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Multiple Antibiotic Resistance, Antibiogram and Phenotypic Detection of Metallo-Beta-Lactamase (MBL) from *Escherichia coli* of Poultry Origin

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Abstract

Background: Bacteria produce antibiotic-degrading enzymes such as carbapenemases. Carbapenemases are a consortium of carbapenem-hydrolyzing enzymes such as metallo- β -lactamase (MBL) that gives Gram-negative bacteria the exceptional ability to degrade and render the carbapenems inefficacious.

Aim: This study evaluated the antibiogram, multiple antibiotic resistance and occurrence of MBL-producing *E. coli* from cloacal swabs of poultry birds in a local poultry farm in Abakaliki, Nigeria.

Materials and methods: A total of 40 cloacal swab samples from the cloacal region of poultry birds were bacteriologically analyzed for the isolation of *E. coli*. *E. coli* isolates were identified using standard microbiology techniques and the antibiogram of the isolates was determined using the disk diffusion technique. The multidrug resistance nature of the *E. coli* isolates was determined using multiple antibiotics resistance index (MARI) protocol while MBL production was phenotypically confirmed using the inhibition based assay.

Results: A total of 29 (72.5%) *E. coli* isolates was recovered from the 40 cloacal swab samples. The *E. coli* isolates were highly resistant to imipenem (31%), meropenem (58.6%), ertapenem (75.9%), cefotaxime (55.2%), ciprofloxacin (89.7%), ceftazidime (93.1%) and ceftazidime (69.0%). MBL production was phenotypically detected in 3 (10.3%) *E. coli* isolates out of the 29 isolates of *E. coli* recovered in this study. The resistant *E. coli* isolates were multiply resistant to antibiotics in the class of fluoroquinolones, cephalosporins, aminoglycosides and carbapenems; and they had a multiple antibiotic resistance of 0.4 on average.

Conclusion: This presumptive study has shown that *E. coli* isolates of poultry origin produce MBL. The emergence and spread of drug resistant bacteria in the community can be contained if we use antibiotics rationally and find alternative measures for promoting animal growth without the use of antimicrobial agents.

Keywords: Metallo- β -lactamase; *Escherichia coli*; Multidrug resistance; Pathogens

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Introduction

In Nigeria, the detection rate of multidrug resistant Gram-negative bacteria in both the community and hospital environment is still at a pitiable state. More worrisome is the fact that the detection of multidrug resistant bacteria such

as metallo- β -lactamase (MBL)-producing bacteria has not yet been institutionalized in our healthcare system. And this could contribute to poor prognosis of the patient as well as lead to inappropriate use or application of antimicrobial therapy. The emergence of new beta-lactamases such as metallo- β -lactamases (MBLs), extended spectrum β -lactamases (ESBLs) and AmpC

enzymes to mention but a few is an important mechanism by which bacteria develop resistance to some available antibiotics [1-3]. Microbial infections caused by antibiotic resistant bacteria lead to increase in the length of hospitalization, severity of illness and the overall cost of treatment. Gram-negative bacteria that produce MBL are widely reported as an emerging threat to the efficacy of the carbapenems including imipenem and meropenem. The increasing frequency of these multidrug resistant organisms from several countries have become of major clinical significance owing to their antibiotic degrading potentials [2,4-11]. MBLs are beta-lactamases that hydrolyze and confer on Gram negative bacteria the singular ability to be resistant to the carbapenems – which are stable against the antibiotic degrading ability of ESBL-producing bacteria [1,2,4,11]. They are encoded by genes that have been procured by bacteria either by mutation or horizontally from other organisms, and they can be chromosomally or plasmid-mediated. MBLs were first formally described from serine beta-lactamases in the 1980s; and since then, MBL-producing Gram negative bacteria is now an increasing problem in many parts of the world [1,2,4-7,12]. Pathogens that produces MBLs can hydrolyze all types of β -lactams including penicillins, cephalosporins, and most especially the carbapenems. However, the monobactams such as aztreonam and serine β -lactamase inhibitors still have antimicrobial activity against the MBLs [1,3,13,14]. The MBLs belong to the class of carbapenemases that depends on zinc ions as cofactors for enzyme activity. Their presence in Gram-negative bacteria can be phenotypically detected in the laboratory using ethylene diamine tetraacetic acid (EDTA) – to which MBLs are sensitive to [1,9,10,11]. The genes that encode MBL production in Gram-negative bacteria are either chromosomally encoded or plasmid encoded as earlier stated; and they can easily be transmitted via mobile genetic elements such as plasmids and transposons in a bacterial population [1,13,14]. The use of antibiotics in livestock production and poultry practices is a good breeding ground for the emergence and spread of antibiotic resistant bacteria in the community [15-17]. According to Wegener [18], the use of antibiotics in animal feeds place a crucial role in the development and spread of antibiotic resistant bacteria in the community. This avenue enables the selection of resistance strains of bacteria including *E. coli* exposed to the antibiotics in the intestinal flora of the birds. In this study, the antibiogram, multiple antibiotic resistance and occurrence of MBL-producing *E. coli* from cloacal swabs of poultry birds was determined.

Materials and Methods

Sample collection and processing

A total of 40 consecutive and non-duplicated cloacal swab samples were collected from the cloacal region of poultry birds from a local poultry farm in Abakaliki metropolis, Ebonyi State, Nigeria using sterile swab sticks. The samples were labeled and transported to the Microbiology Laboratory unit of Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis. Each sample was inserted into sterile test tubes of 5 ml freshly prepared nutrient broth (Oxoid, UK) and incubated at 30°C overnight. Following overnight incubation at 30°C, the test tubes

containing the samples were examined for visible bacterial growth as evidenced by the presence of turbidity.

Bacterial isolation and identification

Test tubes positive for bacterial growth as evidenced by turbidity were aseptically sub-cultured onto freshly prepared MacConkey agar (MAC) and eosin methylene blue (EMB) agar plates for the isolation of *E. coli*. The inoculated agar plates were incubated at 30°C overnight. Suspected colonies of *E. coli* were sub-cultured onto freshly prepared MAC and EMB agar plates for the isolation of pure colonies of *E. coli*. *E. coli* was identified using standard microbiology identification technique including: colonial/morphological appearance on culture media, Gram staining, indole test and methyl red test [19].

Maintenance of bacteria stock culture

Each of the isolated *E. coli* isolates were maintained as stock cultures for further studies on nutrient agar (Oxoid, UK) slants in Bijou bottles. This was done by streaking the isolates on the agar slants, and incubating at 30°C for 18-24 hrs. After incubation, the inoculated slants were stored in the refrigerator at ambient temperature and these stock cultures served as source of *E. coli* isolates for further bacteriological studies.

Antimicrobial susceptibility testing (AST)

AST was performed according to a previously described methodology using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar plates as per the Clinical and Laboratory Standard Institute (CLSI) guidelines [4,20]. Imipenem (10 μ g), meropenem (10 μ g), ertapenem (10 μ g), amikacin (30 μ g), ofloxacin (5 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), cefotaxime (30 μ g), ciprofloxacin (10 μ g) and ceftiofloxacin (30 μ g) (Oxoid, UK) were used for antimicrobial susceptibility testing. Susceptibility test plates were incubated at 30°C for 18-24 hrs; and the ensuing inhibition zone diameters (IZDs) were measured to the nearest millimeter using a meter rule. All the IZDs were recorded and interpreted as percentage susceptible, intermediate and resistant using the CLSI standard antibiotic breakpoints [20].

Screening test for MBL

MBL enzyme-producing *E. coli* isolates was suspected when the test organism(s) was resistant to any of the carbapenems including imipenem and meropenem as was described previously [4,5,9]. Bacterial isolates showing inhibition zone diameter (IZD) of \leq 23 mm to any of the carbapenems were suspected to produce MBL enzyme and these isolates were subjected to phenotypic confirmation test.

Phenotypic detection of MBL-positive *E. coli*

The test bacteria isolates (adjusted to 0.5 MacFarland turbidity standard) were aseptically swabbed on Muller-Hinton (MH) agar plates, and standard antibiotics disks of imipenem (10 μ g) and meropenem (10 μ g) disks impregnated with EDTA (1 μ g) and imipenem and meropenem disks without EDTA was aseptically placed on the MH agar plates. A distance of 25 mm

was maintained between the antibiotic disks. All test plates were incubated at 30°C for 18-24 hours and zone of inhibition were recorded and interpreted as per the CLSI criteria [20]. A difference of ≥ 7 mm between the zones of inhibition of any of the carbapenems with EDTA and disks without EDTA infers MBL production phenotypically [4,9].

Determination of multiple antibiotic resistance index (MARI)

Multiple antibiotic resistance index (MARI) was determined by the method of [21] with little modification. MARI was evaluated to determine the multidrug resistant nature/profile of resistant isolates in our study. To determine resistant profile of these resistant isolates, MARI was evaluated using the formula: $MARI = a/b$, where “a” is the number of antibiotics to which the resistant bacteria was resistant to and “b” is the total number of antibiotics to which the resistant bacteria has been evaluated for.

Results

This study detected the production of metallo- β -lactamase (MBL) from *Escherichia coli* isolates that emanated from the cloacal region of poultry birds in a local poultry farm in Abakaliki metropolis, Ebonyi State, Nigeria. The antibiogram and multiple antibiotic resistance index of the isolated *Escherichia coli* isolates was also evaluated. After the culture of the samples on MacConkey agar and eosin methylene blue agar plates, a total of 29 (72.5%) isolates of *Escherichia coli* was bacteriologically isolated from the cloacal swab samples analyzed in this study. The isolated *E. coli* isolates produced pink-red colonies on MacConkey agar plates as well as green metallic sheen colonies on eosin methylene blue (EMB) agar plates. The result of the identification studies showed that the *E. coli* isolates were positive for methyl red test, indole production and Gram-negative (Table 1). The percentage susceptibility of the isolated *Escherichia coli* isolates to some selected antibiotics is shown in (Figure 1).

The *Escherichia coli* isolates recovered in this study from the cloacal swab samples were highly resistant to the 3rd-generation cephalosporins including cefotaxime (55.2%), ceftriaxone (13.8%) and ceftazidime (69.0%). Ofloxacin had no inhibitory activity against the *E. coli* isolates which were found to be completely resistant to this fluoroquinolone (100%). Similar high level of resistance was also observed amongst the *E. coli* isolates to ciprofloxacin (89.7%), which is also a fluoroquinolone (Figure 1). The *E. coli* isolates were also found to be highly resistant to ceftiofur (93.1%), which is a 2nd-generation cephalosporin. A handful of the *E. coli* isolates also showed reduced susceptibility to the carbapenems including meropenem, ertapenem and imipenem at the rate of 58.6%, 75.9% and 31.0% respectively (Figure 1). Table 2 shows the frequency of MBL-producing *E. coli* isolates in this study.

Out of the 29 *E. coli* isolates that were phenotypically screened for MBL production, only 3 (10.3%) isolates of *E. coli* were phenotypically confirmed to produce MBL (Table 2). The other 26 isolates of *E. coli* did not produce MBL by the inhibition based assay technique used in this study. On average, the resistant *E.*

coli isolates had a multiple antibiotic resistance index of 0.4. They were multiply resistant to antibiotics in the class: aminoglycosides, fluoroquinolones, carbapenems, and cephalosporins (Table 3).

Discussion

The production of metallo- β -lactamase (MBL) by Gram-negative bacteria especially in members of the *Enterobacteriaceae* family is an important resistance mechanism that allows these organisms to resist the antimicrobial onslaught of some potent antibiotics such as the carbapenems. This study was aimed at determining the antibiogram, multiple antibiotic resistance and production of MBL from *Escherichia coli* isolates recovered from cloacal swab samples from poultry birds in a local poultry farm in Abakaliki, Nigeria. A total of 40 consecutive, non-duplicated cloacal swab samples were used for this study. The result of the bacteriological investigation shows that a total of 29 (72.5%) *E. coli* isolates were isolated from the 40 cloacal swab samples. Faecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease; and most *E. coli* serotypes or strains are occasionally responsible for product recalls due to food contamination [22]. All the *E. coli* isolates in this study were completely resistant to ofloxacin (100%), a fluoroquinolone. This resistance rate was followed by ciprofloxacin (89.7%), another fluoroquinolone to which the test *E. coli* isolates also showed reduced susceptibility to. This report of *E. coli* resistance to the fluoroquinolones in this study was reported by [23]. In Iran, [24] also reported multiple antimicrobial resistance in *E. coli* isolates recovered from chickens in Iran as was obtainable in this study. The percentage of *E. coli* isolates that was found to be resistant to ciprofloxacin in our study (89.7%) was lesser than that reported by [25] in Egypt – in which 40% of the tested *E. coli* isolates in their study was found to be resistant to ciprofloxacin. Members of the *Enterobacteriaceae* family including *E. coli* often contain resistance determinants for other classes of antibiotics such as

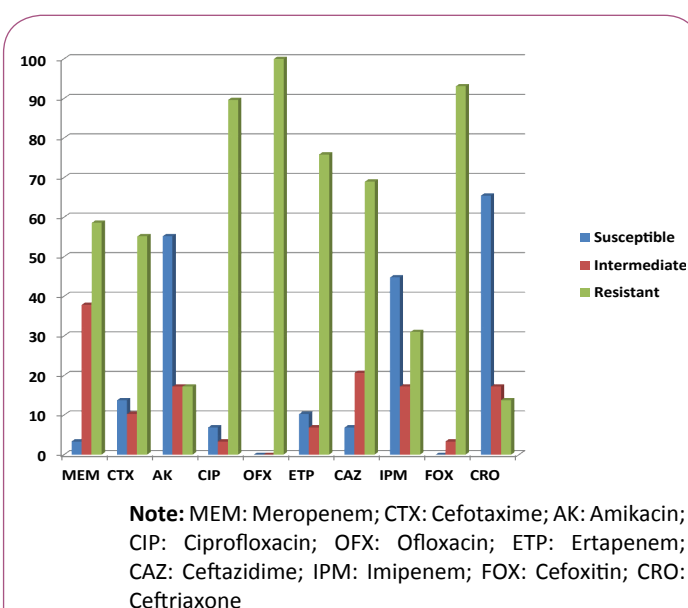


Figure 1 Antimicrobial susceptibility profile of the 29 *Escherichia coli* isolates from cloacal swab samples.

Table 1 Prevalence of *Escherichia coli* isolates.

Organism	Total samples analyzed	Positive samples	Prevalence (%)	Morphological appearance on culture media	Gram reaction	Indole test	MR test
<i>Escherichia coli</i>	40	29	72.5	Lactose-fermenting colonies on MAC; and green metallic sheen colonies on EMB	Gram-negative	Positive	Positive

Note: MAC-MacConkey agar, EMB-Eosin methylene blue agar, MR-Methyl red

Table 2 Detection of MBL in 29 *E. coli* isolates.

Organism	Sample source	Number (%)	MBL positive n (%)	MBL negative n (%)
<i>Escherichia coli</i>	Cloacal swabs of poultry birds	29 (72.5)	3 (10.3)	26 (89.7)

Key: n - number of samples, % - percentage

Table 3 Multiple antibiotics resistance index (MARI) of selected *E. coli* isolates.

Isolate No.	MARI	Antibiotics
E1	0.3	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP
E9	0.3	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP
E3	0.4	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP
E5	0.4	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP
E6	0.3	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP
E8	0.4	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP
E10	0.4	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP
E12	0.3	AK, FOX, CIP, OFX, CAZ, CTX, IPM, MEM, ETP

Note: AK: Amikacin; FOX: Cefoxitin; CIP: Ciprofloxacin; OFX: Ofloxacin; CAZ: Ceftazidime; CTX: Cefotaxime; IPM: Imipenem; MEM: Meropenem; ETP: Ertapenem; MARI: Multiple antibiotics resistance index

the aminoglycosides, sulfonamides, and fluoroquinolones which are readily transmissible from one strain of organism to another and between different species of Gram-negative bacteria [26,27]. The *E. coli* strains in this study were found to be highly resistant to the cephalosporins and carbapenems used in this study especially cefoxitin (93.1%), ceftazidime (69.0%), cefotaxime (55.2%), imipenem (31.0%), meropenem (58.6%) and ertapenem (75.9%). The high resistance of *E. coli* isolates of poultry origin as reported in this study is in line with a similar study carried out in Ibadan, Nigeria, [28] who reported that *E. coli* from poultry frozen foods were resistant to beta-lactam antibiotics and non-beta-lactams alike. In terms of their multiple antibiotic resistance indexes, the *E. coli* isolates were found to be multiply resistant to antibiotics in the class: cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. The average multiple antibiotic resistance index of the isolated *E. coli* in this study was pegged at 0.4. This indicates that the *E. coli* isolates bacteriologically recovered in this study were found to be multiply resistant to antibiotics from four different classes as was aforementioned. This result of ours is in agreement with the report by Tawab AA et al. [25] and Talebiyan R et al. [24] who reported the multiple antibiotic resistance nature of *E. coli* isolates from poultry origin in Egypt and Iran respectively. In China, [29] reported in a similar study that *E. coli* isolates of poultry origin are multidrug resistant, and that they express enzymes that allow them to be multidrug resistant in nature. Out of the 29 isolates of *E. coli* that were bacteriologically recovered from the 40 cloacal swab samples, the production of metallo- β -lactamase (MBL) was phenotypically detected in only 3 isolates of *E. coli*. MBL production was not detected in the other 26 *E. coli* isolates by the inhibition-based assay technique that was used in this study. The prevalence rate of *E. coli* isolates that produced

MBL phenotypically in this study was 10.3%. This result is similar to a previous study that we conducted in Abakaliki metropolis in 2016, in which we phenotypically screened *Enterobacteriaceae* isolates of poultry origin for the production of MBL [4]. However, the prevalence rate of MBL-positive *E. coli* isolates in this current study (10.3%) is lesser than what we obtained in the previous year in which MBL production was detected phenotypically in 9 (23.1%) *E. coli* isolates. Our report of MBL production in *E. coli* isolates of poultry origin is in agreement with the study done by Wadekar et al. [30] who showed that *Enterobacteriaceae* isolates including *E. coli* produce multidrug resistance enzymes such as MBL that allows them to be multidrug resistant in nature. The results of this study contribute to the growing reports of antibiotic resistance in the community. This goes to show that antibiotics are irrationally used in the community. The occurrence of an MBL-positive isolate in a poultry farm portends serious public health implication because this isolate could serve as a route via which resistance traits could spread undetected.

Conclusion

Conclusively, this current study has presumptively shown that *E. coli* isolates of poultry origin are multidrug resistant in nature. Our report also shows that these isolates produce metallo- β -lactamase (MBL) phenotypically, and this allows them to be resistant to the carbapenems. The rapid spread of resistance among bacteria may be due to widespread and inappropriate use of antibiotics in the community. Absolute care should be taken in the treatment and handling of poultry birds. The use of antibiotics in animal husbandry and poultry production is a major driving force that contributes to the development of antibiotic resistant bacteria amongst food-producing animals. Aggressive

action is therefore needed now to nip in the bud, the emergence and spread of drug resistant bacteria in the community.

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