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Microbial Quality and Phenotypic Profile of Extended Spectrum Beta–Lactamase Producing *Escherichia coli* and *Klebsiella* species Contamination in Dressed Chicken Meat in Maiduguri Metropolis, Northeastern Nigeria

[1*]Sai'du, A. S., [1]Apollos, R. P., [1]Mohammed, S., [2]Ejeh, F. E., [1]Tijjani, A. O., [1]Ahmed, B. and [2]Wafar, E.

[1]Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, Nigeria
[2]Department of Veterinary Microbiology, University of Maiduguri, Nigeria

[*] *Author for Correspondence: adamudvm13@gmail.com*

ABSTRACT

A cross sectional study was conducted to detect Extended-Spectrum β-lactams (ESBLs) producing *E. coli* and *Klebsiella* species in dressed chicken meat in Maiduguri Metropolis, Borno State, Nigeria. A total of 384 samples were collected (chicken meat swab and intestinal contents) from two (Abbaganaram and Monday Markets) live bird markets (LBMs) in Maiduguri Metropolitan Council (MMC) and one (Tashan Bama) in Jere Local Government Area (LGA). Colony count, culture, isolation and determination of microbial quality of meat were performed based on standard bacteriological protocols. Biochemical tests were conducted to differentiate the isolates, and antimicrobial susceptibility test was performed using Kirby Bauer disk diffusion method. Tashan Bama LBM had 6.4 x 10^7 CFU/g dressed chicken meat contamination, while, *E. coli* and *Klebsiella* species had the highest number of isolates, 178 (46.4%) 28 (7.3%), respectively. The study also revealed 14 (93.3%) and 5 (71.4%) β-lacta m antimicrobial resistant isolates due to *E. coli* and *Klebsiella* species, respectively. The phenotypic expression of multi_drug resistance (n=10) patterns of those isolates; further revealed the β-lactam producing *E. coli* and *Klebsiella* species in dressed c hicken meat. Critical control points should be established to minimize contamination and the zoonotic risk of multi-drug resistance pathogens in chicken meat in Maiduguri

Keywords: Antimicrobial resistance, ESBL, *E. coli*, Foodborne infections, Klebsiella.

INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) are enzymes in gram-negative bacteria that confer resistance to the majority of beta-lactam antibiotics. The ESBLs hydrolyze most penicillin, extended-spectrum cephalosporins, and aztreonam (Silvia and Jacoby, 2014). These ESBLs producers have been noticed mainly in the *Enterobacteriaceae* family of bacteria which may harbour several antibiotic resistance determinants making treatment of infections caused by these pathogens more difficult challenge to public health (Rottier *et al*., 2012). The ESBLs producers have complex epidemiology; the most prominent bacteria involved include *Escherichia coli* and *Klebsiella pneumoniae Escherichia coli* and *Klebsiella pneumoniae* reservoirs comprise the environment (soil and water), wild animals, farm animals, food, and pets (Carattoli, 2013). In some communities, backyard poultry houses at residential premises may

disseminate antimicrobial-resistant ESBL bacteria in the environment (Nafarnda *et al*., 2012). A study conducted in Spain identified ESBL-producing bacteria in poultry with *E. coli* strains comprising ESBL CTX-M-14, CTX-M-9, and SHV-12 (Briñas *et al.,* 2003). In another study conducted in Britain, 54.5% of CTX-M-carrying *E. coli* was isolated from broiler slaughter-slab and 3.6% from individual broiler cloacal samples (Randall *et al*., 2011). Food animals colonized with ESBL-producing bacteria can enhance the spread of bacteria at the community level (Bortolaia *et al.,* 2010; Bennett et al., 2013). Meat is every edible part of any slaughtered animal, whether the same is in its natural state or has been subjected to freezing, chilling, salting, canning or other preservative processes (Clark and Lubs, 1978). The source of water for abattoir activities is vital to meat hygiene. Water is needed for maintaining the cleanliness of the abattoir's environment and for washing off blood from the carcasses. Other sources of contamination in abattoirs and meat stalls may be unhygienic practices such as poor handling, use of contaminated tables to display meat meant for sale and the use of contaminated knives in cutting operations. Contamination of meat and meat products occur

when raw meat is exposed to contamination or pathogenic microbes (WHO, 1982). The quality of meat and meat products can be degraded due to contamination with digestive enzymes, microbial spoilage and fat oxidation (Berkel *et al*., 2004). Lipid oxidation, protein degradation and the loss of other valuable molecules are the consequence of meat spoilage process. Pre-slaughter handling of livestock and post-slaughter handling of meat plays a significant role in the deterioration of meat quality. The glycogen content of animal muscles is reduced when the animal is exposed to pre-slaughter stress which changes the pH of the meat, to higher levels, depending on the production level of lactic acid (McGaugh and Noor, 2012; Miller, 2002).

Antimicrobial resistance (AMR) is regarded as a major global risk and an emerging public health threat, involving both human and animal health (Schrijver *et al*., 2018). The indiscriminate use of antimicrobials in pets and food animals has led to the development of antimicrobial resistance among pathogens with serious consequences on the efficacy of antimicrobial therapy against infectious disease (Nordmann, 2011; Shaikh *et al*., 2015). This phenomenon has been linked to the indiscriminate use/misuse of antibiotics in human medicine, agriculture and veterinary medicine (Shaikh *et al*., 2015). The transmission of resistant bacteria from animals to humans is a matter of serious concern with the possibility of cross-species transmission (Schrijver *et al*., 2018). Additionally, AMR has been transmitted horizontally between unrelated bacteria through sharing of resistance genes in the form of a plasmid, and this has posed an even greater risk in public health (Caratolli, 2013).

The *Enterobacteriaceae* family contains a large number of genera that are biochemically and genetically related to one another. Many of the traditional or familiar bacteria are found in this family, *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Proteus*, *Yersinia*, *Klebsiella*, and *Citrobacter* among others. Common characteristics of the family *Enterobacteriaceae* are; they are gram-negative, short-rods, non-sporulating, facultative anaerobes. These organisms have simple nutritional requirements and MacConkey agar is used to isolate members of *Enterobacteriaceae* into pinkish and colorless colonies of lactose fermenters and non-lactose fermenters respectively. Lactose fermenters include among others; *Citrobacter*, *Escherichia*, *Enterobacter* and *Klebsiella*. Non-lactose fermenters include; S*higella*, *Yersinia*, *Proteus, Salmonella*, etc.

To the best of our knowledge little or no work done on the phenotypic expression of ESBLs producing in *E. coli* and *Klebsiella* species in chicken meat in Maiduguri, and specifically the MMC and Jere are having the most patronized LBMs. These areas are the major suppliers of chicken meat in Maiduguri Metropolis. The Abaganaram and Monday-market (MMC) LBMs and Tashan Bama (Jere LGA) LBMs were also chosen on the basis of their daily slaughter capacity and major suppliers of chicken meat to the general public. However, previous hospital-based study amongst 439 isolates from clinical human specimens of at the University of Maiduguri Teaching Hospital confirmed the prevalence of ESBLs producing *E. coli* and *Klebsiella* species at 27.3% and 30%, respectively (Mohammed *et al*., 2016).

The hygienic practices of the people working in those LBMs are very poor and this may likely increase the contamination level and serve as sources of foodborne pathogens to the public. Therefore, the present study was designed to determine the prevalence and detect phenotypic expression of ESBLs producing *E. coli* and *Klebsiella* species in dressed chicken meat in live bird markets (LBMs) and slaughter slabs in Maiduguri metropolis.

MATERIALS AND METHODS

Study Area

The study was conducted in Maiduguri Metropolis, in Abaganaram and Monday-market LBMs in Maiduguri Metropolitan Council (MMC) and Tashan Bama LBMs in Jere LGAs of Borno State.

Study Design

A cross-sectional study was designed to cover Maiduguri Metropolitan Council (MMC) and Jere Local Government Areas based on the availability of Live Bird Markets (LBMs) and chicken slaughter slabs. A total of 384 dressed chicken meat samples were collected from three (3) selected live-bird markets, two of which represent the MMC and one represents the Jere LGA. The Abaganaram and Monday-market live-bird markets were selected from the MMC based on the daily slaughter capacity and Tashan Bama live-bird market was chosen from Jere LGA proportionately for the same reason (Mohammed *et al*., 2016; Kwoji *et al.,* 2019). Chicken meat swab and intestinal contents were collected simultaneously from each bird chosen at convenience.

Sample Size Determination

A total of 384 samples were determined based on the 50% prevalence, as presented below:

Using the formula:

$n=Z^2Pq/e^2$ (Thrusfield, 2013)
Where: n =sample size
Z = corresponding value of confidence level (1.96 for 95% CI)
P =estimated value for the proportion of a sample (50%)
q =1-P (1-0.5 = 0.5)
e = desired margin of error (5% = 0.05)

Then, $n=Z^2 *P (1-P)/e^2$
$= (1.96)^2 \times 0.5(1-0.5) / (0.05)^2$
$= 3.842 \times 0.25/0.0025=0.9605/0.0025$, n= 384
Therefore, the calculated number of samples of 384 dressed chicken meats were sampled in this study.

Sample Collection and Transportation

Two (2) visits were conducted for each sample location. Hundred (n=100) samples each for chicken-meat swab and intestinal contents (of each bird) were collected from *Monday Markets* LBM and slaughter slab, 50 samples each for the meat swab and intestinal contents were collected from *Tashan Bama* LBM and slaughter slab, whereas, 42 samples each for the meat swab and intestinal contents of the chicken

meat were collected from the Abaganaram LBM and slaughter slab, this brought the total samples collected to n=384. All samples were collected aseptically using sterile meat swab sticks (HiMedia, India) and plain universal bottle and were transported immediately in an ice packed container to the Veterinary Microbiology Laboratory for further analysis.

Laboratory Procedures

Enrichment

The samples were inoculated into 20ml of peptone water for 24 hours.

Bacterial isolation and Colony Count

One milliliter (1ml) of the broth cultured sample was taken, following serial dilutions of 1:10ml of distilled water with dilution factor of 10^6. Thus, 1ml out of the 10ml of the diluents was dispensed onto the plate (Pour plating method) and 20ml of nutrient agar was poured onto the plate containing 1ml of the diluents and was allowed to solidify. It was incubated at 37°Cfor 24 hours after that the colonies were counted to determine the number of colony-forming units, using a Colony counter (WINCOM®) by manual counting of colonies on plates illuminated by transmitted light. The concentration of bacteria in the original culture was then calculated based on the assumption that each colony was raised from one single bacterium, colony forming unit (CFU). Therefore, only parts of a plate were analyzed and used to estimate the whole plate count after extrapolation (Blodgett, 2008).

Culture and Isolation

All samples collected were inoculated into a peptone water (H/MEDIA®) and incubated aerobically at 37°Cfor 24 hours. The inoculated samples were sub-cultured on prepared Eosin Methylene Blue (EMB) (HiMedia®, India) plate and incubated at 37°Cfor 24 hours and was sub-cultured on Sorbitol MacConkey Agar (SMAC) plate and incubated at 37°Cfor 24 hours to detect *E. coli*. The plates were observed for cultural and morphological characteristics for growth of *E. coli* and *Klebsiella* species.

Biochemical Tests

The conventional four different biochemical tests, indole test, methyl red, Voges-proskauer, and citrate test (IMViC) for the identification and differentiation of enteric bacteria (*Enterobacteriaceae*) were used in this study as described by (Clark and Lubs, 1978; Tille, 2014).

Antimicrobial Sensitivity Tests (AST)

Antimicrobial susceptibility test was performed on Mueller Hinton Agar by the standard Disk diffusion method. The Beta lactams antimicrobials used include: penicillin-G(10µg), amoxicillin(15µg), ampicillin(10µg), cephalothin(30µg), ceftazidime(10µg), ceftriaxone(30µg), vancomycin(30µg), polymyxin-B(300µg), azithromycin(15µg) and ciprofloxacin(5µg). Other classes of antimicrobial agents, such as glycopeptide, polymyxins, and quinolones were used alongside the beta-lactams. The isolated pure cultured samples from the sub-cultured plate were inoculated into nutrient broth (L:S-BIOTECH®) for 24 hours. Pour plating methods was used for Antibiotic Sensitivity Tests (AST) and Kirby Bauer diffusion disk sensitivity test were used for rapid determination of drug, in which 1ml of nutrient broth was poured onto the agar. If the bacterium was sensitive to a particular agent, then a define zone of inhibition occur around the disc after incubation.

Data Analysis

The data obtained were presented in tables and chats using the Microsoft Excel version 2019 for Windows 10. Association between location and sample type was determined using Chi-squared test and presence of *E. coli* and *Klebsiella* species in dressed chicken meats in MMC and Jere LGAs, and odds ratio (OR) on 95% CI were measured to determine the strength of the association. Value of $p \leq 0.05$ was considered significant. The IBM SPSS Statistics version 23.0 was used for the analyses.

Ethical Statement

The study did not involve the use of live animals.

Microbial Quality of Dressed Chicken Meat Sampled in Live Bird Markets in MMC and Jere LGAs

The dressed chicken meat sampled (swab and intestinal contents) from Tashan Bama was the highest and has the average value of microbial contamination 6.4 x 10^7 CFU/g. The average values of the colony count for all the three (3) locations are provided in the Table 1.

Table 1: Microbial Quality of Dressed Chicken Meat in MMC and Jere LGAs Live Bird Markets

Location	Average Microbial Contamination *(CFU/g)	p-value
Monday Market	3.9 x 10^7 CFU/g	0.61
Tashan Bama	6.4 x 10^7 CFU/g	0.62
Abaganaram	2.7 x 10^7 CFU/g	0.16

*CFU/g = Colony forming unit per gram of chicken-meat sample

Prevalence of *Escherichia coli* and *Klebsiella* species in Dressed Chicken Meat Sampled in MMC and Jere LGAs

The *Escherichia coli* had the highest average prevalence 178 (46.4%) of the microbial contaminations than *Klebsiella* species 28 (7.3%) in the study area. The result of this study revealed that, pooled intestinal samples had the highest (52.4%) prevalence rate of *E. coli* compared to pooled swabs with 40.6%. On a contrary side, pooled swab recorded highest (7.8%) prevalence of *Klebsiella* species compared to pooled intestine with 6.8%. Furthermore, pooled swabs are about 1.3 times more likely to have *E. coli* contamination than pooled intestine (OR 95%CI: [1.28(0.9-1.83]). In addition, location-based prevalence revealed that, Tashan Bama had the highest rate (60.0%) of *E. coli* contamination in intestinal samples compared to Monday Market (48%) and

Abaganaram (52.4%). Same pattern was observed in case of swab samples (Table 2). The pooled results (192) represent the chicken meat swab and intestinal contents from same bird, and was also compared with the separate samples (384). However, there was no statistically significant ($p>0.05$) association between sample type (swab and intestine), location, and contamination of chicken meat with *E. coli* and *Klebsiella* species (Table 2). The typical *E. coli* and *Klebsiella* species isolated in EMB and SMAC in this study are presented in figure 1 (A & B) respectively.

Table 2: Prevalence and Distribution of *Escherichia coli and Klebsiella* species base on location in Dressed Chicken Meat Sampled in MMC and Jere LGAs.

Location	Isolate ID	*E. coli* (%)	χ2	OR (95% CI)	p-value
Monday market (n=100)	MmS	41 (41.0)		1.0*	
	MmI	48 (48.0)	0.381	0.85 (0.52-1.41)	0.61
Tashan Bama (n=50)	TbS	25 (50.0)		1.0	
	TbI	30 (60.0)	0.29	0.83 (0.43-1.6)	0.618
Abaganaram (n=42)	AS	12 (28.6)	2.1	1.83 (0.8-4.17)	0.16
	AI	22 (52.4)		1.0	
Pooled swab (n=192)	PS	78 (40.6)	1.86	1.28 (0.9-1.83)	0.17
Pooled Intestine (n=192)	PI	100 (52.4)		1.0	
Subtotal		**178 (46.4)**			
Monday market (n=100)		**Klebsiella (%)**			
	MmS	10 (10.0)		1.0	
	MmI	10 (10.0)	1.00	1.0 (0.4-2.5)	0.01
Tashan Bama (n=50)	TbS	5 (10.0)		1.0	
	TbI	3 (6.0)	0.37	1.6 (0.4-7.4)	0.71
Abaganaram (n=42)	AS	0			
	AI	0			
Pooled swab (n=192)	PS	15 (7.8)		1.0	
Pooled Intestine (n=192)	PI	13 (6.8)	0.133	1.15 (0.5-2.5)	0.84
Subtotal		**28 (7.3)**			

Note: MmS –Monday Market Swab, **MmI**- Monday Market Intestine, **TbS**-Tashan Bama Swab, **TbI**- Tashan Bama Intestine, **AS**- Abaganaram swab, **AI**-Abaganaram Intestine, **PS- pooled** swab, **PI**- pooled intestine. **N**=384. Location (Monday Market and Tashan Bama). *1.0 = Reference value.

Antimicrobials Susceptibility and Resistance Profile for *E. coli* and *Klebsiella* species in Dressed Chicken Meat Isolates in MMC and Jere LGAs

Majority of the *E. coli* and *Klebsiella* isolates show resistance to one or more of the 10 antimicrobial agents used, with high level of the beta-lactam antimicrobial agent resistance 14 (93.3%) and 5 (71.4%) for *E. coli* and *Klebsiella* isolates respectively, and few isolates show resistance to glycopeptide, polymyxins, and quinolones. However, a large proportion of isolates were highly sensitive to ciprofloxacin, azithromycin and polymyxin B, reflecting sensitivity to quinolones, macrolides and polymyxin antimicrobials respectively (Tables 3 and 4).

Antimicrobial Resistance Patterns and Multiple Antimicrobial Resistance (MAR) for *E. coli* and *Klebsiella* Species isolates from dressed chicken meat in MMC and Jere LGAs.

Eight of the isolates in this study were multidrug resistant with the CIP, AMS, PB, VA, CRO, CAZ, KF, AMP, AMC, PG multidrug resistance phenotype being the most (n=10) occurring pattern (Table 5).

DISCUSSION

This study revealed that Tashan Bama has the highest average level (6.4×10^7 CFU/g) of *E. coli* contamination in dressed chicken meat which might not be unrelated to the poor implementation of sanitary measures in poultry industries in Maiduguri, Borno State, Nigeria. Furthermore, the level of *E. coli* contamination found in this area is above the acceptable contamination level of (<105) of microbial set by the Codex Alimentarius Commission -CXG 77-2011 (CAC, 2011). Also, poor practices among meat vendors, live bird markets and slaughter slabs in Maiduguri and environs, lack knowledge of application of hazard analysis of critical control point (HACCP) principles in the chicken-meat industries. This may serve as a predisposing factor of contracting numerous food-borne pathogens especially *E. coli* to the public chicken meat in increased burden of AMR isolates (Bunning et al., 1997; Henry and Xin, 2014).

Table 3: Antimicrobials Susceptibility and Resistance Profile for *E. coli* in Dressed Chicken Meat Isolates in MMC and Jere LGAs.

Class of Antimicrobials	Antimicrobial	Susceptible (%)	Resistance (%)
β-Lactam	P10 (10 ug)	0 (0.0)	9 (100)
Glycopeptide	VA30 (30ug)	0 (0.0)	10 (100)
β-Lactam	AMC30 (30ug)	1 (6.7)	14 (93.3)
β-Lactam	AMP10 (10µg)	0 (0.0)	9 (100)
Polymyxins	PB300 (300µg)	8 (53.3)	7 (46.7)
β-Lactam	KF30 (30µg)	0 (0.0)	9 (100)
Macrolides	AZM15 (15 µ)	9 (60)	6 (40)
β-Lactam	CAZ10 (10µg)	0 (0.0)	9 (100)
Quinolones	CIP5 (5µg)	11 (64.7)	6 (35.3)
β-Lactam	CRO30 (30µg)	1 (6.7)	14 (93.3)

Note: P10=Penicillin G, AZM15=Amoxicillin, AMP10= Ampicillin, KF30= Cephalothin, CAZ10= Ceftazidime, CRO30= Ceftriazone,.VA30= Vancomycin, PB300= Polymyxin-B, AZM15= Azithromycin and CIP5= Ciprofloxacin.

Table 4: Antimicrobials Susceptibility Profile for *Klebsiella* species in Dressed Chicken Meat Isolates in MMC and Jere LGAs

Class of Antimicrobials	Antimicrobial	Susceptible (%)	Resistance (%)
β-Lactam	P10 (10 ug)	0 (0.0)	9 (100)
Glycopeptide	VA30 (30ug)	0 (0.0)	7 (100)
β-Lactam	AMC30 (30ug)	2 (28.6)	5 (71.4)
β-Lactam	AMP10 (10ug)	0 (0.0)	9 (100)
Polymyxins	PB300 (300ug)	5 (71.4)	2 (28.6)
β-Lactam	KF30 (30ug)	0 (0.0)	9 (100)
Macrolides	AZM15 (15ug)	7 (50.0)	7 (50.0)
β-Lactam	CAZ10 (10ug)	0 (0.0)	9 (100)
Quinolones	CIP5 (5ug)	7 (63.6)	4 (36.4)
β-Lactam	CRO30 (30ug)	0 (0.0)	15 (100)

Note: P10=PenicillinG, AZM15=Amoxicillin, AMP10= Ampicillin, KF30= Cephalothin, CAZ10= Ceftazidime, CRO30= Ceftriazone, VA30= Vancomycin, PB300= Polymyxin-B, AZM15= Azithromycin and CIP5= Ciprofloxacin.

The study recorded phenotypic expression of multidrug-resistant (n=10) occurring pattern among the *E. coli* and *Klebsiella* spp isolates. An isolate is said to be a multiple drug resistant type, only if it is resistant to at least one or more antimicrobials from more than 3 different classes of antimicrobials (resistant to ≥3 antimicrobials of different classes). Based on that, most of the isolates appeared to be resistant to one or more ESBLs antimicrobials, indicating that, they are β-lactam producing *E. coli* and *Klebsiella* species. This may be attributed to indiscriminate use of antimicrobials in poultry industries which is implicated in development of resistance. This finding agrees with that of Rasheed *et al.* (2014) who reported that multidrug resistance is on the increase in foodborne pathogens. As such food-handler training, food premise inspections, and community-based education programs promoting proper food handling and preparation techniques are effective components in reducing public exposure to AMR due to food-borne pathogens (Karmali et al. 2010).

The study also revealed that *E. coli* had the highest prevalence of 46.4% as compared to that of *Klebsiella* species (7.3%). This might be attributed to the fact that *E. coli* is an environmental contaminant, coliforms detected in water sources, hands of poultry handlers and containers. In this line, similar higher prevalence rates (48.4%) of *E. coli* have been reported in poultry and poultry products in Morocco (Cohen *et al*., 2007), 98% in India (Sharma and Chattopadhyay, 2015). This may likely to be due to the increased use of reused water during the processing of chicken carcasses in Northern Nigeria. Consequently, good water quality and close monitoring of water are required during the entire processing of chicken meat. This can only be achieved by employing a stringent water management system from the source to the processing steps. This is because water is a potential vehicle for direct transmission of bacteria in most developing countries, and serve as a leading cause of water borne-disease (WHO, 2015). On the contrary, Adzitey *et* al. (2011) had reported a lower prevalence of 16% in Tamale, Ghana, indicating the possible risk of food-borne infections in poorly cooked chicken meat.

The sources of these *E. coli* and *Klebsiella* species in this study may be due to faecal contamination during processing by endogenous *E. coli*, and exogenous by cross-contamination from the environment, fomites such as knives, clothes of butchers and meat display tables, water used for washing carcasses and hands of butchers, meat-retailers and buyers. Similarly, the same scenario of meat contamination was reported by Okorie-Kanu *et al*. (2020) in the southeastern part of Nigeria. Contamination of carcasses do occur through skin-to-carcass or faecal-to-carcass transfer of the pathogen during the slaughter process at processing plants which is the major risk factor for human infection. Additionally, cross-contamination can occur during further processing of carcasses in the processing plants, during distribution and storage of beef and chicken meat as described by Abdissa *et al*. (2017).

The critical control points in the chicken-meat production and certain cross-contamination events have been described by various authors (Agerso *et al*., 2014; Börjesson *et al.,* 2016; Steve, 2017). The study had established that *E. coli* is one of

the major contaminants in broiler chickens at all critical control points compared with *Klebsiella* species as earlier reported by Mohamed-Noor *et al.* (2012) in Khartoum, Sudan. The rate at which *E. coli* isolates are detected in ready-to-eat meats in the study area solely depends on management practices of the deep litter system and processing of chicken meat. Goksoy *et al.* (2004) reported that chickens are susceptible to infection by a variety of bacteria especially those that are pathogenic. This may partially be attributed to their rearing systems, especially the deep litter system, which allows chicken droppings to be soaked within the deep litter system allowing enteric microbial contamination within the poultry houses.

In this study, the majority of *E. coli* and *Klebsiella* isolates show resistance to one or more of the antimicrobials used, with high-level resistance to the beta-lactam antimicrobial agents. This may likely be due to the principles of biofilm and induction gene transfer in the bacterial population. In concordance with the above findings, Projan, (2010), reported that wastewater from processing facilities contributes to the spread of multidrug-resistant bacteria into the environment. This consequently potentiates the increase in AMR in food animals and to public through consumption of such meats containing those antibiotic residues (NAP-AMR, 2017).

Table 5: Antimicrobial Resistance Patterns and Multiple Antimicrobial Resistance (MAR) index profile for the *E. coli* (swab) isolates from dressed chicken meat in MMC and Jere LGAs.

Swab/Intestinal Isolates	No. of Antimicrobials each Isolate was resistance to (a)	Multi-drug Resistance Patterns	MARS index (a/b)
MmS 1	9	AZM,PB,VA.CRO,CAZ, KF,AMP,AMC,PG	0.9
MmS5	10	CIP,AMS,PB,VA,CRO,CAZ, KF,AMP,AMC,PGAZM,PB,	1
MmS 12	9	VA,CRO,CAZ, KF,AMP,AMC,PG	0.9
TbS 2	10	CIP,AMS,PB,VA,CRO,CAZ, KF,AMP,AMC,PG	1
TbS 3	9	AZM,PB,VA,CRO,CAZ,KF, AMP,AMC,PG	0.9
TbS 4	8	AZM,PB,VA,CAZ,KF,AMP, AMC,PG	0.8
AS 8	10	CIP,AMS,PB,VA,CRO,CAZ, KF,AMP,AMC,PG	1
AS 11	10	CIP,AMS,PB,VA,CRO,CAZ, KF,AMP,AMC,PG	1
AS 12	10	CIP,AMS,PB,VA,CRO,CAZ, KF,AMP,AMC,PG	1

Notes: a= No. of antimicrobials each isolate was resistant to. b= No. of antimicrobials used. MAR=Multiple Antimicrobial Resistance, MARS index= (a/b)

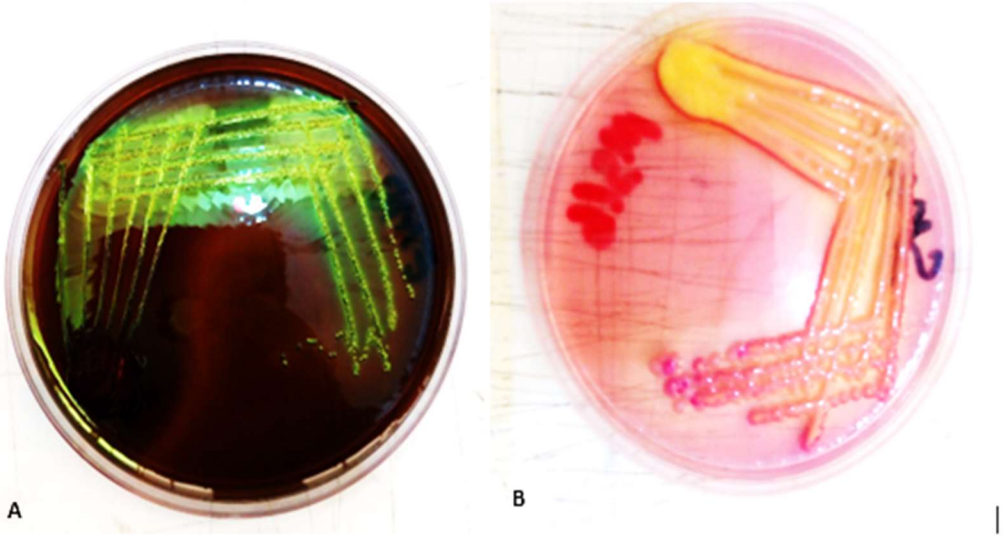

Figure 1: (A) A Photograph depicting *E. coli* isolates on Eosine Methylene Blue (EMB) med-ium. (B) *Klebsiella* species on Sorbitol MacConkey Agar (SMAC) medium.

Resistance to antimicrobial agents was observed in this study, to many classes of antimicrobials used, such as glycopeptides, polymyxins, and quinolones. But some of the isolates had shown a considerable number of susceptibilities to ciprofloxacin, azithromycin and polymyxin B, of the

quinolones, macrolides and polymyxins families respectively.

Previous researches had reported on the exchange of genetic materials, particularly the resistance and virulence gene under the concept of the animal-human-environmental interface (Iramiot *et al*., 2020). Furthermore, the study revealed that, a total eight (8) of the *E. coli* and *Klebsiella* isolates in this study showed multidrug-resistant expression with multiple patterns on antibiogram profile, signifying the challenges in the emergence of MDR species with consequent relapse in the clinical treatment of many foodborne diseases and other complications. These findings would therefore, raise awareness which could contribute to the development of effective policies and interventions for antibiotic stewardship in poultry production in Nigeria.

Similarly, Katakwebe *et al.* (2012) find out that there has been a low knowledge of antibiotic stewardship in countries such as Tanzania and Nigeria. In Nigeria, it was found that the majority of farmers were neither aware of nor comply with the mandatory withdrawal period after administering antibiotics (Nsofor and Iroegbu, 2013), and that majority of farmers do not seek veterinary advice for disease diagnosis or an antibiotic prescription but, relied on personal experience, advice from other farmers or folklore. In contrast, most high-income countries have established national monitoring programs to control the spread of antibiotic-resistant bacteria.

Conclusion

The average value of microbial quality of dressed chicken meat found in this study was 6.4 x 10^7 CFU/g. This suggests that dressed chicken meat may be a potential source of food-borne pathogens and a threat to public health if not properly processed. The study also recorded a high prevalence of pathogenic *E. coli* 178 (46.4%) and *Klebsiella* spp. 28 (7.3 %) and majority of the *E. coli* and *Klebsiella* isolates expressed high level of resistance to the beta-lactam antimicrobial agents used in this study, as high as 14 (93.3%) and 5(71.4%) respectively.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

ASS designed the work, supervised and drafted the manuscript; ARP and ASS conducted the research work, collected the samples and carried out the laboratory analysis; MS helped in the acquisition, analysis and interpretation of data for the work; EFE revised the contents of the draft critically and intellectually; BA and EW contributed immensely in the laboratory, especially the microbiological analysis. All authors read and approved the final version.

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