Incidence and Antibiotic Resistance Pattern of *Klebsiella* Species Isolated From Poultry Feaces in Owo Metropolis

ADELUWOYE-AJAYI OLAYEMI AMOS[1] BELLO, ADEDOYIN AYOWOLE[2]
[1, 2] *Department of Science Laboratory Technology (Microbiology Unit), Faculty of Applied Sciences, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria*

Abstract- ***The wrong and excessive use of antibiotics in the poultry industry poses a threat to public health due to the release of antibiotic-resistant bacteria into the environment and its impact on consumers of poultry products. This research aimed to investigate the occurrence and patterns of antibiotic resistance found in Klebsiella species isolated from poultry faeces in Owo metropolis. Fecal samples were collected from healthy broilers in three privately-owned poultry farms in Owo, using sterile universal bottles and spatulas. Klebsiella species were isolated using MacConkey Agar, and their characteristics on Nutrient Agar were also observed. The isolated Klebsiella species exhibited different characteristics on different media types. All samples from the three farms contained Klebsiella species that were resistant to AUG and CAZ were susceptible to GEN, IMP, AZT, CIP, and NIT. A MARI score of 0.29 was obtained which indicates the presence of multidrug-resistant Klebsiella species in the bird excrement from poultry farms in Owo. Consequently, poultry breeders in Owo should exercise caution when employing antibiotics.***

I. INTRODUCTION

The demand for poultry meat is increasing, primarily as a result of its acceptability by most societies, its comparatively low cholesterol content, and its egg products (Bolan et al., 2010). The poultry business includes a wide range of products, such as eggs, meats, goose fat and feathers which contribute to its growth (Wang and Orsi, 2013). In Nigeria, the poultry industry has become increasingly important in providing employment opportunities and animal food production (Emokaro et al., 2016; Mshelia et al., 2016). In 2013, the industry had approximately 180 million birds and produced 300,000 and 650,000 tonnes of meat and eggs, respectively (FAOSTAT, 2018). Of all the agricultural sub-sectors in Nigeria, the poultry sub-sector is the most commercialized, with poultry meat and eggs being a primary source of protein in many households (Awogbemi et al., 2018). Enteric bacterial pathogens in the poultry industry pose a threat to public health and can contribute to the transmission of zoonotic diseases (Anderson et al., 2016). One of these zoonotic diseases is foodborne illness, which is caused by agents that enter the body through contaminated food and is a significant public health concern (Tan et al., 2013). It remains a major problem worldwide as it affects people's well-being and has economic impacts (Akbar and Anal, 2013). Due to the high consumption of chicken meat, it is essential to take great care to ensure the safety of the industry against potential hazards (van der Sluijs et al., 2010).

Urinary tract infections (UTIs) of bacterial origin are a leading cause of morbidity and comorbidities, resulting in frequent visits to healthcare institutions worldwide (Gebremariam et al., 2019; Odoki et al., 2019; Susethira and Uma, 2016). Studies have shown that the most common causes of UTIs are gram-negative bacteria like *E. coli*, *Pseudomonas* species, *Klebsiella* spp., *Proteus* species, and Gram-positive bacteria like coagulase-negative staph (CoNS) and *S. aureus* (Odoki et al., 2019; Agalu et al., 2014; Chander and Shrestha, 2014; Sule and Kumurya, 2016).

Klebsiella spp. are a type of Gram-negative, rod-shaped, non-motile bacteria belonging to the family Enterobacteriaceae (Janda and Abbott, 2006; Abbot, 2007). They are the second most prevalent species of Enterobacteriaceae and can be found in soil, water, and food habitats as well as on the mucosal surfaces of mammals like humans and dogs (Nordmann et al., 2009). Typically, these bacteria often produce lysine decarboxylase but do not produce ornithine

decarboxylase and they test positive for the Voges-Proskauer test. The size of members of the Enterobacteriaceae family ranges from 0.3 to 1.0 mm in width to 0.6 to 6.0 m in length, and they are frequently facultative anaerobic (Abbot, 2007). *Klebsiella* species are frequently present in mucoid colonies. (Janda and Abbott, 2006; Euzeby, 2010). The species have 77 capsular antigens (K antigens), which set the serogroups apart.

Apart from being acknowledged as important prevalent causative agents in cases of nosocomial pneumonia, septicemia, urinary tract infections, wound infections, occurrences within intensive care units (ICUs), and instances of neonatal septicaemia, *Klebsiella* species are Hazard category 2 organisms (Janda and Abbott, 2006). A common nosocomial and community-associated pathogen, *Klebsiella pneumoniae* causes a variety of diseases in both people and animals (Cheng et al., 2018; Ripabelli et al., 2018). According to Janda & Abbott (2006) damage can only be caused by 108 *Klebsiella* organisms per gram of faeces.

Studies have reported on the widespread application of antibiotics in poultry farming for preventative measures, treating infections, and growth enhancement (Agyare *et al*., 2018; Boamah *et al*., 2016; Gregova *et al*., 2012). However, d ue to this practise, the number of antibiotic-resistant bacterial strains in the food chain has increased, which poses a significant threat to global health (Agyare et al., 2018; Boamah et al., 2016; Gregova et al., 2012; Guetaba et al., 2015; Adelowo et al., 2014; Donkor et al., 2012). Despite the availability of antibiotics, bacteria that are resistant to antibiotics can thrive. Moreover, the extensive spread of resistant genes through plasmids among different bacterial species in the digestive system has exacerbated this issue (Agyare et al., 2018; Davis et al., 2018; Hedman et al., 2020). It has also been reported that resistance plasmids being transferred between unrelated bacteria is a major contributor to the increase in antibiotic resistance (Agyare *et al*., 2018; Davis *et al*., 2018; Hedman *et al*., 2020).

According to Boeckel et al. (2015), the global use of antibiotics in livestock will increase from 63,000 tons to 105,000 tons by 2030. It is estimated that by 2050, antibiotic resistance will cause more than 10 million fatalities and close to $100 trillion USD in economic losses worldwide (Maestre-Carballa *et al*., 2019). Klein et al. (2018) have reported that these practises contribute to the broad spread of germs that are resistant to antibiotics in humans, cattle, and the environment. This has serious negative effects on patients' length of hospital stays, societal costs, and even deadly outcomes. Multidrug-resistant bacterial infections are linked to greater mortality, morbidity, and healthcare expenses (Ndir et al., 2016).

Antibiotics are widely used in poultry as growth enhancers, therapeutics and prophylaxis(Manyi-Loh *et al*., 2018). Antibiotic-resistant bacteria have been discovered in poultry waste, products, and the entire poultry environment (Davis et al., 2018; Hedman et al., 2020; Eibach et al., 2018; Sung et al., 2017). Antibiotics are frequently administered to chicken in order to treat diseases and for prophylaxis (Muthuma et al., 2016). Strains of bacteria resistant to antibiotics found in the digestive system of poultry easily contaminate poultry carcasses or eggs, and their consumption could potentially disrupt the natural balance of bacteria in the human digestive tract.The discharge of poultry products into the environment, inadequate cleanliness, and the persistence of bacteria in the environment can all be seen as contributing factors to the spread of drug-resistant variant strains (Manyi-Loh et al., 2018).

This present study places great importance on the occurrence and antibiotic resistance pattern of *Klebsiella* species found in the faeces of poultry, as these bacteria are considered opportunistic pathogens that pose a risk to the health of poultry, as well as humans who ingest them.Currently in Owo metropolis, there is a paucity of information on the incidence and antibiotic resistance pattern of *Klebsiella* species from poultry feaces hence the aim of the present study is to investigate the occurrence and antibiotic susceptibility profile of *Klebsiella* species. This particular objective was accomplished by isolating *Klebsiella* species from poultry faeces, determining the isolated *Klebsiella* species, calculating the frequency of the isolated *Klebsiella* species in the poultry faeces, and estimating the antibiotic resistance pattern and Multiple Antibiotic

Resistance Index (MARI) score of the isolated *Klebsiella* species.

II. MATERIALS AND METHODS

Study Area: The study areas were Tayo farm located in Iselu, Eric farm situated at Opomulero junction, and Museli farm in Oke-Ogun, all of which are located in Owo, Ondo State, Nigeria (Table 3.1).

Table 3.1: Description of the Sampling Stations.

SITE CODE	DESCRIPTION
A	It is a privately owned poultry farm situated in Iselu, Owo, where 100 broiler birds were raised using a deep litter system. The antibiotics administered included Augmentin, Ciprofloxacin, and Amoxicillin.
B	It is a privately-owned poultry farm situated at Opomulero, Owo. The farm housed a total of 150 broiler birds that were raised in enclosures. The antibiotics administered to the birds included Augmentin, Ciprofloxacin, and Gentamicin.
C	It is a privately-owned poultry farm situated in Oke-ogun, Owo, where 120 broilers were reared using a deep litter system. The administration of antibiotics including Augmentin, ofloxacin, and Amoxicillin was noted.

Sample Collection: Between October to November 2022, aseptic collection of freshly passed fecal samples was conducted from nine visibly healthy broilers. The samples were placed into properly labeled sterile capped universal bottles using a sterile spatula. These broilers were from two distinct privately-owned poultry farms located in Owo. Following collection, the samples were kept cool with ice packs and conveyed to the Microbiology unit laboratory in the Department of Science Laboratory Technology at RUGIPO for prompt bacteriological investigation.

Ethical Approval and Informed Consents: Although ethical approval was not necessary, verbal consent was obtained from both the farm owners and workers during the sample collection process.

Isolation of *Klebsiella* species: To prepare the poultry fecal samples for analysis, 1g was mixed with 10ml of de-ionized water to create a stock solution. The stock was then subjected to tenfold serial dilution, and 1ml of each dilution (10-2, 10-4, and 10-6) was dipensed on sterile Petri dishes labeled accordingly. Eosin methylene blue (EMB) and MacConkey (MAC) agar cooled to about 45^{o}Cwere dispensed separately into the aliquots of samples aseptically and gently swirled. The plates were then allowed to solidify and incubated for 24-48 hours at 35-370C to promote the growth of Klebsiella species (Egea et al., 2012). Subsequently, distinct colonies were sub-cultured on freshly prepared MAC, and repeated streaking was performed to obtain pure cultures of Klebsiella species for further analysis. Klebsiella species colonies typically appear as pink or red on MAC agar. Finally, all the suspected Klebsiella species isolates were identified using standard microbiological techniques, as outlined by Cheesbrough (2010).

Morphological Characterization of Isolates: Upon obtaining a 24-hour old pure culture of the isolates, the different morphologies were morphologically characterized and noted and recorded, according to established procedures (Sohani and Sanjeeda, 2012).
Gam Staining: A smear was made on a clean microscope slide using the 24-hour old culture of the test isolate, which was then heat-fixed. The slide was stained with crystal violet solution for a duration of 1 minute, rinsed with water, and Lugol's iodine solution was applied for another 1 minute. After draining off the Lugol's iodine solution, the slide was rinsed with water and subsequently decolorized using a few drops of 95% ethanol for 20 seconds. The slide was then counter-stained with safranin and allowed to dry. The stained slide was viewed using oil immersion magnification. Following the established principles, Gram-negative cells were observed to be decolorized by alcohol and appeared pink to red in color, while Gram-positive cells appeared purple in color (Sohani and Sanjeeda, 2012).

Biochemical Characterization of the Isolates: The bacterial isolates underwent further identification

through a battery of biochemical tests following standard protocol. The panel of tests conducted comprised of motility, catalase, citrate, indole, Methyl Red, and Voges-Proskauer test.

Antimicrobial susceptibility test of the *Klebsiella* species:The Kirby-Bauer disk diffusion method, as described by Jayabarath (2015), was employed to carry out antimicrobial susceptibility testing on *Klebsiell*a species. Bacteria that had recently been grown were mixed with 5 ml of sterile nutrient broth to create the bacterial inoculum. The turbidity of this inoculum was then adjusted to 0.5 McFarland standards.The antimicrobial susceptibility testing was executed on Mueller-Hinton agar, using antibiotics such as beta-lactam combination agent (augmentin 20/10µg), cephem (ceftazidime 30µg), carbapenem (imipenem 10µg), aminoglycosides (gentamicin 10µg), fluoroquinolone (ciprofloxacin 5µg), monobactam (aztreonam 30µg) and nitrofurans (nitrofurantoin 300µg). The plates were then incubated aerobically at 37°C for 24 hours. The areas where bacterial growth was suppressed (zone of inhibition) were assessed using a meter rule, and the results were recorded and analysed according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) (2020).

Multiple Antibiotic Resistance Index of isolates: As described by Ekwealor et al. (2016), the calculation of the Multiple Antibiotic Resistance Index (MARI) involved employing the provided equation below. This index was derived by assessing whether a zone of inhibition was present or absent. The MARI value was determined by dividing the total count of antibiotics to which an isolate demonstrated resistance by the total count of antibiotics administered to each strain.

$$\text{MARI} = \frac{\text{Number of antibiotics strain resistant}}{\text{Number of antibiotics strain subjected}}$$

III. RESULTS AND DISCUSSION

Results

Morphological, Cultural and Staining Characteristics of the Isolates*:* The isolates were identified using the colony characteristics on the various bacteriological media shown in table 4.1.1.

Gram Reaction and Biochemical Characterization of the Isolates: The isolates were all rod-shaped, Gram-negative bacteria. They were positive for catalase, citrate, and Voges-Proskauer, but negative for motility, indole, and methyl red (table 4.1.2).

Incidence of the Isolates in the Poultry Feaces: The prevalence of Klebsiella species in the poultry faeces collected from the three poultry farms was displayed in Table 4.1.3. There were *Klebsiella* species present in every sample from each of the three farms.

Antibiotic Susceptibility Patterns of *Klebsiella* speciesIsolated from Poultry Feaces and their Multiple Antibiotic Resistance Index (MARI) Score: The rate of resistance and susceptibility to the test antibiotics displayed by Klebsiella species isolated from poultry faeces was shown in Table 4.1.4. The Klebsiella species isolates from the poultry faeces obtained from the three farms were sensitive to GEN, IMP, AZT, CIP, and NIT, but resistant to AUG and CAZ, respectively. Their MARI score was 0.29.

Table 4.1.1: Morphological, Cultural and Staining Characteristics of the Isolates from the Poultry Feaces

S/N	Media Used	Colony Characteristics	Morphology (Staining Characters)
1	MacConkey Agar	Large, circular, mucoid, and pink to red in colour colonies	Gram-negative, pink colour, small rod shaped
2	Nutrient Agar	Large, circular, mucoid, and white in colour colonies	appearance, arranged in single or paired short

KEY: S/N = Serial number

Table 4.1.2: Biochemical Characteristics of the Isolates from the Poultry Feaces

S/N	Isolates Code	MOT	CAT	CIT	IND	MR	VP	Probable Organism

1	FA	-	+	+	-	-	+	*Klebsiella* species
2	FB	-	+	+	-	-	+	*Klebsiella* species
3	FC	-	+	+	-	-	+	*Klebsiella* species

KEY: S/N = Serial number, + = Positive, - = Negative, MOT = Motility, CAT = Catalase, CIT = Citrate, IND = Indole, MR = Methyl red, VP = Voges-proskauer

Table 4.1.3: Incidence of the *Klebsiella* species in the Poultry Feaces Collected from the three Poultry Farms

Farm Code	Incidence
A	+
B	+
C	+

KEY: + = Presence of *Pseudomonas* species

Table 4.1.4: Antibiotic Susceptibility Patterns of *Klebsiella* speciesIsolated from Poultry Feaces and their Multiple Antibiotic Resistance Index (MARI) Score:

S/N	Isolate Code	Antibiotics							MARI	Identified Isolates
		Zone of Inhibition (mm)								
		GEN	AUG	IMP	NIT	CAZ	ATM	CIP		
1	FA	(18.50) S	(11.50) R	(25.20) S	(18.00) S	(13.20) R	(22.20) S	(32.00) S	0.29	*Klebsiella* species
2	FB	(16.20) S	(12.40) R	(26.30) S	(19.20) S	(14.10) R	(23.20) S	(33.20) S	0.29	
3	FC	(17.30) S	(11.70) R	(30.30) S	(20.10) S	(15.20) R	(24.30) S	(34.40) S	0.29	

KEY: GEN = Gentamicin, AUG = Augmentin, IMP= Imipenem, NIT = Nitrofurantoin, CAZ = Ceftazidime, ATM = Aztreonam, CIP = Ciprofloxacin, R = Resistance, S = Susceptible

- Discussion

Throughout the years, it has been well-documented that the poultry industry and its products can harbor antibiotic-resistant bacteria (Blaak et al., 2015; Blaak et al., 2014; Rasmussen et al., 2015; García-Vello et al., 2020), which can be transmitted to humans via contact with contaminated products or surfaces (Agyare et al., 2018; Hedman et al., 2020; Aniokette et al., 2016). This presents a significant public health concern, as antibiotic-resistant bacteria can cause severe illness or even death, and can be challenging to treat (Blaak et al., 2015; García-Vello et al., 2020). Therefore, the spread of antibiotic-resistant bacteria from poultry to humans and the environment is a major global issue. The goal of this research is to determine the prevalence and antibiotic resistance profile of Klebsiella species found in the faeces of poultry in the Owo metropolis.

The isolated Klebsiella species in this investigation had big, round, mucoid colonies that were pink to red in color on MacConkey agar and large, circular, mucoid colonies that were white on Nutrient agar. These findings agreed with the traits of Klebsiella species that had previously been proposed by other researchers (Omeike et al., 2022)

All the isolates tested positive for catalase, citrate, and voges Prauskauer but negative for motility, leading to the identification of the isolates as Klebsiella species using indole methyl red and methyl red. These results are consistent with the distinctive biochemical traits of Klebsiella species that Omeike et al. had previously proposed (2022).

The results of this study, which revealed the presence of Klebsiella species in all three farms visited, contradict the findings of Omoeike et al. (2022), who reported the absence of Klebsiella species in one of the farms sampled. This variation in results could be attributed to the discrepancy in the number of farms from which samples were collected. The present research gathered poultry fecal samples from three farms, while Omeike et al. collected their samples from five farms.

Based on the findings of this study, all Klebsiella species obtained from the fecal samples collected from the three poultry farms were susceptible to GEN, IMP, NIT, CIP, and ATM, but resistant to AUG and CAZ. These findings are consistent with a previous study (Omeike et al., 2022).

The Multiple Antibiotic Resistance Index (MARI) determines the level of an isolate's resistance to the antibiotics tested, and a value of ≥ 0.2 indicates a high risk of contamination from sources with high antibiotic use (Thenmozhi et al., 2014). The MARI index of 0.29 observed in this study is comparable but lower than the 0.73 reported in a previous study on poultry farms in another location in Nigeria (Omeike et al., 2022).

CONCLUSION AND REOMMENDATION

Conclusion

The research suggests that poultry faeces can harbour *Klebsiella* species, which have the potential to transfer antibiotic resistance genes to humans. Moreover, the utilization of poultry droppings as fertilizer raises apprehensions because it poses health hazards to the public if the crops and vegetables grown with it are ingested.

Recommendation

The study calls for close monitoring of antibiotic resistance in our environment and controlled use of antibiotics in poultry since the birds serve as a source of protein and a staple in many homes.

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