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Occurrence of ESBL Producing *Klebsiella* sp. in Selected Broiler Farms in Afikpo, Ebonyi State Nigeria

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***Abstract*: The study was aimed at examining the occurrence of ESBL producing *Klebsiella* sp. in selected broiler farms in Afikpo. Fecal droppings from randomly selected five (5) poultry farms were collected, labeled and sent to the microbiology laboratory for analysis. 5g weighed out, ground in mortar and dissolved in 10ml distilled water and used to prepare ten-fold serial dilution. By spread plate technique, 0.1ml was inoculated onto prepared media; incubated for 24 hrs at 37ºC. by standard laboratory protocol, isolates where characterized as *Klebsiella* sp. isolates were subjected for antibiotics Susceptibility Testing by disc diffusion and zone of inhibition recorded. All the strains with diameter less than 27mm for cefotaxime and less than 25mm for ceftriaxone, were selected for checking ESBL production and by Double Disc Synergy Test (DDST) method placed at a distance of 15 mm. increase of zone of inhibition above 5 mm in the presence of amoxicillin (20 µg) and clavulanic acid (10µg) indicated presence of ESBL producer. Result showed high incidence of ESBL producing *Klebsiella* sp. with high resistance to the tested antibiotics. Only six Tetracycline (TE), Levofloxacin (LE), Amoxyclav (AMC), out of the 13 antibiotics inhibited the growth of *Klebsiella* sp. and three out of the five samples showed the presence of ESBL *Klebsiella* sp. producers. From the foregoing, we recommend that there was need to do periodic surveillance at each poultry farm to monitor the prevalence of ESBL producers and take measures to contain their spread.**

***Keywords*: ESBL, Klebsiella, Broiler, Farms.**

1. Introduction

The main causes of cephalosporin resistance are the production of extended spectrum β-lactamase (ESBL) and AmpC β-lactamase (Jacoby, 2009; Bevan *et al.,* 2017). The emergence of bacterial resistance is not only due to the evolution of microorganisms, but also because of excessive misuse of antimicrobial agents, which have speed up this process (WHO, 2015). Antibiotic resistance genes appeared to originate from environmental bacteria, which effect microbiota in the environment. Excessive use of antibiotics for prophylaxis and curative treatment and release of human and animal microbiota containing resistance genes aggravate this situation (Martinez, 2009).

ESBLs catalyze the hydrolysis of penicillins and cephalosporins. Gram-negative enteric bacteria that belong to the family Enterobacteriaceae have become resistant to this class of β-lactam agents by acquiring the ESBL gene and produce related enzymes. ESBL is widespread throughout the world, with more than 1.5 billion people colonized with ESBL-producing Enterobacteriaceae (Woerther *et al.,* 2013). *K. pneumonia* shows high resistance to a broad spectrum of antibiotics including β-lactam antibiotics, fluoroquinolones, and aminoglycosides (Fair and Tor, 2014; Dsouza *et al.,* 2017). There are many modes of transmission which may be either of transfer between bacteria from one host to another, by transfer of clones, or transfer of resistance genes, which are located in a mobile genetic material, between bacterial species that involve horizontal gene transfer, including pathogenic and non-pathogenic strains. These processes are influenced by the use of antibiotics in human medicine and veterinary medicine (Smet *et al.,* 2019).

It has been reported by researchers that infections caused by Multidrug-resistant (MDR) *K. pneumoniae* have also become a major issue, because of higher morbidity, longer hospitalizations, increased mortality, and excessive health care costs compared with infections related to antibiotics, because of susceptible microorganisms (Correa *et al.,* 2013; Ma and Wang, 2013). Pet contact is related to ESBL-E transmission has been mentioned in previous studies (Meyer *et al.,* 2012), but little is known about mode of transmission. In this study we aimed to identify the ESBL-producing *Klebsiella pneumonia* and MDR in companion animals.

Klebsiella pneumonia being a pathogen cause animal and human infections throughout the world, and these infections are linked with resistance to very important antimicrobial agents (Harada *et al.,* 2016; Marques *et al.,* 2019). Recently, the World Health Organization (WHO) categorized the ESBL produced by *K. pneumonia* as a top priority pathogen. The genus Klebsiella belongs to the family Enterobacteriaceae and consists of Gram-negative pathogens with mucoidal aspects. The digestive tract of hosts from both animals and humans work as a reservoir and is often function as a source of infection (Fertas-Aissani *et al.,* 2013). The genus Klebsiella is categorized into four species: *Klebsiella pneumoniae*,

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Klebsiella oxytoca, *Klebsiella terrigena*, and Klebsiella planticola, with *Klebsiella pneumoniae* further consists of three subspecies, *Klebsiella pneumoniae* subsp. pneumoniae, *Klebsiella pneumoniae* subsp. ozaenae, and *Klebsiella pneumoniae* subsp. rhinoscleromatis (X-Li *et al.,* 2004).

Poultry products have become another source of income for many families especially in this present time that job is difficult to find. Many graduates and locals have taken to poultry farming as it seems to secure prompt earnings. This is due to the fact that many families have realized the importance of white meat and its other products like egg. But this all important product has been met with problem affecting humanity. Most of this product has led to the introduction of antibiotic resistant bacteria such as extended – spectrum β-lactamase (ESBL) in the lives of man who depend on these products for his protein supply. This may be due to the antibiotics that are used in rearing of these birds. It is on the basis of this that this study is undertaken to examining the occurrence of ESBL producing *Klebsiella* spp. in selected broiler farms in Afikpo.

2. Materials and Method

A. Sample Collection

Fecal droppings from randomly selected five (5) poultry farms were collected into sterile containers, labeled and sent to the microbiology laboratory for analysis.

B. Samples and Media Preparations

Each of the collected dried feaces samples was ground and 5g weighed out and mixed with distilled water. This was then used in preparing 10-fold serial dilutions up to 10^{-3} dilution factor. All media (EMB, Nutrient agar, MacConkey agar) used for this study were prepared based on manufacturer's instruction.

C. Media Inoculation

Using spread plate technique, 0.1ml aliquot of 10^{-3} dilution of each sample was aseptically inoculated on the solidified media, evenly spread by glass spreader and incubated for 24 hours at 37^{o}C.

D. Identification of Isolates

The suspected *Klebsiella* spp. isolate was characterized using conventional/standard microbiology techniques such as:

- Cultural morphology (pigmentation, shape and size)
- Cellular features (gram reaction and shape - rod)
- Biochemical features (catalase, coagulase, urease, Methyl red, oxidase test, indole test, citrate utilization test, H_2S production test, Voges-Proskauer test and sugar fermentation test).

E. Antimicrobial Susceptibility Testing

The isolate was tested for antimicrobial susceptibilities by the disc diffusion method according to the CLSI guidelines. The confirmed *Klebsiella* sp. was streak on solidified Muller Hilton Agar and incubated for 24 hours at 37^{o}C. The zone of inhibition was observed and recorded in millimeter (mm). All the strains which showed a diameter of less than 27mm for cefotaxime and less than 25mm for ceftriaxone, were selected for checking ESBL production.

F. ESBL Determination by Double Disc Synergy Test (DDST) Method

This was determined between a disc of betalactamase inhibitor (amoxicillin (20 µg) and clavulanic acid (10 µg) and antibiotic disc of third generation cephalosporins (ceftazidime (30 µg) and cefotaxime (30 µg) placed at a distance of 15 mm apart on a culture of the test isolate on Muller-Hinton Agar.

The test isolate was considered to produce ESBL if the inhibition zone size around the antibiotic disc increased above 5 mm in the presence of a beta-lactamase inhibitor disc (amoxicillin (20 µg) and clavulanic acid (10 µg). This increase occurs because the clavulanic acid inactivates the ESBL produced by the test organism resulting in the formation of extended inhibitory zone.

3. Result

Cultures morphology of isolates from the five sampled droppings show the presence of large white muciod colonies on nutrient agar and large pink muciod colonies on MacConkey agar indicating the possible presence of *Klebsiella* sp (table 1).

Biochemical characterization of the isolates indicate the presence of *Klesiellla* sp. from the samples collected from five poultry farms.

The antibiotics were of varying concentration. These were as follows: Cefalexin (CN) 10mcg, Erythromycine (E) 15mcg, Tetracycline (TE) 30mcg, Levofloxacin (LE) 5mcg, Amoxyclav (AMC) 10mcg, Clindamycin (CD) 2mcg, Co-Trimoxazole (Sulpha/Trimethoprim) COT 25mcg, Cloxacillin (Cox) 1mcg, Ceftraxone (CTR) 30mcg, Ofloxacin (OF) 5mcg, Netillin (Netilmicin Sulphate) (NET) 30mcg, Ciprofloxacin

Table 1
Cultural feature on nutrient and MacConkey agar

Sample	NT	MacC	Susp. Organism
A	Large White mucoid colonies	large pink mucoid colonies	*Klebsiella* sp
B	Large White mucoid colonies	large pink mucoid colonies	*Klebsiella* sp
C	Large White mucoid colonies	large pink mucoid colonies	*Klebsiella* sp
D	Large White mucoid colonies	large pink mucoid colonies	*Klebsiella* sp
E	Large White mucoid colonies	large pink mucoid colonies	*Klebsiella* sp

Table 2
Biochemical features isolates from the sampled poultry droppings

Sample	Gram	Ind	MR	VP	Moti	Cit	Capsule	spore	urase	Glu	Lac	Mannit	Susp Orgnis
A	-	-	-	+	-	+	+	-	+	+	+	+	*Klebsiella* sp
B	-	-	-	+	-	+	+	-	+	+	+	+	*Klebsiella* sp
C	-	-	-	+	-	+	+	-	+	+	+	+	*Klebsiella* sp
D	-	-	-	+	-	+	+	-	+	+	+	+	*Klebsiella* sp
E	-	-	-	+	-	+	+	-	+	+	+	+	*Klebsiella* sp

Table 3
Antibiotics susceptibility pattern of the *Klebsiella* sp. isolates

Antibiotics	% sensitive	% resistance
Cefalexin (CN)	100%	0%
Erythromycine (E)	100%	0%
Tetracycline (TE)	0%	100%
Levofloxacin (LE)	0%	100%
Amoxyclav (AMC)	0%	100%
Clindamycin (CD)	100%	0%
Co-Trimoxazole (Sulpha/Trimethoprim)	0%	100%
Cloxacillin (Cox)	47%	53%
Ceftraxone (CTR)	59%	41%
Ofloxacin (OF)	9%	91%
Netillin (Netilmicin Sulphate)	71%	29%
Ciprofloxacin (CIP)	23%	77%
Gentamycin (GEN)	86	14%

(CIP) 10mcg, Gentamycin (GEN) 10mcg.

Isolates recorded 100% resistance to Cefalexin, Erythromycine, Clindamycin (CD). However, Tetracycline, Levofloxacin, Amoxyclav recorded 100% sensitivity to the isolates. Also, Ofloxacin, Ciprofloxacin recorded 91% and 77% sensitivity respectively. The isolates recorded 86% resistance against Gentamycin (table 3).

ESBL producing *Klebsiella* sp. among five samples indicated that three samples showed the presence of ESBL production as the inhibitory zone size around the antibiotic disc increased above 5 mm in the presence of a beta-lactamase inhibitor disc (amoxicillin (20 µg) and clavulanic acid (10 µg) (table 4).

Table 4
Detecting ESBL Production among *Klebsiella* isolates

Number Samples	Number Positive For *Klebsiella* sp	Number Negative For *Klebsiella* sp
5	3	2

4. Discussion

The present study examined the occurrence of ESBL producing *klebsiella* sp. in selected broiler farms in Afikpo. Cultures morphology of isolates from the five sampled droppings show the presence of large white muciod colonies on nutrient agar and large pink muciod colonies on MacConkey agar indicating the possible presence of *Klebsiella* sp (table 1). Isolates were tested against such antibiotics as Cefalexin (CN) 10mcg, Erythromycine (E) 15mcg, Tetracycline (TE) 30mcg, Levofloxacin (LE) 5mcg, Amoxyclav (AMC) 10mcg, Clindamycin (CD) 2mcg, Co-Trimoxazole (Sulpha /Trimethoprim) COT 25mcg, Cloxacillin (Cox) 1mcg, Ceftraxone (CTR) 30mcg, Ofloxacin (OF) 5mcg, Netillin (Netilmicin Sulphate) (NET) 30mcg, Ciprofloxacin (CIP) 10mcg, Gentamycin (GEN) 10mcg.

Isolates recorded 100% resistance to Cefalexin, Erythromycine, Clindamycin (CD). However, Tetracycline, Levofloxacin, Amoxyclav recorded 100% sensitivity to the isolates. Also, Ofloxacin, Ciprofloxacin recorded 91% and 77% sensitivity respectively. The isolates recorded 86% resistance against Gentamycin (table 3).

ESBL producing *Klebsiella* sp. among five samples indicated that three samples showed the presence of ESBL production as the inhibitory zone size around the antibiotic disc increased above 5 mm in the presence of a beta-lactamase inhibitor disc (amoxicillin (20 µg) and clavulanic acid (10 µg) (table 4).

The presence of ESBL producing *Klebsiella* sp. among poultry farms in this present study agrees with the study conducted by Akila *et al.* (2016) who noted a high prevalence of ESBL *Klebsiella* spp in India in a retrospective study

undertaken to identify the prevalence of ESBL producing Klebsiella spp. and their susceptibility pattern. They found out that of 209 Klebsiella spp., 80(38.28%) were found to be ESBL producers. All ESBL producing *Klebsiella pneumoniae* strains were susceptible to imipenem.

The isolates in this present study were 100% resistant to Co-Trimoxazole (Sulpha/Trimethoprim). This result is higher than that recorded elsewhere by Akila (2016) in India. They reported that in their study about 45% of isolates were resistant to co-trimoxazole.

The high use of antibiotics in breeding of birds such as poultry farm may be attributed to this high incidence of ESBL producing *Klebsiella* sp. in this present study as antibiotics use is known to select for resistance bacteria. Our position agrees with the report of Medeiros (2007), who opined that it is well known that antibiotic use selects for resistant bacteria and the indiscriminate and excessive use of beta-lactam antibiotics is in itself a driving force for clinically significant increase in the incidence of ESBL producing bacteria.

The occurrence of ESBL is a serious public issue as it is associated with many consequences not just health related but also economic related. Our position agrees with the observation of researcher like Kollef, (2000) and Cosgrove (2006) who in their separate studies remarked that the consequences of ESBL production, mainly in bloodstream infection (BSI), are well characterized, including notable delays in receipt of appropriate antibiotic therapy, prolonged length of stay (LOS), and increased cost of care. Their presence is equally associated with mortality. Importantly, others have found higher rates of mortality (Lautenbach *et al.,* 2001; Tumbarello *et al.,* 2007; Schwaber and Carmeli, 2007; Tumbarello *et al.,* 2010, Rottier *et al.,* 2012).

5. Recommendations

1) There is need to do periodic surveillance at each poultry farm to monitor the prevalence of ESBL producers and take measures to contain their spread.
2) The use of antibiotics in poultry farm should be regulated and standardized by authorities.
3) Seminar should be organisms to educate poultry farmers on the impact of antibiotics use on birds and human consumers.

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