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Occurrence of Carbapenem – Resistant Escherichia Coli Carrying Bla NDM and Bla Kpc Genes from Commericlal Poultry Farm Environments in Aba Abia State.

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Abstract- ***Carbapenem antibiotics are the referred to as the last resort treatment against bacteria. The aim of this research was to determine the occurrence of carbapenem resistant E.coli carrying bla$_{NDM}$ and bla$_{KPC}$ from commercial poultry farms environment in Aba. Three commercial poultry farms which had not less of 5000 birds and not less than 3 years were randomly selected from major areas in Aba (Aba north, Aba south and Osisioma) for the study. 50 samples each from the soil, drinking water and droppings from the three different farms were taken using new sterile containers and transported to the laboratory for further analyses. A tenfold serial dilution of the three samples were done and plated out on freshly prepared sterile Eosin methylene blue (EMB) agar, MacConkey agar, at 44^{o}C and 37^{o}C for 24hours. Purification of isolates was done by subculturing colonies with the green metallic sheen on to fresh EMB agar plates. These colonies were then tested against Imipenem and meropenem and the resistance recorded. The resistance isolates we screened for Cabarpenemase (KPC and NDM) using molecular detection techniques. Result showed that, Green metallic sheen had an occurrence of 100% on the soil, droppings 96.7% and drinking water 84%. Highest resistant E.coli were isolated from the soil samples and the occurrence of the resistance genes showed that in farm A and C, bla$_{NDM}$ was the higher than bla$_{KPC}$ with a difference of $p \leq 0.0021$ and $p \leq 0.0565$ among the samples, while in farm B bla$_{KPC}$ was higher with $P \leq 0.0051$.This study has shown a high level of resistance genes(bla$_{KPC}$ and bla$_{NDM}$) from these commercial poultry farms and hence the need for public health interventions.***

I. INTRODUCTION

Antibiotic resistance is a global health concern, and the use of antibiotics in food animals is a leading contributing factor to this problem. Due to the lucrative market for chicken meat, and it is a popular alternative source of animal protein, many individuals venture into it both on a commercial scale and on a small scale. Carbapenem resistance in Enterobacteriaceae is a serious emerging antimicrobial resistance (AMR) issue that has been escalating and posing challenges in treating infections caused by the resistant pathogen(Akilu et al.,2021). Enterobacteriaceae are inhabitants of the intestinal flora and are among the most common human pathogens that cause cystitis and pyelonephritis with fever, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections (Nordmann et al.,2011). The bacteria are transmitted easily between human and animals, especially via fomites, food, and water. During the transmission, genetic materials are transferred through horizontal gene transfer, mediated mostly by plasmids and transposons. However, the inappropriate use of antibiotics to treat infectious diseases and promote growth in poultry flocks is a common practice in developing countries such as Nigeria. As a result, the misuse of antibiotics creates selection pressure and leads to the emergence of resistant poultry pathogens. These pathogens not only cause significant production and economic losses but are also associated with public health diseases. Carbapenem is a broad-spectrum β-lactam antibiotic that is regarded as the last-line antibiotic, especially to be used in critically ill patients who have developed antimicrobial-resistant bacterial infections. Unfortunately, Enterobacteriaceae of which E. coli is one of them, have developed resistance against this last resort drug and made it ever challenging to treat infections caused by these pathogens. Carbapenems are commonly used to combat multiple resistant bacteria which cannot be treated by other therapeutic

options. However, there is concern that these carbapenemases will penetrate the food chain due to the recent discovery of this resistance in agricultural animals and poultry farms (Aya et al.,2023). Therefore, to maintain their effectiveness, the development and spread of resistance mechanisms against carbapenems have to be prevented. One benefit of this class of antibiotics is that carbapenems are comparatively resistant to hydrolysis by most -lactamases. They are however inactivated by carbapenemases, which also confer resistance to β-lactams (Garcia –Graella et al.,2020). The genes coding for carbapenemases are frequently located in mobile genetic elements, facilitating their dissemination horizontally among different bacteria (Monteiro et al., 2012; Aya et al., 2023).

The aim of this research was to determine the presence of carbapenem resistant genes from E.coli isolated from the soil, drinking water and droppings of the birds in the poultry farms in Aba Abia State.

II. MATERIALS AND METHODS

Media used and preparation: The media for the microbial analyses were Eosin Methylene Blue agar(EMB) (Rapid Labs, UK) for identifying E. coli, and Muller hinton agar (Rapid Labs, UK) for sensitivity testing. All these were prepared according to the manufacturer's instructions and autoclaved at 121ºC for 15mins at 15psi. They were then be allowed to cool to 45^0C and then dispensed into sterile plastic disposable petri dishes.

Sample collection:
Sample collected were soil, droppings and drinking water from 3 commercial poultry farms in Aba, Abia State. A total of 50 samples each were collected from the different farms at different points, using a clean new polyethene bags and disposable bottles which were properly labeled. The soil sample was collected using soil auger, the droppings were collected from fresh litter using a new and sterilized spatula, while water was taken from their drinking cans using new sterile disposable bottles.

Bacteria identification
The samples were prepared using the serial dilution method as described in Cheesbrough (2006). From each site per sample, a gram (1g) of the soil sample was weighed into a test tube and 9mls of normal saline was added. Ten (10) test tubes standing on a rack was also filled with 9mls of normal saline. Using a sterile pipette, 1ml was taken from the first tube containing the soil sample into the next tube then from there also 1ml was taken to the next tube until the 9^{th} tube, the seventh tube was used for the inoculation. This same procedure was repeated for the dropping and drinking water samples, respectively. The plates were then incubated at 44ºC for 24 hours. The plates were then inspected for the green metallic sheen and then sub-cultured further on freshly prepared EMB agar plates for pure colonies. The further production of the green metallic sheen was a positive result for E.coli (Cheesbrough, 2009). Results were further interpreted using ABIS online microbiology software for bacterial identification (Mohammed et al., 2015).

Phenotypic characterization of CRE isolates
The pure cultures of bacterial isolates were screened for carbapenemases using the macConkey meropenem supplemented medium methods described by (Amjad et al.,(2011) . Standard discs of Imipenem(10µg) and meropenem (10µg) were used following the CLSI. Antibiotic discs were placed on the surface of Inoculated Muller hinton agar (MHA) plates using sterile forceps. The discs were 30mm apart and incubated for 24 hrs at 37^0C. After that, zones of inhibition were read using a metre rule and recorded in (mm). Isolates that showed a zone of inhibition ≤21mm in diameter for meropenem and / or Imipenem were considered as suspected carbapenemase producers, there were then stored in slants for further confirmation analysis.

DNA Extraction and amplification of carbapenemase producing E.coli: DNA extraction was done using the thermal lysis method as described by (Nordmann, et al.,2011) with slight modification. Single colony of pure isolates was inoculated in to sterile 2 ml nutrient broth and incubated at 37ºC for 24 hr after which1 ml was taken and centrifuged at 12,000 rpm for 15 min. The pellets were collected and resuspended in to 200

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µl TBE buffer, vortexed properly and boiled in a water bath for 10 min followed by another centrifugation at 12,000 rpm for 15 min. The supernatants were transferred into sterile Eppendorf tubes to determine their concentration and DNA purity using a (UV-Vis Thermo Scientific TM Nanodrop Life Spectrophotometer, (Model S-22, Boeco, Germany).

Previously described methods for amplification and detection of carbapenem encoding genes namely blaKPC, blaNDM, in the Enterobacteriaceae was employed in this study (2, 15, 22). The primers used were E. coli specific 16srRNA gene fragment of Ec16 primer pairs (F 5' GACCTCGGTTAGTTCACAGA-3' and R 5'-CACACGCTGACGCTGACCA-3') (Islam et al., 2016) while PCR was done using Master cycler gradient thermocycler with a final volume of 25 µl master mix consisting of12.5 µl 2X universal PCR master mix, 2 µl of primers (10 µM) each of forward and reverse primers, 5.5 µl nuclease-free water and 5 µl of DNA template. The PCR protocol include: initial denaturation at 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at appropriate temperature for each carbapenemase genes as depicted in table 1 for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 10 min. Amplicons were visualized on 1.5% agarose.

III. WRITE DOWN YOUR STUDIES AND FINDINGS

RESULTS

TABLE 1: Occurrence of the E. coli from the different sample sites

SITES	SOIL	DROPPINGS	WATER
FARM A	50(33.3)	50 (34.5%)	46 (36.5%)
FARM B	50 (33.3)	50 (34.5%)	40 (31.74%)
FARM C	50 (33.3)	45(31.0%)	40 (31.74 %)
TOTAL	150 (100%)	145 (100%)	126 (100%)

Key: numbers in ()* are the percentages of the number outside the bracket.

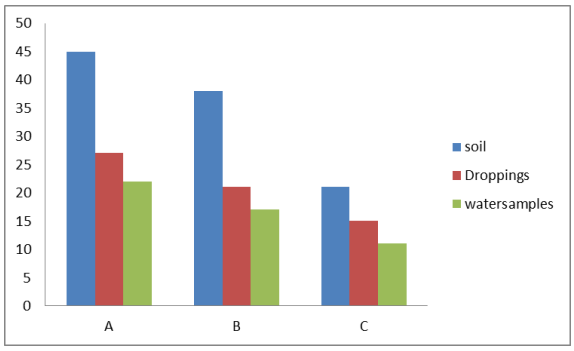

Fig 1 showing the occurrence of the resistance genes per farm site

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OCCURRENCE OF CARPEBENEMS GENES FROM THE RESISTANT E.COLI FROM FARM A

SAMPLES	NO. EXAMINED	KPC (%)	NO. EXAMINED	NDM (%)
SOIL	25(100)	23 (92.0)	25(100)	24 (96.0)
WATER	21(100)	18(85.7)	21(100)	21 (100)
DROPPINGS	21(100)	20 (95.2)	21(100)	21 (95.2)
TOTAL	67(100)	61 (91.1)	67(100)	66 (98.5)
Lower 95% CL of mean		14.08		17.70
Upper 95% CL of mean		26.58		26.30
One sample t test P value		0.0051		0.0021

Key: numbers in ()* are the percentages of the number outside the bracket.

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OCCURRENCE OF CARBAPENEMS GENES FROM THE RESISTANT E.COLI FROM FARM B

SAMPLES	NO. EXAMINED	KPC (%)	NO. EXAMINED	NDM (%)
SOIL	17 (100)	14 (82.4)	17 (100)	14 (82.4)
WATER	16(100)	11(68.8)	16(100)	9 (56.3)
DROPPINGS	12(100)	12(100)	12(100)	9 (82.2)
TOTAL	45(100)	37 (82.2)	45(100)	32 (71.1)
Lower 95% CL of mean		8.539		3.496
Upper 95% CL of mean		16.13		17.84
One sample t test P value		0.0051		0.0236

Key: numbers in ()* are the percentages of the number outside the bracket.NO-Number

OCCURRENCE OF CARPABENEM GENES FROM THE RESISTANT E.COLI FROM FARM C

SAMPLES	NO. EXAMINED	KPC (%)	NO. EXAMINED	NDM (%)
SOIL	23 (100)	21 (91.3)	23 (100)	23 (100)
WATER	17(100)	15 (88.2)	17(100)	17 (100)
DROPPINGS	9(100)	7 (77.8)	9(100)	9 (100)
TOTAL	49 (100)	43 (87.8)	49 (100)	49 (100)
Lower 95% CL of mean	-	3.115	-	1.115
Upper 95% CL of mean		31.78		33.78
One sample t test P value		0.0716		0.0565

Key: numbers in ()* are the percentages of the number outside the bracket. NO-Number

IV. DISCUSSION

The occurrence of E.coli from the different samples sites showed that the materials sampled from sample Site A , B, and C were Soil, droppings and water, and result showed that the all the soil samples from A(33.3%),B(33.3%),C had E. coli , making it a total of 150(100%) . Escherichia coli is a ubiquitous organism that can often lead to fatal infections in immunocompromised humans (Karmali et al.,2010). The propensity for E. coli to serve as a reservoir for antimicrobial determinants genes has been previously demonstrated (Bailey et al., 2010). in a study conducted by Al et al. (2019) reported that all 400 swabs obtained from chickens across 50 broiler farms were found to be positive for E. coli and had the presence of resistance genes in them. Another work conducted by Ayana et al.,(2025) showed high microbial load of E. coli. In the study conducted by Abdelraouf et al., (2018) and another group of researchers, Chika et al. (2017) also reported that Escherichia spp. were the most frequently isolated bacterial specie from the Poultry samples.

It was also observed that there was higher resistance from the soil farm A followed by B and least was C (Fig 1). This could be because of the different farm setting environment; farm was cited on a farm soil and also had other farm animals and an orchard within the same vicinity hence they use of antibiotics was higher, again the workers didn't have schedules for cleaning and usually dumped their wastes in a heap with the disposed weekly and this has been a practice for years, this was also same for farm B, however farm C was cemented and had paid cleaners

as workers too, hence the hygienic practices carried out in farm C was daily and hence higher than that of farm A and B .The E.coli isolated from fresh poultry droppings were also evaluated for the presence of resistance and it was observed that there were presence of resistance E.coli from the dropping, however farm A(54%) had a higher count than farm B(42%) and C(33.3%). The drinking water of these birds were as evaluated, since the antibiotics are mostly introduced into their drinking water, the sanitary levels of these containers are considered very important as these containers are made of plastic materials that could encourage the build-up of biofilms, the organisms are always introduced while the birds are trying to drink water with their beaks which have been used on the soils during feeding, these could also be normal flora or opportunistic pathogens which could become pathogenic and pick up the resistance genes from constant exposure to antibiotics in those water containers. The result showed that the isolates from the drinking water, farm A(45%) had the highest occurrence, followed by farm B (42%) and least was C (33.3%). Using the pearsons correlation coefficient analysis, it was observed that the correlation between Resistant isolates from soil and droppings in farm A was 0.972, and between the soil and water was 0.983. In farm B similar relationship in a correlation coefficient between the soil and droppings but between the droppings and water samples in farm B r^2=0.999. The same trend was observed between water samples in farm C and the soil (r^2=0.983) while between the water samples and droppings r^2=0.999. This simply means that similar antibiotic interactions were happening in these farms.

The distribution of KPC (Klebsiella pneumoniae carbapenemase) genes detected in Escherichia coli isolated from droppings, soil, and water samples collected from three poultry farms (A, B, and C) was also investigated. In farm C, 25 Resistant E. coli samples examined from the soil showed that 23 (92%) of the resistant E. coli isolates had KPC while 24 (96.0%) had NDM. E. coli isolated from the water samples showed that Out of 21 isolates, only 18 (85.7%) of the isolates had KPC genes, however they all had NDM genes. Out of 21 isolates examined, resistant isolates from the droppings showed that only 20 (95.2%) carried the KPC genes while all the 21(100%) carried the NDM genes. Again, it was also observed that the most resistant Carpabenem genes isolated from the farm A was NDM genes. When this was statistically compared, result showed that there were significant differences between the soil, water and droppings with a P-value of 0.0051 for KPC and 0.0021 for NDM. In farm B, 17 E.coli samples examined from the soil showed that 14 (82.4%) of the resistant E. coli isolates had SHV while 14 (82.4.0%) had NDM. E. coli isolated from the water samples showed that Out of 16 isolates, only 11 (68.8%) of the isolates had SHV genes, however they all had NDM genes. Out of 12 isolates examined, resistant isolates from the droppings showed that all 12 (100%) isolates carried the KPC genes while 9 (82.2%) carried the NDM genes. It was also observed that KPC genes were mostly present. When this was statistically compared, result showed that there were significant differences between the soil, water and droppings with a P-value of 0.0051 for KPC and 0.00236 for NDM genes.

The KPC gene encodes an enzyme capable of hydrolysing carbapenem antibiotics, which are considered last-resort drugs for treating multidrug-resistant bacterial infections. The detection of this gene in a poultry farm environment is therefore a serious public health concern, indicating the possible dissemination of carbapenem-resistant organisms beyond clinical settings into agricultural ecosystems. it was evident that soil samples exhibit the highest levels of KPC gene detection across all three farms, with Farm A showing the most prominent peak, followed by Farm C and Farm B. This trend suggests that the soil acts as a major environmental reservoir for KPC-producing E. coli within the poultry production environment. The elevated detection in soil can be attributed to the repeated deposition of poultry droppings and litter containing resistant bacteria, as well as the persistence of resistance genes in organic matter-rich soil. Soil conditions favour the survival of resistant bacteria and promote horizontal gene transfer through mobile genetic elements such as plasmids and transposons, facilitating the long-term maintenance and spread of carbapenemase genes among environmental microbes

CONCLUSION

The research highlights the urgent need for improved biosecurity, the management on the use of antibiotic drugs to the barest minimum and waste management measures in poultry farms to limit environmental contamination with resistance genes. Strengthening antibiotic stewardship, properly treating poultry manure before its disposal, and ensuring clean water supplies are essential to break the cycle of resistance transmission. Public health policies can be put in place to help ensure compliance as this remains a public health threat.s

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