; while that of beta lactamase producing *E. coli* and *K. pneumoniae* were respectively 55.4 and 2.0%. The occurrence of ESBL producing *E. coli* and *K. pneumoniae* were, respectively 23.0 and 0.5%. The occurrence of ESBL positive *E. coli* in chickens, chickens' environment and chicken rearers were respectively 15.7, 6.3 and 1.5% ($P>0.05$). Also, the occurrence in Madam Fibi's farm (2 days old white cockerels) and Baltic farm Sabon line (3 weeks old white cockerels' farm) were respectively 3.9 and 5.4% ($P<0.05$). The antibiotic susceptibility profile revealed that ESBL producing *E. coli* and *K. pneumoniae* were 100% resistant to ampicillin, ceftriazone and ceftazidime. They also showed resistance to other antibiotics like ciprofloxacin, tetracycline, etc. The curing rate of the *E. coli* and *K. pneumoniae* were 18.8 and 0%, respectively. Chicken farms harbor ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*, some of which can be cured.

Keywords: Antimicrobials, beta lactamase, chickens, curing, ESBL, plasmids

Introduction

Bacteria have different mechanisms of resisting the activities of antimicrobials. One of such mechanisms is by secreting enzymes that destroy the antibiotics. Extended spectrum beta lactamase (ESBL) is one of such enzymes secreted by bacteria that destroy antibiotics. ESBL is secreted by some strains of *Escherichia coli* and *Klebsiella pneumoniae* and this attribute confers on this strain the characteristic of being antibiotic resistant (Brooks *et al.*, 1998).

Antibiotic resistance is a threat to public health. It threatens the ability to treat common infectious diseases, resulting in death and disability of individual. As an example, the treatment failures for patients with blood infections caused by bacteria that produce enzymes capable of hydrolyzing third generation cephatosorin (called extended spectrum lactamase (ESBL) like ESBL producing *Klebsiella pneumoniae* infected group was almost as twice as high as that of the non-ESBL producing *Klebsiella pneumoniae* infected group (Tumbarello, 2006).

The achievements of modern medicine are put at risk by antimicrobial resistance. Without effective antimicrobial for prevention and treatment of infections, the success of organ transplantation, cancer chemotherapy and major surgery would be compromised (WHO, 2014). WHO (2014) report on global surveillance of antimicrobial resistance revealed that antibiotic resistance is no longer a prediction for the future. It is putting at risk the ability to treat common infections in the community and hospitals.

The use and misuse of drugs accelerates the emergence of drug resistant strains (Cheesbrough, 2010). Poor infection control and inappropriate food-handling encourage the further spread of antimicrobial resistance.

ESBL producing gram negative organisms in which *Escherichia coli* and *Klebsiella* species are the chief culprits limit therapeutic options as a result of their multidrug resistance (Anago *et al*, 2013). This is because they possess resistant plasmids which not only makes them resistant to third generation cephalosporin but also makes them resistant to other antibiotics (Nathisuwan *et al.*, 2001).

Some of the ESBL genes located on plasmids can be easily transferred between and within bacterial species. As a result of this, ESBL genes can be found in nearly all the species of enter obacteriaceae (Jacoby and Medeiros, 1991).

ESBL producing *Escherichia coli* can be isolated from chicken feed (Oyinloye and Ezekiel, 2011). It can also be isolated from humans (clinical specimen) (Folasage *et al.*, 2014), from animal farms (Dahms *et al*, 2012). Gao *et al.* (2014) reported having isolated ESBL producing *Escherichia coli* from the environment close to a poultry farm.

ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* are major concern for everybody all over the world because of their multidrug resistance. Therefore, this study is to find out the occurrence of *E. coli* and *K pneumoniae* in chicken farms and ways of remedying the ugly situation.

Materials and Methods

Study area

Jalingo was the study area. It is the head quarter of Jalingo Local Government Area and the capital of Taraba State which is located in the North-East geopolitical zone of Nigeria. Jalingo is in the Northern Guinea Savanna zone of the vegetative cover of Nigeria. It located between latitude 8^0 47′ North and $90^01'$ North; longitude $11^09'$, East and $11^030'$ East. It has a population of approximately 118,000 people (2006 census) and a land mass of 3,871 sq km. The annual precipitation fall is 1053 while the temperature averages 27.3^0C (KÖpper Geiger, 1936).

Collection of samples

Samples were collected from 4 poultry farm. The poultry farms studied were: Bello's farm, Ijaja's farm, Madam Fibi's farm and Baltic farm. Bello and Ijaja's farms constitute of broilers 7 or less than 7 weeks old while Madam Fibi's farm and Baltic farm (Sabon Line) were 2 days old and 3 weeks old white cockerels, respectively.

The samples collected were cloacal swabs, floor and wall swabs, drinker and feeder swabs. Others were, stool and urine of the poultry farmers as well as the drinking water and feed of the chickens. The samples were grouped into three. They are: chicken (comprised of the cloacal swab), chicken environment (comprised of swabs from floors, wall, drinkers, feeder and also the drinking water and the feed) and chicken rearers (comprised of stool and urine samples).

Bacterial isolation and identification

Samples collected were cultured within 2 h of collection on MacConkay agar and EMB agar (Oxoid CM 516, UK) and incubated at 37°C for 18–24 h. *Escherichia coli* and *Klebsiella pneumoniiae* were identified using standard microbiological techniques (Cheesbrough, 2010)

ESBL detection

Isolates were tested for beta-lactamase production using acidometric method as described earlier by Cheesbrough (2010). All positive *β*-lactamase isolates were screened for ESBL production by double disk diffusion test according to Liofichem (2010). An aliquot of a 0.5 McFarland equivalent standard of the test organisms were sreaked on the surface of a sterile Mueller Hinton agar plate using a sterile swab stick. After 20 min of pre-incubation, a combination disc Augmentin (30 µg) (Amoxicillin 20 µg/clavulanic acid 10 µg combination) was placed 15 mm apart from the center of ceftriaxone disk (30 µg) and ceftazidime disk (30 µg) .This was incubated for 18 – 24 h at 37°C.

Antimicrobial susceptibility testing

This test was done using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI 2012) guidelines. The Modified Kirby-Bauer standardized disc diffusion testing was done using the direct colony suspension method. A suspension was made from a 24 h growth of the organism in saline to match the 0.5 McFarland turbidity standard. This was seeded on the entire surface of a Mueller-Hinton agar plate while rotating the plate at an angle of 60° three times. 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 (35–37°C) for 18–24 h, after which the diameter of the zones of growth inhibition around the discs was measured with a ruler. These results were further interpreted using the Performance Standards for Antimicrobial Susceptibility Testing, CLSI 2012.

Plasmid curing

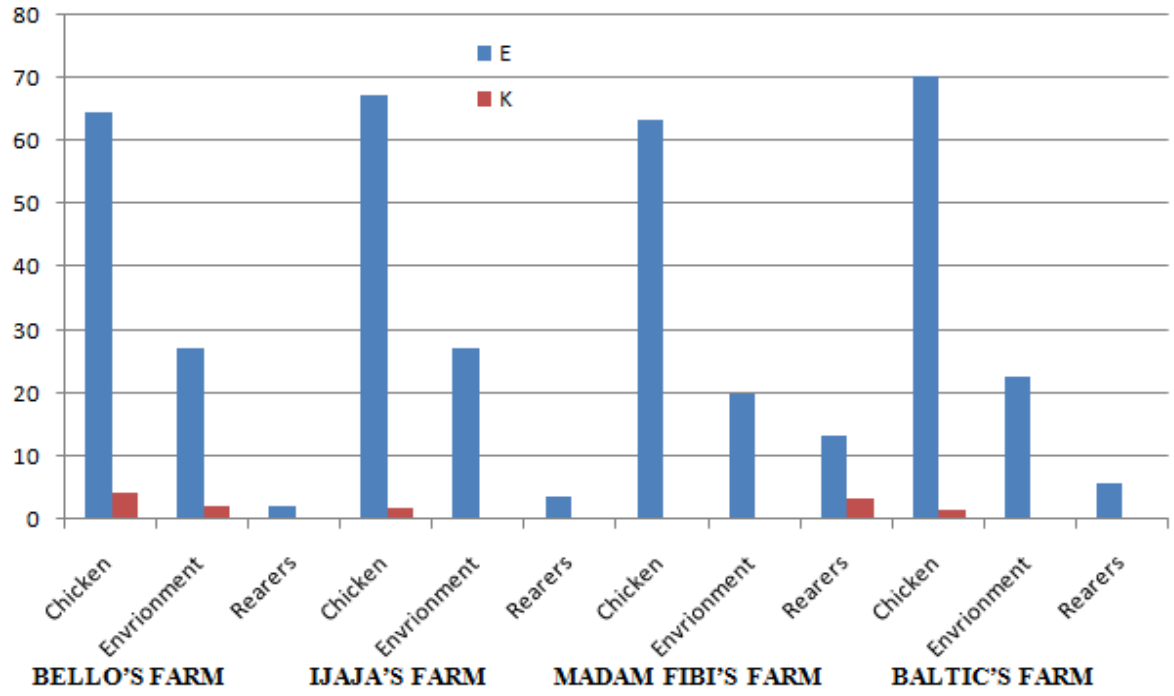
ESBL positive isolates were selected and subjected to acridine orange as described by Iroha *et al.* (2010). Each tested organism was grown in a solution of 5ml double strength nutrient broth supplemented with 0.1 ng/ml of acridine orange and incubated at 37^0C for 24 h. After incubation the test organisms were retested for ESBL production using double disk synergy test.

Statistical analysis

The data obtained during the investigations were subjected to analysis of variance and chi-square and inferences made at $p \leq 0.05$ using statistical package for social sciences (SPSS) version 21.0.

Results and Discussion

Escherichia coli and *Klebsiella pneumoniae* are Enterobacteriaceae that can be found in chicken farms. The findings in Fig. 1 revealed that E*scherichia coli* and *Klebsiella pneumoniae* were isolated from all the farms but their occurrence differs; *Escherichia coli* had a higher occurrence than *Klebsiella pneumoniae*. This is because most of the samples were of faecal origin especially those from the chickens. In some of the farms, the poultry floor, drinkers and feeders were heavily soiled with chicken dropping. Dadheech *et al.* (2016) on his work with clinically sick chickens reported 100% occurrence of *E.coli* which is higher than the high occurrence (97.1%) also obtained in this study. The low occurrence (2.9%) of *Klebsiella pneumoniae* obtained in his study is lower than 73.3% reported by Younis *et al.* (2016).



E = *Escherichia coli;* **K** = *Klebsielal pneumoniae*

Fig. 1: The occurrence of *Escherichia coli* and *Klebsiella pneumoniae* in Chicken farms

Table 1: Occurrence of β-lactamase producing *Escherichia coli* and *Klebsiell apneumoniae* in chicken farms

Farms	β-Lactamase Producers				
	Escherichia coli			*Klebsiell apneumoniae*	
	Number of Isolate	Number Isolated	Frequency of Isolates (%)	Number Isolated	Frequency of Isolates (%)
Bello **(broiler)**	48	27	13.2	3	1.4
Ijaja **(broiler)**	55	32	15.7	0	0
Baltic, Sabon line (3 weeks old)	71	36	17.6	1	0.6
Madam Fibi (2 days old white cockerels)	30	18	8.8	0	0
Total	**204**	**113**	**55.4**	**4**	**2.0**

Beta lactamases are ancient enzymes that existed even in the absence of the therapeutic antibiotics. The ability to produce them confers to such bacteria the attribute of being antibiotic resistant. The findings in Table 1 revealed that some of the *Escherichia coli* and *Klebsiella pneumoniae* strains obtained were capable of secreting beta lactamases. In broilers' farms, a higher occurrence (15.7%) was obtained from IJaja's farm while 13.2% was obtained from Bello's farm. This was because a higher level of hygiene was observed in Bellos's farm. Observations made from Table 1 revealed that in cockerels' farms, the occurrence of beta lactamase producing *Escherichia coli* is higher in Baltic farm than in Madam Fibi's farm. Also there was no occurrence of beta lactamase producing *Klebsiella pneumoniae* in Madam Fibi's farm while there was only 0.6% in Baltic farm. This is because Madm Fibi's farm was a new farm. The farm had not been exposed to a lot of contaminations. The floor and walls were very clean; likewise the feeders and the drinkers. In fact, a high level of hygiene was observed in the farm.

The findings in Fig. 2 revealed that the zone of inhibition around ceftazidime and ceftriaxone containing discs augmented towards the disc containing clavulanic acid (augmentin) in culture that contains ESBL producing bacteria. This agrees with the report by Folasage *et al.* (2014).

Fig. 2: Synergy of clavulanic acid containing disk with ceftazidime and ceftriaxone in double disk synergy test (DDST) for *ESBL* phenotypic confirmation test

Observations in Table 2 revealed that ESBL producing *E coli* and *K. pneumoniae* had the occurrence of 23 and 0.5%, respectively. This is lower than 65.9% reported by Hiroi *et al*. (2012) but higher than 14.2% occurrence reported by Nwakaeze *et al.* (2013) in Abakaliki, Ebonyi State. Table 2 also revealed that, the occurrence of ESBL producing *E. coli* in Bellos, Ijaja's, Baltic and Madam Fibi's farms were respectively 5.4, 8.3, 5.4 and 3.9%. ESBL producing *K. pneumoniae* was obtained only in Bellos's farm and the occurrence was 0.5%. In broilers' farms, a lower occurrence (8.3%) was obtained from Bellos's farm. This was because the environment (feeders, drinkers, floor and walls) of Bello's farm was cleaner than that of Ijaja's farm. Also, in Bello's farm, there was restriction on entry into the farm. In cockerels farms, a higher occurrence (5.4%) of ESBL producing *Escherichia coli* was obtained from Baltic farm while 3.9% was obtained from Madam Fibi's farm. This is because in Baltic farm, the floor and the walls were not clean. A very low level of hygiene was observed in the farm.

The findings in Table 3 revealed that 23.5% occurrence of ESBL producing *Escherichia coli* and *K. pneumoniae* was obtained from chickens, chicken rearers and chicken environment. Only one ESBL producing *Klebsiella pneumoniae* was obtained from the chicken environment. None was obtained from the chicken rearers and chickens. This occurrence (23.5%) is lower than 79.8% reported by Overdevest *et al.* (2011) but higher than 9.4% reported by Chah and Oboegbulem (2007). Observations made from Table 3 also revealed that the highest occurrence (15.7%) of ESBL producing *E. coli* was obtained from chickens while the least (1.5%) was obtained from the rearers. This is because cloacal swabs were used as the samples from chickens and *E. coli* are coliforms.

1-ceftazidime,2-ceftraixone,4-augmentin

Table 2: Occurrence of ESBL positive *E. coli* and *K. apneumoniae* in chicken farms

Farms	ESBL Positive Isolates				
	E. coli			*K. apneumoniae*	
	Number of Isolates	Number Isolated	Frequency of Isolates (%)	Number Isolated	Frequency of Isolated (%)
Bello's (broilers)	48	11	5.4	1	0.5
Ijaja's (broilers)	55	17	8.3	0	0
Madam Fibi's (2 days old white cockerels)	30	11	5.4	0	0
Baltic, Sabon Line (3 weeks old white cockerels)	71	8	3.9	0	0
Total	**204**	**47**	**23.0**	**1**	**0.5**

Table 3: Occurrence of ESBL positive *E. coli* and *K. apneumoniae* from various farm samples

Sample	ESBL Positive Isolates				
	E. coli			*K. apneumoniae*	
	No. of Isolates	Number Isolated	Frequency of Isolates (%)	Number Isolated	Frequency of Isolated (%)
Chickens	143	32	15.7	0	0
Chickens' Environment	50	12	5.8	1	0.5
chicken rearers	11	3	1.5	0	0
Total	**204**	**47**	**23.0**	**1**	**0.5**

Table 4: Antibiotics susceptibility profile of ESBL positive *E. coli* and *K. pneumoniae*

Antibiotics	(Ug/disc)	*Escherichia coli*						*Klebsiella pneumoniae*					
		S	%	I	%	R	%	S	%	I	%	R	%
Ampicillin	10	0	0	0	0	47	100	0	0	0	0	1	100
Chloramphenicol	30	14	29.8	11	23.4	22	46.8	1	50	0	0	0	0
Ciprofloxacin	5	12	25.5	2	4.3	33	70.2	0	0	0	0	1	100
Nitrofurantoin	300	42	89'4	4	8.5	1	2.1	1	100	0	0	0	0
Gentamicin	10	32	68.1	4	8.5	11	23.4	1	100	0	0	0	0
Tetracycline	30	11	23.4	8	17.0	28	59.6	0	0	0	0	1	100
Ceftriaxone	30	0	0	0	0	47	100	0	0	0	0	1	100
Ceftazidime	30	0	0	0	0	47	100	0	0	0	0	1	100

Observations made from Table 4 revealed the antibiotic susceptibility profile of the ESBL producing *Escherichia coli* and *K. pneumonia*e. The isolates showed absolute resistance to ampicllin; this is because of the overuse of ampicilliin in animal production in Nigeria. They also showed absolute resstance to ceftriaxone and ceftazidime as a result of the high use of these drugs in clinical practice. They also showed high resistance to chloramphenicol (46%), tetracycline (59%) and ciprofloxacin (70.2%). This is because of the abuse of these drugs in self medication. They showed minimal resistance (2.1%) to nitrofurantoin. This multi drug resistance observed in these ESBL producing strain supports previous reports by other researchers which includes Gundagon and Avci (2003) that ESBL producing *E. coli* and *K. pneumoniae* showed high resistance (77.8 and 69.8%) respectively to tetracycline. Afunwa *et al.* (2011) also reported a high resistance (40%) to ciprofloxacin. Nakamura e*t al*. (2012) reported high resistance (70%) to ciprofloxacin. Sheikh *et al.* (2014) reported minimal resistance to nitrofurantoin. Tadesse *et al.* (2018) reported moderate resistance (25%) to chloramphenicol.

Table 5: Plasmid curing rate of the ESBL producing *E. coli* and *K. apneumoniae*

Isolate	No. of isolates with plasmid	No. of isolates with no plasmid after curing	% of isolates cured
Escherichia coli	47	9	18.8
Klebsiella pneumonie	1	0	0
Total	**48**	**9**	**18.8**

Resistant plasmids are those extrachromosmal DNA that carry the genes for resistant to antibiotics (Brooks *et al.*, 1998). Observations from Table 5 revealed that 18.8% of the ESBL producing *E. coli* was cured while none of the ESBL producing *K. pneumoniae* was cured. Though there have never been any report on the curing rate of ESBL producing bacteria to the best of the knowledge of the researchers but a similar study by Folasge *et al.* (2014) and Iroha *et al.* (2014) on clinical samples reported curing rates of 13.5 and 100%, respectively.

Conclusion

Chicken farms are reservoirs of ESBL producing *E. coli* and *K. pneumonia;* the occurrence of the ESBL producing *K. pneumoniae* was minimal. ESBL producing bacteria are multidrug resistant but they can be inhibited by clavulanic acid. Therefore, everybody needs to work together to ensure that this strain of bacteria is eliminated.

Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

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