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Studies on the Occurences of Extended-Spectrum Beta-Lactamase (ESBL) Producing *Klebsiella pneumonia* in Chicken Environments.

Lebechukwu Ogechukwu Rapuruchukwu and Obodo Nathanael C.

Department of Medical Microbiology, Applied Microbiology and Brewing Enugu state University of Science and Technology (ESUT). Faculty of Applied Natural Science.

ABSTRACT

Samples were collected from 70 poultries and 20 slaughters in Enugu north Local Government Area (L.G.A), Udi L.G.A and Enugu south L.G.A areas of Enugu State. A total number of 125 samples were analyzed in this research with the aim of screening for ESBL producing *Klebsiella pneumonia* and objectives of isolating ESBL producing *Klebsiella pneumonia* in chicken feed, chicken water, chicken excretes, chicken slaughters and chicken environment in the poultries located in the above mentioned areas. Twenty samples were analysed each for the chicken feeds, waters, excretes, slaughters and environment and a total of 100 samples of *Klebsiella pneumonia* were tested for ESBL production. Sixty eight samples (68%) of *Klebsiella pneumonia* were confirmed positive for ESBL production resulting from 12% ESBL positive samples from chicken feeds, 10% ESBL positive samples from chicken water, 17% ESBL positive samples from chicken excrete, 17% ESBL positive samples from chicken slaughters and 12% ESBL positive samples from chicken environment. To confirm ESBL production due to plasmid mediated origin or genetic mediated origin, a total of 68 samples of *Klebsiella pnemoniae* were cured or treated of plasmid using Acridine orange dye and retested for ESBL production. Out of the 68 samples of *Klebsiella pneumonia* cured, 59 samples (86.7%) were cured of plasmid and do not show ESBL production after retest and they are referred to as plasmid mediated ESBL while 9 samples (13.2%) were cured of plasmid and still show resistant to penicillin and cephalosporin after retest due to other reasons such as lack of penicillin binding protein (PBP) and not due to plasmid and they are called Genetic mediated ESBL.

Keywords: Occurences, Extended-Spectrum Beta-Lactamase *Klebsiella pneumonia*

INTRODUCTION

ESBLs (Extended Spectrum βeta Lactamases) are enzymes produced by Gram-negative bacteria; which has the ability to break down commonly used antibiotics, such as penicillins and cephalosporins and render them ineffective for treatment [1].

B-lactamases may be Plasmid mediated or Chromosomally encoded and universally present in a species. ESBLs are often encoded by genes located on large plasmids, and these carry genes for resistance to penicillins and cephalosporins. The chromosomal enzymes are believed to have evolved from PBPs with which they show same-sequence homology.[2], [3] This was probably a result of the selective pressure exerted by β-lactam–producing soil organisms found in the environment [4].

If ESBL-producing bacteria cause an infection, a different antibiotic may need to be used to treat the infection. [5], [6] People who carry ESBL-producing bacteria cause an infection, without any signs or symptoms of infection and are said to be colonized [7].

At present, however, organizations such as the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) provide guidelines for the

detection of ESBLs in *Klebsiella pneumoniae, Escherichia coli, K. oxytoca, and Proteus mirabilis*. In common to all ESBL-detection methods is the general principle that the activity of extended-spectrum cephalosporins against ESBL-producing organisms will be enhanced by the presence of Clavulanic acid. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant isolates have recently been reported [8], [9]

ESBLs represent an impressive example of the ability of gram-negative bacteria to develop new antibiotic-resistance mechanisms in the face of the introduction of new antimicrobial agents.

Extended spectrum beta lactamase producing *Klebsiella pneumonia* can be isolated from infected chicken environment, chicken feed, chicken water troph, poultry droppings and poultry workers [10].

Aim Of The Study

To study the occurrence of B-lactamase producing *Klebsiella pneumonia* in chicken environment.

Objectives:

The aims and objectives of this project are

- To isolate *Klebsiella pnemoniae* from chicken feeds
- To isolate *klebsiella pnemoniae* from chicken water samples
- To isolate *klebsiella pnemoniae* from chicken excretes
- To isolate *klebsiella pnemoniae* from commercial chicken slaughters
- To isolate *Klebsiella pnemoniae* from chicken environment.
- To test for susceptibility using B lactam drugs in order to isolate ESBL producing organisms (*Klebsiella pnemoniae*)
- To conduct Phenotypic confirmatory test to confirm ESBL production using B-lactam drug plus B-lactamase inhibitor (clavanic acid) in order to distinguish the isolate with ESBL and those that are resistant for other reasons
- To carry out Plasmid curing
- To Retest for ESBL production

Research Questions:

- Can ESBL producing *Klebsiella pneumoniae* be isolated from chicken feed?
- Can ESBL producing *Klebsiella pneumoniae* be isolated from chicken water samples?
- Can ESBL producing *Klebsiella pneumoniae* be isolated from chicken excretes?
- Can ESBL producing *klebsiella pneumoniae* be isolated from chicken slaughter environment?
- Can ESBL producing *klebsiella pneumoniae* be tested for B lactam antibiotic susceptibility?
- Can ESBL producing *Klebsiella pneumoniae* undergo phenotypic confirmatory test using clavanic acid in order to test for those resistant for other reasons?
- How can plasmid be cured?
- How can *Klebsiella pneumoniae* be retested for ESBL production after plasmid curing.

Research Hypothesis

ESBL producing *K. pneumonia* are susceptible to the action of ampicillin and cephalosporin in chicken environment.

Research Population

A total number of 70 poultries
and 20 chicken slaugthers were used
in this research.

Scope Of The Research

The scope of the research where these samples were collected was from Enugu North L.G.A (Ngwo), Enugu south L.G.A and Udi L.G.A of Enugu State.

Research Design

The research design used in this research was experimental research design.

Research Setting

The major setting or locations where these samples were collected were from Enugu North L.G.A (Ngwo) Enugu South L.G.A and Udi L.G.A of Enugu State.

MATERIALS AND METHODS

Samples Used Include

Chicken feed samples, Chicken water samples, Chicken excrete samples, Chicken Slaughter environment, Chicken environment from different sources in Enugu State, Nigeria.

Sterilization Of Glass Wares

All glass wares used were sterilized in a hot air oven at 160°c for one hour.

Sample Collection

Different sample of chicken feeds, chicken water, chicken excrete and swab from chicken Slaughters, Chicken environment were collected from different sources (poultries and Slaughters) in Enugu State. These specimens were labeled correctly accordingly and are stored in refrigerator in readiness for use.

Preparation Of Media

The media used were Nutrient agar, Macconkey agar. They were prepared according to the manufacturer's instructions and sterilized in an autoclave at 121oc for 15 minutes.

Isolation Of Primary Culture

The samples were inoculated into Macconkey Agar and incubated at 37oc for 24 hours

Identification Of The Isolates (*Klebsiella Pneumonia*).

The colonial appearance of the growth were noted and described.

Gram Staining Identification Procedure

After 24hrs, many colonies appeared and *klebsiella pneumonia* were identified with Gram staining procedure using an Aseptic wire loop, the colonies of the organisms were collected, and smears were made on microscopic slides, these slides are allowed to dry and were heat fixed then they were flooded with a primary stain called crystal violent dye and were allowed to stay for about 60 seconds after which the slides were washed with clean tap water and drained, after this step, the slides were flooded with Gram's Iodine (Mordant) and were allowed to stay for about 1 minute before washing off with clean tap water. [11] After this step the slides were tilt with 95% alcohol (Decolourizer) until the alcohol draining from the slides became colourless and the slides were washed again with clean tap water and drained, then the slides were flooded with a counter stain called Neutral red stain and are left to stay for about 3 minutes before washing with a clean tap water and are blot dryed. These slides were observed under oil immersion (×100) objective lens.

Microbiological Confirmation Test

Sugar Test

REAGENT: Phenol red broth, (differential for gram negative organism)

This is a test used to identify gram negative enteric bacteria. The phenol red broth is prepared according to the manufacturer's instruction and poured in different test tubes and the isolated organisms were introduced into the test tube containing the phenol red broth and colour changes were observed from red to yellow indicating positive to sugar test.

Catalase Test

REAGENT: Hydrogen peroxide

This test is used to know or dictate the presence of catalase enzyme in a test isolates using hydrogen peroxide. In this test, drops of hydrogen peroxide were placed on the slides, and the isolates were introduced to the hydrogen peroxide and oxygen bubbles were observed for positive results

Oxidase Test

REAGENT: N, N,N^1, N^1 tetramethyl P-phenylenediamine (TMPD)

This is a test used in Microbiology to determine isolates that produces certain cytochrome C oxidase; if positive, it means that the isolate contain cytochrome C Oxidase and can use oxygen for energy production by converting O_2 to H_2O_2 or H_2O with an electron transfer chain. If negative it means isolate does not contain cytochrome C Oxidase.

The disks of this reagent were prepared using impregnation method and the disk were allowed to dry. After this, each of these disk were wet with about 4 drops of distilled water and using an aseptic wire loop, large mass of the isolated bacteria were transferred to the disk and color changes were observed after 3 minutes, if the area of the inoculation turns dark blue-maroon or black, then result is positive, if light pink- Negative.

Indole Test

REAGENT: Tryptophan broth, Kovac reagent.

This test is used to determine the ability of an organism to split amino acid tryptophan to form the compound indole. The tryptophan broth was prepared according to the manufacturers instruction and poured into test tubes, and the broths in test tubes were inoculated each with the isolated bacteria and were incubated at 37oc for 28 hours, after the incubation, 0.5ml of Kovac's reagent were added to each of the test tubes and colour changes were observed.

Methyl Red Test

REAGENT: MR-VP (methyl red-Voges proskauer) broth used for both methyl red and VP tests. This test determines whether the isolates perform mixed acid fermentation when supplied glucose. The broth was prepared according to the manufacturer's instruction and was poured into many test tubes, after this, each test tube was inoculated with the isolated bacteria and they were incubated at 35°c for 4 days. After the incubation, 5 drops of methyl red indicator were added to each test tube and the color changes were observed

Voges-Proskauer Test

REAGENT: MR-VP broth, alpha naphthol, potassium hydroxide.

This is a test for identification of enterobacteriaceae, usually performed along side with methyl red test. It is used to detect acetoin in bacteria broth culture. [12] The broth was prepared according to the manufacturer's instruction and was poured into many test tubes, after this, each test tube was inoculated with the isolated bacteria and they were incubated at 35°c for 4 days. After the incubation, Alpha naphthol and potassium hydrogen were added and the color changes were observed in the test tubes, for positive acetoin is converted to diacetyl, naphthol is catalysed to bring out red.

Serial Dilution of The Antibiotics

A serial dilution is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. Serial dilutions are widely used in experimental sciences, including biochemistry, pharmacology, and microbiology [13]. A 5 fold Serial dilution was used in preparation of antibiotic disc dilution. Serial dilutions are used to accurately create highly diluted solutions as well as solutions for experiments resulting in concentration curves with a logarithmic scale.

Production Method

Preparation of Paper Disks

Using an ordinary office two hole puncher, paper disks with approximate diameter of 6.3mm were punched out one by one from sheets of Whatman paper, precaution were taken to avoid overlapping of the holes.

Preparation of antibiotic solutions

Antibitiotics used were powders and tablets obtained as pure antibiotics from commercial sources. These Powders were accurately weighed and dissolved in the appropriate diluents to yield the required concentration, using sterile glassware. Stock solutions were prepared using the formula (1000/P) X V X C=W, where

i. P= potency of the antibiotic base,
ii. V=volume in ml required,
iii. C=final concentration of solution and
iv. W=weight of the antimicrobial to be dissolved in V..W=weight of the antimicrobial to be dissolved in V. Standard strains of stock cultures were used to evaluate the antibiotic stock solution. [14] The antibiotic powder were dissolved in their appropriate solvents and further diluted in distilled water.

Impregnation of disk

Immersion method was employed; blank disks were soaked in known concentration of antibiotics and then allowed to dry for 2 hours.

Drying

Without covering the Petri dishes, the disks were allowed to dry in a clean incubator at 35°C for 2-3 hours.

After drying

50 to 100 disk were placed in small sterile air tight labeled containers with a desiccant at the bottom. A layer of sterile cotton or foam was placed over the desiccant to avoid contact with the disks. The disks were stored in a freezer at 14oc. [15]; [16] Unopened containers were removed from the freezer 1 or2 hours before use to equilibrate to room temperature before this

opened to minimize the amount of condensation that may occur when warm room air reaches the cold containers

Screening or Susceptibility Testing For B- Lactamase Production Using B-Latam Antibodies Such As Penicillin And Cephalosporin Disks

Step 1: Disk diffusion for antibodies susceptibility testing can screen for ESBL producing *K. pneumonia* by noting specific zone diameter which indicate high level of suspicion for ESBL production using

- Ampicillin, disks
- Ceftriaxone disks
- Ceftazidime disks
- Cefotaxime disks
- Ampiclox disks

Phenotypic Confirmatory Test For Esbl Production

Step 2: The second tests, tests for synergy between an oxyimino cephalosporin and clavulanate distinguishing isolates with ESBL from those that are resistant for other reasons. [17] The clinical and laboratory standard institute advocate the use of cefotaxime (30mc g) or ceftazidime (30 mcg) disks with and without clavulanate (10mc g) for the phenotypic confirmation of the presence of ESBL *in klebsiella pneumonia.*

A difference of ≥ 5mm between the zone diameters of either the cephalosporin and their respective cephalosporin/clavulnate disk is taken to be phenotypic confirmation of ESBL production

Phenotypic confirmation = Cephalosporin Vs Cephalosporin + Clavulnate.

Plasmid Curing

Step 3: This is the treatment of cells with a substance that interferes with plasmid replication. [18] The elimination of a plasmid from a cell culture by treatment with Acridine Orange or Ethidium or Sodium dodecyl sulfate at a concentration insufficient to inhibit bacteria chromosome replication but sufficient to inhibit plasmid replication.

Reagents required:

Acridine orange dye, nutrient broth, Macconkey agr

- Acridine orange
- Nutrient broth medium
- Macconkey agar

Plasmid curing procedure:

The nutrient broth was prepared according to the manufacturer's instruction and 5ml of the nutrient broth was poured into many test tubes then 50µl (0.10mg/ml) of Acridine orange dye was added into each of the test tube containing the nutrient broth, after this, ESBL producing *Klebsiella pneumonia* cultures were inoculated to nutrient broth having acridine orange and were incubated in an incubator for 24 hours [19].

Retest For Esbl Production Using Screening And Confirmation Test Method

SCREENING TEST**:** After the incubation, the nutrient broth containing the Acridine dye and the organisms were collected and inculated in a petridish for sensitivity or screening test using disk diffusion method, the plates are incubated for 24 hours at 37°c. Disk diffusion for antibiotic susceptibility testing can screen for ESBL producing *K. pneumonia* by noting specific zone diameter which indicate high level of suspicion for ESBL production using:

- Ampicillin, disks
- Ceftriaxone disks
- Ceftazidime disks
- Cefotaxime disks
- Ampiclox disks

CONFIRMATION TEST**:** This is the second tests, these tests for synergy between an oxyimino cephalosporin and clavulanate distinguishing isolates with ESBL from those that are resistant for other reasons [20]. The clinical and laboratory standard institute advocate the use of cefotaxime (30mc g) or ceftazidime (30 mcg) disks with and without clavulanate (10mc g) for the phenotypic confirmation of the presence of ESBL *in klebsiella pneumonia.*

A difference of ≥ 5mm between the zone diameters of either the cephalosporin and their respective cephalosporin/clavulnate disk is taken to be phenotypic confirmation of ESBL production

Phenotypic confirmation = Cephalosporin Vs Cephalosporin + Clavulnate.

Phenotypic confirmation = Penicillin Vs Penicillin + Clavulanate.

RESULTS

TABLE 1: MICROOBIOLOGICAL IDENTIFICATION TEST

MICROBIOLOGICAL TESTS	*K.PNEUMONIAE*
Colony Appearance	Large,circular,Smooth spreading
Colony Shape	Circular colony
Gram stain	-ve (Negative)
Motility	- ve (Negative)
Flagella	-ve (Negative)
Capsules	+ve (Positive)
Sugar test	+ve (Positive)
Carbohydrate	+ ve (Positive)
Catalase	+ve (Positive)
Oxidase	- ve (Negative)
Indole	-ve (Negative)
Lactose	+ve (Positive)
Voges proskauer	+ve (Positive)
Methyl red	- ve (Negative)
Gas production	+ve (Positive)

TABLE 2: Screening and Confirmation for *Klebsiella pneumonia,* result from Chicken Feeds

Antimicrobial Agents	Screening Test	Confirmation test (B lactam plus Augumentine)	Esbl Production ≥5mm Zone Diameter With Clavulanic Acid	Positivity/ Negativity
Cefotaxime 30ug	5mm	9mm	4mm	Negative
	6mm	11mm	5mm	Positive
	6mm	10mm	5mm	Negative
	6mm	12mm	8mm	Positive
Ceftriazone 30ug	5mm	13mm	7mm	Positive
	6mm	14mm	8mm	Positive
	4mm	8mm	4mm	Negative
	3mm	12mm	9mm	Positive
Cefotazidime 30ug	4mm	10mm	6mm	Positive
	6mm	10mm	4mm	Negative
	3mm	9mm	6mm	Positive
	5mm	11mm	6mm	Positive
Ampicillin 30ug	4mm	7mm	3mm	Negative
	4mm	8mm	5mm	Negative
	3mm	9mm	6mm	Positive
	5mm	9mm	4mm	Negative
Ampiclox 30ug	5mm	10mm	5mm	Positive
	6mm	11mm	5mm	Positive
	5mm	10mm	5mm	Negative
	4mm	9mm	5mm	Positive

POSITIVE FOR ESBL PRODUCTION: 12
NEGATIVE FOR ESBL PRODUCTION: 8

TABLE 3: Screening and Confirmation for *Klebsiella pneumonia,* result from chicken water

Antimicrobial Agents	Screening Test	Confirmation test (B lactam plus Augumentine)	Esbl Production ≥5mm Zone Diameter With Clavulanic Acid	Positivity/ Negativity
Cefotaxime 30ug	5mm	9mm	4mm	Negative
	6mm	11mm	5mm	Positive
	5mm	9mm	4mm	Negative
	6mm	12mm	8mm	Positive
Ceftriazone 30ug	5mm	13mm	7mm	Positive
	6mm	14mm	8mm	Positive
	4mm	8mm	4mm	Negative
	6mm	10mm	4mm	Negative
Cefotazidime 30ug	4mm	10mm	6mm	Positive
	6mm	9mm	5mm	Negative
	3mm	7mm	4mm	Negative
	5mm	11mm	6mm	Positive
Ampicillin 30ug	4mm	9mm	5mm	Positive
	6mm	10mm	4mm	Negative
	3mm	9mm	6mm	Positive
	5mm	8mm	3mm	Negative
Ampiclox 30ug	5mm	9mm	4mm	Negative
	6mm	11mm	5mm	Positive
	5mm	10mm	5mm	Positive
	4mm	8mm	4mm	Negative

POSITIVE FOR ESBL PRODUCTION: 10
NEGATIVE FOR ESBL PRODUCTION: 10

TABLE 4: Screening and Confirmation for *Klebsiella pneumonia*, result from chicken excretes

Antimicrobial Agents	Screening Test	Confirmation test (B lactam plus Augumentine)	Esbl Production ≥5mm Zone Diameter With Clavulanic Acid	Positivity/ Negativity
Cefotaxime 30ug	5mm	9mm	4mm	Negative
	6mm	11mm	5mm	Positive
	5mm	10mm	5mm	Positive
	6mm	12mm	8mm	Positive
Ceftriazone 30ug	5mm	13mm	7mm	Positive
	6mm	14mm	8mm	Positive
	4mm	8mm	4mm	Negative
	3mm	12mm	9mm	Positive
Cefotazidime 30ug	4mm	10mm	6mm	Positive
	3mm	11mm	5mm	Positive
	3mm	9mm	6mm	Positive
	5mm	11mm	6mm	Positive
Ampicillin 30ug	4mm	9mm	5mm	Positive
	4mm	9mm	5mm	Positive
	3mm	9mm	6mm	Positive
	4mm	9mm	5mm	Positive
Ampiclox 30ug	5mm	10mm	5mm	Positive
	6mm	11mm	5mm	Positive
	5mm	10mm	5mm	Positive
	4mm	8mm	4mm	Negative

POSITIVE FOR ESBL PRODUCTION: 17
NEGATIVE FOR ESBL PRODUCTION: 3

TABLE 5: Screening and Confirmation for *Klebsiella pneumonia*, result from chicken slaughter environment

Antimicrobial Agents	Screening Test	Confirmation test (B lactam plus Augumentine)	Esbl Production ≥5mm Zone Diameter With Clavulanic Acid	Positivity/ Negativity
Cefotaxime 30ug	5mm	9mm	4mm	Negative
	6mm	11mm	5mm	Positive
	5mm	10mm	5mm	Positive
	6mm	12mm	8mm	Positive
Ceftriazone 30ug	5mm	13mm	7mm	Positive
	6mm	14mm	8mm	Positive
	4mm	8mm	4mm	Negative
	3mm	12mm	9mm	Positive
Cefotazidime 30ug	4mm	10mm	6mm	Positive
	3mm	11mm	5mm	Positive
	3mm	9mm	6mm	Positive
	5mm	11mm	6mm	Positive
Ampicillin 30ug	4mm	8mm	4mm	Negative
	4mm	9mm	5mm	Positive
	3mm	9mm	6mm	Positive
	4mm	9mm	5mm	Positive
Ampiclox 30ug	5mm	10mm	5mm	Positive
	6mm	11mm	5mm	Positive
	5mm	10mm	5mm	Positive
	4mm	9mm	5mm	Positive

POSITIVE FOR ESBL PRODUCTION: 17
NEGATIVE FOR ESBL PRODUCTION: 3

TABLE 6: Screening and Confirmation for *Klebsiella pneumonia*, result from chicken environment

Antimicrobial Agents	Screening Test	Confirmation test (B lactam plus Augumentine)	Esbl Production ≥5mm Zone Diameter With Clavulanic Acid	Positivity/ Negativity
Cefotaxime 30ug	5mm	9mm	4mm	Negative
	6mm	11mm	5mm	Positive
	5mm	10mm	5mm	Positive
	6mm	12mm	8mm	Positive
Ceftriazone 30ug	5mm	13mm	7mm	Positive
	6mm	14mm	8mm	Positive
	4mm	8mm	4mm	Negative
	3mm	6mm	3mm	Negative
Cefotazidime 30ug	4mm	10mm	6mm	Positive
	6mm	10mm	4mm	Negative
	3mm	9mm	6mm	Positive
	5mm	9mm	4mm	Negative
Ampicillin 30ug	4mm	9mm	5mm	Positive
	6mm	9mm	3mm	Negative
	3mm	9mm	6mm	Positive
	4mm	9mm	5mm	Positive
Ampiclox 30ug	7mm	10mm	3mm	Negative
	6mm	10mm	4mm	Negative
	5mm	10mm	5mm	Positive
	4mm	9mm	5mm	Positive

POSITIVE FOR ESBL PRODUCTION: 12
NEGATIVE FOR ESBL PRODUCTION: 8

TABLE 7: SUMMARY 1: FROM CHICKEN FEED

ESBL CONFIRMATION	NUMBER TESTED	ESBL PRODUCING *KLEBSIELLA PNEUMONIA*
Cefotaxime and Augumentine	4	2
Ceftriazone and Augumentine	4	3
Ceftazidime and Augumentine	4	3
Ampicillin and Augumentine	4	1
Ampiclox and Augumentine	4	3
Total	**20**	**12**

TABLE 8: SUMMARY 2: FROM CHICKEN WATER

ESBL CONFIRMATION	NUMBER TESTED	ESBL PRODUCING *KLEBSIELLA PNEUMONIA*
Cefotaxime and Augumentine	4	2
Ceftriazone and Augumentine	4	2
Ceftazidime and Augumentine	4	2
Ampicillin and Augumentine	4	2
Ampiclox and Augumentine	4	2
Total	**20**	**10**

TABLE 9: SUMMARY 3: FROM CHICKEN EXCRETES

ESBL CONFIRMATION	NUMBER TESTED	ESBL PRODUCING *KLEBSIELLA PNEUMONIA*
Cefotaxime and Augumentine	4	3
Ceftriazone and Augumentine	4	3
Ceftazidime and Augumentine	4	4
Ampicillin and Augumentine	4	4
Ampiclox and Augumentine	4	3
Total	**20**	**17**

TABLE 10: SUMMARY 4: CHICKEN SLAUGHTERS

ESBL CONFIRMATION	NUMBER TESTED	ESBL PRODUCING *KLEBSIELLA PNEUMONIA*
Cefotaxime and Augumentine	4	3
Ceftriazone and Augumentine	4	3
Ceftazidime and Augumentine	4	4
Ampicillin and Augumentine	4	4
Ampiclox and Augumentine	4	3
Total	**20**	**17**

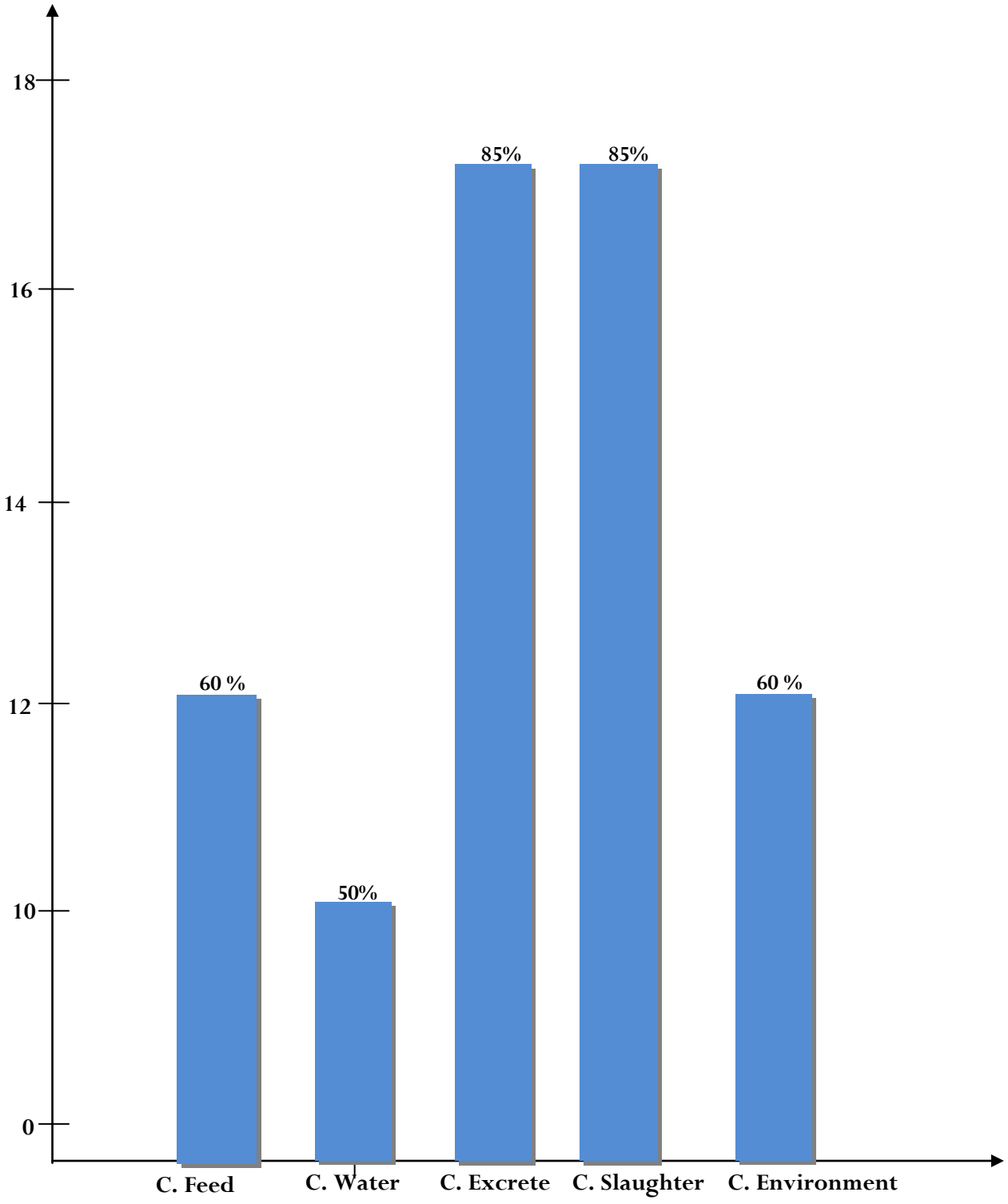
TABLE 11: SUMMARY 5: FROM CHICKEN / POULTRY ENVIRONMENT

ESBL Confirmation	Number Tested	ESBL Producing *Klebsiella Pneumonia*
Cefotaxime and Augumentine	4	2
Ceftriazone and Augumentine	4	3
Ceftazidime and Augumentine	4	3
Ampicillin and Augumentine	4	1
Ampiclox and Augumentine	4	3
Total	**20**	**12**

TABLE 12: PERCENTAGE ESBL POSITIVE *KLEBSIELLA PNEUMONIAE* FROM DIFFERENT SOURCES IN ENUGU

Sources	No positive	No tested	Evaluation	Percentage
K.pneumonia from Chicken Feed	12	20	12/20 (100)	60%
K.pneumonia from Chicken water	10	20	10/20 (100)	50%
K.pneumonia from Chicken execretes	17	20	17/20 (100)	85%
K.pneumonia from chicken Slaugther	17	20	17/20 (100)	85%
K.pneumonia from poultry environment	12	20	12/20 (100)	60%
Total	**68**	**100**	**68/100 (100)**	**68%**

FIGURE 1: BAR CHART REPRESENTATION OF THE OCCURENCES



SOURCES OF *Klebsiella pneumoniae*

TABLE 13: MEASUREMENT OF VIABILITY OF THE DISTRIBUTION of ESBL PRODUCTION

Sources	ESBL +ve Klebsiella pneumonia	X	F	FX (x)
Chicken feed	12	10	1	10
Chicken water	10	12	2	24
Chicken Excretes	17	17	2	34
Chicken Slaughter	17			
Chicken environment	12			
TOTAL	**68**		**5**	

Mean = $\Sigma \frac{X}{N}$ **or** $\frac{FX}{F}$

ESBL positive Klebsiella pneumoniuae

= $\frac{68}{5}$ **= 13.5**

TABLE 14: MEASUREMENT OF STANDARD DEVIATION OF ESBL DISTRIBUTION

Xb *K. pneumonia*	x $X - \overline{X}$	$\overline{Xb}$
12	-1.5	13.5
10	-3.5	
17	2.5	
17	2.5	
12	-1.5	

$$Xb \quad \sqrt{\frac{\Sigma(X-X)^2}{N}}$$

$$Xb\ (K.pneumoniae) = \sqrt{\frac{2.25}{5}} = \sqrt{0.45} = 0.6708$$

TABLE 15: *Klebsiella pneumonia RETEST* AFTER PLASMID CURING

Sources	No of ESBL Positive	No cured (ESBL Negative) Plasmid-mediated ESBL production	No cured but still show resistance. (Genetic-Mediated ESBL production)
Chicken feed	12	12	0
Chicken water	10	8	2
Chicken excretes	17	15	3
Chicken Slaughter	17	15	2
Chicken environment	12	9	3
	TOTAL 68	**TOTAL 59**	**TOTAL 9**

TABLE 16: TOTAL PERCENTAGE OF PLASMID MEDIATED AND GENETIC MEDIATED ESBL PRODUCTION

ESBL producing organisms	Total No For Plasmid Curing	Plasmid-mediated ESBL production (ESBL Negative)	Genetic mediated ESBL production (ESBL Positive)
Klebsiella pneumonia	**68**	**59 (86.7%)**	**9 (13.2%)**

CONCLUSION

Clinically, ESBLs limit the efficacy of β-lactams. including extended-spectrum cephalosporins, and are associated with high morbidity and mortality with high number of hospitalize cases originating from poultry and chicken environment [21].

Plasmids are the major cause of the ESBL resistance to cephalosporin and penicillin derivatives respectively, this is because 86.7% of *Klebsiella pneumonia* tested were cured of plasmids using acridine orange dye and do not show resistance to cephalosporin and penicillin derivatives after retest showing that the presence of the enzyme ESBLs were produced by the plasmids while 13.2% showed resistance to penicillin and cephalosporin due to heritable genetic traits inherited from their parents cells e.g. lack of penicillin binding protein (PBP) and thus are genetic or chromosomal encoded ESBL production [22].

Plasmids are highly transmissible from one bacterium to another and also from one specie to another and they are replicons capable of replicating autonomously within a suitable host cell.

Plasmids destroys the action of cephalosporin and penicillin derivatives by hydrolyzing the B-Lactam rings of these antimicrobials rendering their actions ineffective [23].

Moreover, the indiscriminate use of carbapenems antibiotics may select resistance to these key drugs (penicillin and cephalosporin), thus sowing seeds for significant therapeutic problems to arise in the future.

RECOMMENDATION

There are limited numbers of oral and intravenous antibiotics that are effective against these bacteria. The key is quick diagnosis so that any ineffective antibiotic treatment is stopped immediately. It is very important that the right antibiotics are prescribed at the correct dose for the appropriate length of time and only when required. Drug charts are monitored regularly and any unnecessary antibiotics are stopped

It is recommended that patients infected with ESBLs are isolated from other patients in hospital to try and prevent the cross infection of other patients where possible. There is no doubt that the ESBLs are becoming increasingly complex and diverse and their detection is becoming increasingly challenging for clinical microbiology laboratories. Thus there is need for efficient infection-control practices for containment of outbreaks starting from chicken environment, chicken meat to vegetable production using organic manure from the poultry droppings

There should be intervention strategies, e.g., antibiotic rotation, to reduce further selection and spread of these increasingly resistant pathogens. Effective treatment against ESBLs is Clavulanic acid (β-lactamase inhibitor) which can be used in

combination with the β- lactam drugs for the treatment of urinary tract infections and blood stream poising. ESBLs are not effective against Augumentine, cephamycins, such as cefotetan carbapenems, such as imipenem or meropenem.

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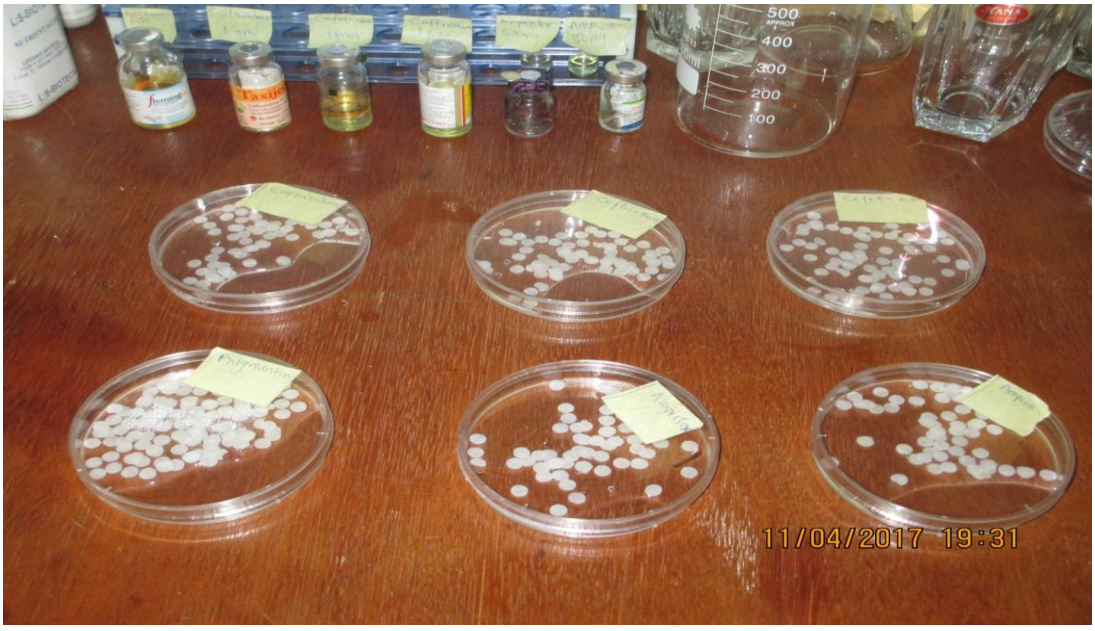
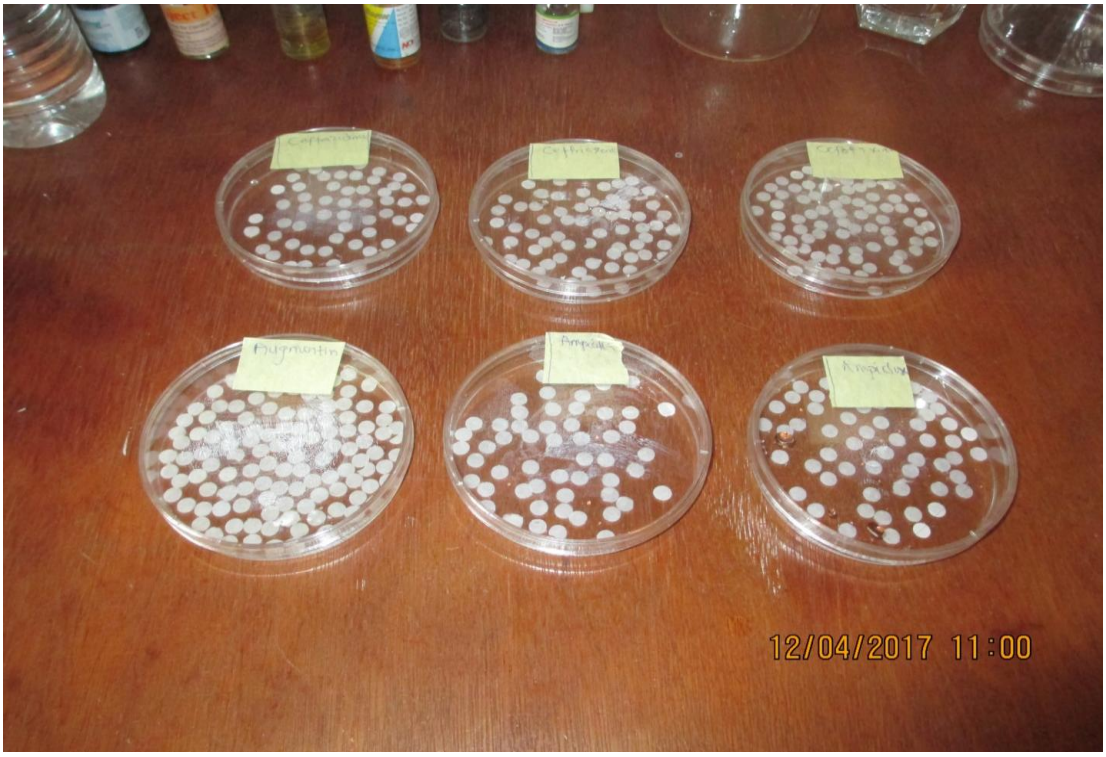
PLATES

PREPARATION OF ANTIBIOTICS DISKS

(Ceftriaxone, Cefotaxime, Ceftazidime, Ampicillin, Ampiclox)





STERILIZATION OF ALL MATERIALS