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Antibiogram and Detection of Metallo-β-Lactamase Producing *Escherichia coli* Isolated From Cow Dung in Owo Metropolis

Olayemi Amos Adeluwoye-Ajayi [a*], Adedoyin Ayowole Bello [a], Folarin Moses Thomas [a] and Oluwasegun Victor Omoniyi [a]

[a] *Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to determine the antibiotic susceptibility profile and the production of metallo-β-lactamase (MBL) by *Escherichia coli* isolated from cow dung in Owo metropolis. The isolation of *Escherichia coli* was done using MacConkey agar and Eosin Methylene Blue Agar and was conventionally characterized. Antimicrobial susceptibility test of the isolates were by disc diffusion method against ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg) and ciprofloxacin (5 µg). Detection of MBL producing isolates was by imipenem-EDTA combined disc test. The isolates showed highest resistance to augmentin (97.8%) and least resistance to nitrofurantoin (20.0%). Out of the 45 *Escherichia coli* isolated from cow dung, 8 (17.8%) produced MBL and were all multidrug resistant. The production of MBL and the high prevalence of antibiotic resistance observed among the *Escherichia coli* in this study infer that cow dung does not only serve as a reservoir for MBL-producers but also as source for the growth and dissemination of clinically significant antibiotic resistant species. Hence, the use of antibiotics as growth enhancers in cow production should be discouraged to help prevent the spread of antibiotic resistant bacteria and thus, preserve the efficacy of available antibiotics.

**Corresponding author: Email: ajayisamson86.@yahoo.com;*

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1. INTRODUCTION

The emergence and rapid dissemination of antibiotic resistance in bacteria is now a global concern. The antibiotic resistance acquired by organisms in one ecosystem can easily be transferred among organisms in different ecosystems [1]. This, in turn, is responsible for wide-scale epidemic and endemic spreads of multidrug-resistant bacteria. Now, it is evident that resistant microbes are found in the different environmental compartments and reservoirs due to misuse and overuse of antibiotics and poor health-care infrastructures [2,3]. Surprisingly, antibiotic-resistant bacteria were found in the pristine environments where there was no direct human influence like habitation, farming, and hospitals [4]. Antibiotic-resistant Bacteria were even found in the soil when raw cow dung and manure were used extensively as fertilizers [5] and ultimately spread into natural and drinking water sources [6].

Escheichia coli has been noted as a very important foodborne pathogen and as the etiological agent of different extra intestinal infections, like urinary tract infection and septicemia [7,8]. Salyers et al. [9] opined that *E. coli* of faecal origin may function as a reservoir of genes coding for drug resistance, and are usually regarded a potential indicator of acquired antibiotic resistant genes in an environment. There are reports of bacterial resistance to commercially available and frequently administered antibiotics in the developing nations around the world. Moreover, variation exists in the pattern of resistance exhibited by *E. coli* isolated from the faecal samples of apparently healthy children [10-14].

Carbapenems generally are presently used as last line antibiotics for the management of infections caused by Gram negative bacteria particularly those suspected to be multidrug-resistant [15,16,17]. Before now, members of the family *Enterobacteriaceae* were not resistant to carbapenems, albeit this has changed because of the appearance of some carbapenem resistant enteric bacteria in the last decade thereby making them a serious public health issue [17]. The 2013 report of the United States Center for Disease Control and Prevention (CDC) shows that the emmergence of enteric bacteria resistant to carbapenem, in the past few years remains the main cause of hard-to-manage infections in patients admitted in hospitals, and they are referred to as considered a pressing danger to human health [15,16].

Metallo-β-lactamase enzymes also known as carbapenemases are enzymes which hydrolyze carbapenems, and possess powerful action on other antibiotics in the class of beta-lactam apart from monobactams. They are also recognized to bestow different level of resistance to beta-lactam antibiotics and their availability in Gram negative organisms particularly those of clinical importance has subjected the usage of carbapenems under risk [17]. Metallo-β-Lactamase (MBLs)-producing *E. coli* are of paramount concern because they are multidrug resistant, limit therapeutic options, lead to treatment failures and increase the cost for treatment and duration of hospitalization.

Currently, there is paucity of information on MBL-producing *E. coli* from animals and the possible contribution of these resistant species to the ever growing antimicrobial resistance observed in humans. Hence, this study was carried out to determine the antibiogram and the production of MBL by *E. coli* isolated from cattle dung in Owo metropolis.

2. MATERIALS AND METHODS

2.1 Sample Collection

Between January and February 2022, freshly passed cattle dung were aseptically collected at the Central Abbattoir, Owo in Ondo State from apparently healthy cattle which were about to be slaughtered into appropriately labelled sterile capped universal bottles with sterile spatula, preserved in ice packs and transported to Microbiology unit of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic Owo for immediate analyses.

2.2 Isolation of *Escherichia coli*

One gram of the cow dung sample was weighed into 10 ml of de-ionized water to make a stock solution. From the stock, tenfold serial dilution was carried out. 1ml each of the serial diluents (10^{-2}, 10^{-4} and 10^{-6}) was dispensed into appropriately labeled sterile Petri dishes. Aseptically, MAC and EMB Agar cooled to about 45 °C was separately dispensed into the aliquots of samples in the Petri dishes and swirled gently, allowed to solidify and incubated at 35-37 °C for 18-24 hours. Distinct colonies were sub-cultured on newly prepared nutrient agar plates; repeated streaking was done to obtain pure culture of *Escherichia coli* prior to biochemical tests [18].

2.3 Preparation and Standardizing Inoculum Suspension

The inoculum suspension was prepared by picking 2-3 colonies of a 24-hour culture with a sterile wire loop and was suspended in 5M normal saline, the suspension was mixed with a vortex mixer. The turbidity of the suspension was standardized to match the 0.5 McFarland's standard which corresponds to approximately 1.5 x 10^8 cfu/ml and this was done by comparing the test suspension with barium sulphate suspension by placing the tubes in front of a white paper with black lines.

2.4 Antimicrobial Susceptibility Test of the Isolated *Escherichia coli*

Antibiotic susceptibility test on the *Escherichia coli* was done using disc-agar diffusion technique [19]. The test antibiotics employed were the following classes of antibiotic agent β-lactam combinations (augmentin otherwise called amoxicillin/clavulanate 20/10 µg), cephem (cefotaxime 30 µg, ceftazidime 30 µg, cefixime 5 µg), carbapenem (imipenem 10 µg), aminoglycosides (gentamicin 10 µg), fluoroquinolone (ciprofloxacin 5 µg, ofloxacin 5 µg), and nitrofurans (nitrofurantoin 300 µg). After incubation period, zones of inhibition were measured in millimeter while the protocols in CSLI [19] guidelines were used for the interpretation. The zones of inhibition obtained were compared with reference for proper classification of the organisms as sensitive, intermediate or resistant to specific antibiotics [19]. Any isolate that showed resistance to minimum of two different classes of antibiotics was taken as a multidrug resistant strain [20].

2.5 Isolates' MBL Production Screening

All isolates that were resistant to imipenem by recording a zone of inhibition diameter less than 23 mm were suspected of producing the enzyme metallo-β-lactamase [21]. They were further subjected to confirmation test phenotypically.

2.6 Phenotypic Confirmation of MBL-Positive Isolates

The turbidity of a culture of the isolated *E. coli* was adjusted to 0.5 MacFarland standard, then inoculated aseptically on freshly prepared Muller-Hinton agar plates. Later, antibiotic disk containing 10 µg imipenem and infused with 1 µg of EDTA as well as another imipenem disc free of EDTA were placed aseptically on the freshly inoculated agars. The dishes were incubated for 24 h at 30^{o}C, then inhibition zones were taken and interpreted using the criteria set by CLSI [19]. According to Ejikeugwu et al. [24], a variation of 7 mm or more recorded in the zones of inhibition between the imipenem disc infused with EDTA and disc without EDTA connfirmed the phenotypic production of MBL.

3. RESULTS

3.1 Identification of the Isolates

Table 1 showed that all the isolates were Gram-negative rods. They were motile, catalase, indole, methyl red, glucose and lactose positive but were citrate, Voges-Proskauer and maltose negative.

3.2 Antibiotic Susceptibility Patterns of the Isolated *E. coli* from Cow Dung

Table 2 showed the varying levels of susceptibility and resistance exhibited by *E. coli* isolated from cow dung to the test antibiotics. The *E. coli* isolates from the cow dung showed highest resistance to augmentin (97.8%) and least resistance (20.0%) to nitrofurantoin.

3.3 Distribution of MBL-Producing *E. coli* Isolated from Cow Dung

Table 3 showed that from the 9 cow dung samples, 45 *Escherichia coli* isolates were obtained. Out of these, 13 (28.9%) were suspected to be MBL-producers, 8 (17.8%) were confirmed to be actual producers of MBL while 37 (82.2%) were non-producers of MBL.

3.4 Antibiotype of the MBL-Producing *E. coli*

The antibiotypes of the MBL-producing *E. coli* showed that three (37.5%) of the eight isolates that produced MBL resisted six classes of antibiotics (NIT-CIP-IMP-CAZ-GEN-AUG), another three (37.5%) resisted five classes (CIP-IMP-GEN-CRX-AUG) and two (25%) were resistant to four (CIP-CAZ-GEN-AUG) different classes of antibiotics, hence they were considered multidrug resistant.

Table 1. Gram reaction and biochemical characterization of the isolates from the cow dung

Isolates	Shape	Gram Reaction	MOT	CAT	CIT	IND	MR	VP	GLU	LAC	MAL	Morphological Appearance on Culture Media	Probable Organism
1-45	Rod	-	+	+	-	+	+	-	+	+	-	Lactose-fermenting (pinkish) colonies on MAC; and green metallic sheen colonies on EMB	*Escherichia coli*

KEY: + = Positive, - = Negative, MOT = Motility, CAT = Catalase, OXI = Oxidase, CIT = Citrate, IND = Indole, MR = Methyl red, VP = Voges-proskauer, GLU = Glucose, LAC = Lactose, MAL = Maltose, MAC = MacConkey Agar, EMB = Eosin Methylene Blue Agar

Table 2. Antibiotic susceptibility patterns of all the *E. coli* isolated from cow dung

S/N	Antibiotics (µg)	No. (%) susceptibility	No. (%) resistance
1	AUG (30)	1 (2.2)	44 (97.8)
2	OFL (5)	13 (28.9)	32 (71.1)
3	CAZ (30)	2 (4.4)	43 (95.6)
4	CRX (30)	4 (8.9)	41 (91.1)
5	GEN (10)	15 (33.3)	30 (66.7)
6	CXM (5)	6 (13.3)	39 (86.7)
7	NIT (300)	36 (80.0)	9 (20.0)
8	CPR (5)	9 (20.0)	36 (80.0)
9	IMP (10)	32 (71.1)	13 (28.9)

KEY: S/N = Serial number, No. = Number, AUG = Augmentin, OFL = Ofloxacin, CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamisin, CXM = Cefixime, NIT = Nitrofurantoin, CPR = Ciprofloxacin, IMP = Imipenem

Table 3. Distribution of MBL-producing *E. coli* isolated from cow dung

Bacteria	Sample	No. of samples	No. of Isolates screened	No of suspected MBL isolates N (%)	MBL positive N (%)	MBL negative N (%)
E. coli	Cow dung	9	45	13 (28.9)	8 (17.8)	37 (82.2)

KEY: N = number of isolates, % = percentage, MBL = Metalllo-Beta-Lactamase

Table 4. Antibiotype of MBL-producing *E. coli* isolated from cow dung

Antibiotype	Classes of Antibiotics	No. (%) MBL+ve *E. coli*
NIT-CIP-IMP-CAZ-GEN-AUG	6	3 (37.5)
CIP-IMP-GEN-CRX-AUG	5	3 (37.5)
CIP-CAZ-GEN-OFL-AUG	5	0 (0.0)
NIT-CIP-CXM-GEN-AUG	5	0 (0.0)
CIP-CRX-GEN-OFL-AUG	5	0 (0.0)
IMP-CXM-GEN-OFL	4	0 (0.0)
NIT-CRX-IMP-AUG	4	0 (0.0)
CIP-CAZ-GEN-AUG	4	2 (25.0)
CIP-CXM-OFX-AUG	4	0 (0.0)
CIP-CAZ-GEN-AUG	4	0 (0.0)
CAZ-OFX-AUG	3	0 (0.0)
CXM-GEN-AUG	3	0 (0.0)
CAZ-AUG	2	0 (0.0)
CIP-AUG	2	0 (0.0)
TOTAL		**8**

NIT:Nitrofurantoin; CIP; Ciprofloxacin; IMP: Imipenem; CAZ: Ceftazidime; GEN: Gentaicin; AUG: Amoxicillin-Clavulanic acid; CRX: Cefuroxime; OFL: Ofloxacin; CXM: Cefixime

4. DISCUSSION

Different bacteria strains use various mechanisms to resist the action of antibiotics, one of these mechanisms is the production of MBL particularly some species in the family *Enterobacteriaceae*. This kind of resistance mechanism has great implication on public health because it limits available treatment alternatives for infections caused by bacteria that are multidrug resistant [22].

The result of the Gram reaction and characterization of the isolates in this study is in accordance with the characteristics of *Escherichia coli* as previously reported by other researchers [23]. The result of the bacteriological investigation showed that a total of 45 *E. coli* isolates were gotten from the cow dung samples. This result varies a little with the report of Ejikeugwu et al*.* [24] who isolated 32 *E. coli* from 40 cloacal swab samples of cow from a local abattoir in Abakaliki metropolis. This disparity may be as a result of difference in sampling techniques. Feacal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease and most *E. coli* strains are occasionally responsible for product recalls due to food contamination.

All the isolates gotten in this study revealed different degrees of resistance to the tested commercially available antibiotics. Out of all the test antibiotics, the isolated *E. coli* showed high resistance to augmentin (97.8%), Ceftazidime (95.6%), Cefuroxime (91.1%), cefixime (86.7%), Ciprofloxacin (80.0%), Ofloxacin (71.1%) and gentamicin (66.7%). However, 71.1% of the isolates showed susceptibility to imipenem, an example of carbapenems. The reasonably high level of activity of the imipenem against the *E. coli* isolates in this study may help to explain why carbapenems have remained the standard drug for the management of infections occasioned by some Gram negative bacteria including those provoked by extended-spectrum-β-lactamases [25]. The E. *coli* showed highest susceptibility to nitrofurantoin (80.0%), a nitrofuran. Similar findings have been reported by other researchers about the pattern of antibiotic resistance of *E. coli* isolated from different environments [26,27].

The production of MBL was identified in 8 (17.8%) of the isolated *E. coli* from the total 45 isolates obtained in this research. There have been reports of the incidence of MBL producing *E. coli* in various settings; and these organisms are responsible for the wide spread of antibiotics resistance genes [28]. In Owo local government area of Ondo State, Nigeria, the location of this present study, information on MBL positive *E. coli* isolated from animals is scarce. The challenge of widespread indiscriminate use and deposition of antibiotics in the environment may have been responsible for the spread of the resistance among many bacteria species. Moreover, other uses of antibiotics in animal production, fishery and other aquaculture as feed additives as well as their use for treating some diseases of plant may have contributed to the wide spread of antibiotic resistance among various microorganisms in our ecosystem [29].

The observation from this study that showed the MBL-producing *E. coli* exhibiting multiple drug resistance to a combination of six (NIT-CIP-IMP-CAZ-GEN-AUG), five (CIP-IMP-GEN-CRX-AUG) and four (CIP-CAZ-GEN-AUG) different classes of antibiotics respectively and this is similar and comparable to the report of a study carried out by Egbule and Yusuf [30] on *E. coli* isolated from cattle and poultry feaces in Abraka, South-South Nigeria which shows multidrug resistance among the isolates, which may have been due to their expression of some enzymes that inactivate these drugs.

5. CONCLUSION

In conclusion, this present study has shown that the *E. coli* strains isolated from cow dung showed varying levels of susceptibility and resistance to different classes of antibiotics tested. The report of this study showed that some of the isolated *E. coli* produced metallo-beta-lactamase and this may have allowed them to resist the action of carbapenems. Hence, there is a need to create awareness about the danger inherent in indiscriminate use of antibiotics among the local people and particularly among the cattle rearers. Strict antibiotic policy and alternative measures for rearing and production of food-producing animals (that does not include the use of antibiotics) is required now than ever to protect and sustain the efficacy of available antibiotics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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